

Developmental Programming, Life-History Trade-Offs, and the Mechanisms that Drive them: Insights from Australian Skinks

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I, Dalton C. Leibold, declare that this thesis is based on my own original research. When collaborative research was conducted, the extent of my contribution and the contributions of the collaborators is properly indicated.

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Keep moving forward.

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Abstract

Conditions experienced during embryonic development can have long-lasting, “programmatic” effects on an organism’s phenotype and fitness. Developmental programming may be initiated through the direct influence of the developmental environment or mediated via maternal phenotypes like resource investment, nesting behaviour, or hormone transfer. The individual effects of the developmental environment and maternal phenotypes on offspring traits have long been appreciated. However, developing individuals are simultaneously exposed to variation in both the environment and maternal phenotypes, and how these effects interact is poorly understood. Additionally, while developmental effects are frequently examined early in life, relatively few studies track their long-term consequences. Furthermore, developmental effects are context-dependent, with the magnitude and directionality of effects potentially differing among species or under varying postnatal conditions. Consequently, recent work in the developmental programming space has called for multi-species, longitudinal studies, that simultaneously quantify key fitness-associated traits alongside physiological or behavioural traits that may facilitate developmental effects.

My thesis work aimed to answer this call by testing how the developmental thermal environment and maternally-derived stress hormones (corticosterone) jointly influence the growth and survival of two congeneric lizards that differ in reproductive investment strategy (*Lampropholis delicata* and *Lampropholis guichenoti*). I also quantified organismal metabolism, cellular physiology, and cognitive performance as potential physiological and behavioural mechanisms underlying developmental effects. To rapidly and simultaneously quantify multiple biomarkers of cellular physiology, I developed functional assay panels for flow cytometric analysis that measure cellular morphology (cell size, mitochondrial mass), mitochondrial function (mitochondrial membrane potential, mitochondrial superoxide), and oxidative damage (lipid peroxidation, 8-OHdG). I applied these functional assay panels to test the effects of developmental CORT and incubation temperature on lizards’ liver, heart, and brain cells, which are associated with growth, longevity, and cognition, respectively. This thesis, therefore, addresses the questions:

- 1) Do multiple developmental conditions interact to affect early life fitness (hatching success) and phenotypes (hatchling body size)?
- 2) Do developmental effects have long-term effects on key life-history traits and tradeoffs, like growth and longevity?
- 3) Are developmental effects on life-history traits driven by changes in organismal metabolism, cellular physiology, or cognitive ability?
- 4) Are the effects of maternal stress and incubation temperature mediated by variation in maternal reproductive investment strategy, both within- and between- species?

Exposure to elevated corticosterone impaired hatching success, but only in *L. delicata*, which lays smaller but more numerous eggs than *L. guichenoti*. Corticosterone and incubation at warm temperatures induced hatchlings of both *L. delicata* and *L. guichenoti* to hatch at reduced body mass, though the negative effects were more pronounced in *L. delicata*. However, variation in egg mass was the strongest predictor of hatching success and hatchling size in both species. The negative effects of developmental corticosterone and incubation temperature on body size not only persisted, but grew more pronounced, in adulthood. Both *L. delicata* and *L. guichenoti* treated with corticosterone, incubated at warm temperatures, or both were smaller at adulthood than conspecifics receiving an ethanol control or incubated at cool temperatures. These reduced adult body sizes persisted despite corticosterone and warm incubation inducing *L. delicata* and *L. guichenoti* to grow more rapidly in early life. In contrast to general expectations of life-history theory, the rapid growth induced by corticosterone and warm incubation did

not trade off against long-term survival, and indeed survival was high in both species regardless of developmental treatments. The effects of developmental corticosterone and incubation temperature on body size and growth were seemingly not driven by underlying changes in metabolism, as the resting metabolic rates of gas exchange in juvenile *L. delicata* and *L. guichenoti* were independent of developmental treatments. Likewise, corticosterone and incubation temperature had limited influence on cellular morphology, mitochondrial function, or oxidative damage in liver and heart cells, and this was true both when lizards were juveniles and as adults. Developmental corticosterone and incubation temperature also did not affect the cognitive performance of juvenile *L. delicata* in spatial learning tasks, nor cellular physiology in the medial cortex region of the brain associated with spatial learning. However, superoxide levels increased with mitochondrial membrane potential in the medial cortex, without a concomitant increase in oxidative damage markers.

Longitudinal studies on *L. delicata* and *L. guichenoti* found that developmental corticosterone and incubation temperature both affect key life-history traits like hatching success, body size, and growth but that their effects are additive rather than interactive. Lizards exposed to elevated corticosterone or warm incubation temperatures hatched at reduced sizes, grew rapidly early in life, but then ultimately reached reduced sizes at adulthood, consistent with a pattern of partial compensatory growth. Lizards may, therefore, grow rapidly to improve survival early in life, at the cost of reduced performance in adulthood. However, this pattern of partial compensatory growth did not trade off against survival to late adulthood, and overall high survival rates suggest that mortality in these species is driven primarily by external factors like predation or food scarcity rather than longevity. The effects of developmental treatments on growth and body size were not mediated by changes in organismal metabolism or the programming of mitochondrial and oxidative physiology in liver or heart cells. Variation in maternal investment strategy, both within- and among- species, was an important mediator of developmental effects in early life, with greater maternal investment improving hatching success and buffering offspring from the negative effects of corticosterone and incubation temperature on hatchling size. However, the growth trajectories of both *L. delicata* and *L. guichenoti* responded similarly to developmental stressors, suggesting conserved effects across closely related species. Developmental treatments also did not affect spatial learning or physiology in brain cells of juvenile *L. delicata*, suggesting a limited role of cognitive performance in facilitating the rapid growth observed. Yet, there remain alternative mechanistic pathways and ecological contexts that may reveal a more complex relationship between developmental environments and life-history trajectories, which warrants further investigation.

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Introduction

Early developmental environments have long-lasting impacts on organisms' physiology and fitness

A central goal of evolutionary ecology is to identify the origins of phenotypic variation not only among, but within species. Phenotypic variation arises through the interactions between an individual's genotype and the environment in which it is expressed (Via and Lande 1985, Bull 1987). For most organisms, perhaps no environment is as important as the one in which they develop during embryonic stages. Developmental environments experienced early in life are a critical period during which key biological systems (e.g., immune function, metabolism, cellular physiology) and fitness-associated traits (e.g., body size, developmental rate, or sex) are shaped. Early developmental periods are also highly sensitive to environmental variation and lead to organizational effects that impart long-lasting changes to organism phenotype (Pigliucci 2001, West-Eberhard 2003, Moczek 2015). Consequently, variation in developmental conditions can induce phenotypic plasticity (Pigliucci 2001, "developmental plasticity" *in sensu* West-Eberhard 2003), which may have fitness consequences that persist into adulthood (Mousseau and Dingle 1991). Developmental plasticity has been linked to variation in fitness-related traits, including growth, metabolism, immune function, ageing, and reproductive senescence across diverse taxa (Lindstrom 1999, Monaghan 2008, Cooper and Kruuk 2018). However, developmental plasticity may be either adaptive or maladaptive depending on environmental context (Ghalambor et al. 2007, Forsman 2014). Plastic responses can be adaptive if they are predictive of the environment experienced later in life (e.g., "predictive adaptive responses" – Gluckman et al. 2005, Bateson et al. 2014, "thrifty phenotype" – Hales and Barker 1992, Wells 2010). However, mismatches between traits and environments later in life also are known to result in non-adaptive responses – particularly when novel environmental stressors affect development (reviewed in Bateson et al. 2004, Rickard and Lummaa 2007).

Environmental conditions, such as temperature (Noble et al. 2017, While et al. 2018), moisture (Brown and Shine 2006, Bodensteiner et al. 2014, Bell et al. 2025), oxygen availability (Sun et al. 2014, Cordero et al. 2017, Parker and Dimkovikj 2018), and even predation pressure (Chivers et al. 2001, Coslovsky and Richner 2011) can act directly on developing individuals to shape key life-history traits. Environmental developmental conditions are especially influential for oviparous species, for whom embryos develop externally to the mother and have limited capacity to buffer themselves from environmental variation (Telemeco et al. 2016, Cordero et al. 2018, Du et al. 2013, but see Du and Shine 2014). Incubation temperature, in particular, is recognized as a critical determinant of developmental plasticity in oviparous taxa (reptiles: Deeming 2004, Booth 2006, 2018, birds: DuRant et al. 2013), owing to the pervasive influence of temperature on biochemical reaction rates, metabolic activity, and the timing of development (Gillooly et al. 2002, Angilletta 2009). Consequently, incubation temperature can generate variation in fitness-associated traits such as growth rates, morphology, physiological performance, and — in some taxa — sex (Noble et al. 2017, meta-analyses: While et al. 2018, Massey and Hutchings 2021, Raynal et al. 2022, Zhang et al. 2025). Although developmental environments like incubation temperature act directly on embryos, the phenotypes they produce

in offspring may vary by context (e.g., mean temperature, variability or fluctuation) and are simultaneously influenced indirectly by the environment via “maternal effects” (*in sensu* Wolf and Wade 2009).

Mothers shape developmental environments of their offspring as much as direct environmental experiences of offspring themselves

Beyond the direct effects of the environment, mothers can influence offspring development through maternal effects – a form of non-genetic inheritance in which the maternal phenotype shapes the phenotype of the offspring (Mousseau and Fox 1998, Wolf and Wade 2009). Historically, maternal effects were viewed in quantitative genetics as a source of nuisance variation that obscured patterns of genetic inheritance (Wolf and Wade 2009), but they are now recognized as a major source of developmental plasticity and phenotypic variation (Rasanen and Kruukt 2007, Badyaev 2008, Badyaev and Uller 2009). Because the maternal phenotype itself depends on the environment in which it is expressed, maternal effects provide an indirect pathway through which the maternal environment shapes offspring developmental environment and therefore offspring phenotype. Mothers may influence the developmental environment of their offspring either during embryonic development, or the early postnatal environment. Maternal effects may arise through altered allocation of resources (Bernardo 1996) or hormones (Schoech et al. 2011) to eggs, modification of nesting behaviour (e.g., nest-site selection or burrowing - Mitchell et al. 2013, Li et al. 2017, Pruett et al. 2019), or changes in postnatal care (Royle et al. 2016, e.g., guarding or provisioning - reviewed in Royle et al. 2025). Maternally-mediated hormone transfer – particularly of glucocorticoids – has received considerable attention as a mechanism by which mothers can transmit information about their environment to developing offspring (Groothuis et al. 2005, Meylan et al. 2012, Crino and Breuner 2015, Crino et al. 2025).

In vertebrates, circulating glucocorticoids become elevated in response to environmental stressors such as food limitation, predation pressure, or temperature extremes (Sapolsky et al. 2000, Wingfield and Kitaysky 2002, Romero 2004). Glucocorticoids primarily function in mediating the physiological stress response following activation of the hypothalamic–pituitary–adrenal (HPA) axis (Sapolsky et al. 2000). These hormones mobilize energy stores and alter physiology and behaviour to help organisms cope with changing conditions (Wingfield et al. 1998, McEwen and Wingfield 2003, Romero et al. 2009). Thus, circulating glucocorticoid levels fluctuate with the maternal stress environment. In viviparous species, circulating glucocorticoids can be transferred to developing offspring via placental connections, whereas in oviparous species they are deposited into yolk during vitellogenesis (Hayward and Wingfield 2004, Itonaga et al. 2011). Elevated maternal corticosterone (CORT) – the primary glucocorticoid in birds and reptiles – therefore provides a physiological route by which the maternal stress environment influences offspring development.

Experimental manipulations of maternal or yolk CORT generally show that increased CORT exposure leads to reduced offspring size or slower growth but can also enhance performance under stressful postnatal conditions (e.g., Meylan and Clobert 2005, Warner et al.

2009, Palacios et al. 2022). Thus, maternally transferred CORT may function as both an anticipatory signal, preparing offspring for environments similar to those encountered by their mothers (“predictive adaptive response,” Gluckman et al. 2005), or as a developmental constraint that impairs fitness-associated traits in offspring. The long-term effects of developmental CORT exposure also depend on the timing, magnitude, and duration of exposure, as well as how it may interact with other environmental factors, like incubation temperature, to drive phenotypic plasticity.

Interactions between maternal effects and offspring environmental conditions are seldom measured in tandem, leaving an important knowledge gap

While the independent roles of developmental CORT and incubation temperature on offspring phenotypes have been studied extensively in nature, embryos are simultaneously exposed to variation in both, yet the two are nearly always treated separately. CORT is known to be influenced dramatically by thermal environments mothers experience, making it likely that interactions between CORT and early offspring thermal environments are prevalent in nature (Jessop et al. 2016, Crino et al. 2025). For example, fence lizards (*Sceloporus undulatus*) subjected to higher temperatures had higher CORT levels (Racic et al. 2020). Likewise, female Jacky dragons (*Amphibolurus muricatus*) held under prolonged basking regimes (i.e., hotter temperatures) had greater circulating CORT during gravidity and produced smaller offspring (Liu et al. 2020). Simultaneous treatment of delicate skink (*Lampropholis delicata*) eggs with CORT and incubation at warm temperatures induced reduced hatchling body sizes and impaired mitochondrial efficiency in adulthood (Crino et al. 2024). Thus, variation in both maternally-derived CORT and environmental temperatures is likely to co-occur in nature and carry phenotypic consequences for offspring, potentially via shared mechanisms.

Mechanistically, both CORT and incubation temperature regulate metabolism and energy use: elevated CORT stimulates gluconeogenesis and mobilizes energy reserves (Sapolsky et al. 2000), while higher incubation temperatures accelerate metabolism and biochemical reaction rates (Schulte 2015). As CORT and temperature act on similar energetic pathways, and are experienced concurrently during development, they may interact to alter growth, performance, and physiological condition in complex, unpredictable ways. In oviparous taxa, where embryos develop outside the mother, cues from maternal and developmental environments may not always align. Mismatches between the maternal stress environment and incubation temperature may provide conflicting developmental signals, producing phenotypes that are poorly suited to the postnatal environment [‘developmental mismatch’; Gluckman et al. (2019)]. Conversely, maternal CORT and incubation temperature may act additively or buffer each other’s effects—compounding costs when energetic demands are high or producing compensatory responses under some thermal regimes (Jackson et al. 2021, Crino et al. 2025). In some cases, the phenotypic effects of interacting maternal effects and developmental environments may not manifest until later life, and their expression is dependent on the postnatal environment (‘hidden phenotypic plasticity,’ Metcalfe 2024). Despite extensive research on the independent roles of maternal CORT and incubation temperature, few studies have tested their interactive effects or followed resulting phenotypes across ontogeny (but see Crino et al. 2024, Recio et al. 2025a,

2025b). Understanding how these developmental environments interact is thus crucial for predicting offspring performance, survival, and the life-history trade-offs that emerge from early-life stress, particularly by revealing the physiological mechanisms that mediate developmental effects.

Developmental plasticity in response to early environments are underpinned by physiological changes that affect life history and fitness

Life-history theory predicts that organisms face fundamental trade-offs in allocating limited energetic resources toward growth, reproduction, and self-maintenance (Stearns 1989, 1992). These trade-offs arise because the physiological processes that support rapid growth and performance often incur costs to long-term maintenance and survival. Metabolic rate, mitochondrial function, and oxidative balance form the physiological basis of these trade-offs, linking cellular bioenergetics to life-history trajectories (Brown et al. 2004, Speakman et al. 2015, Koch et al. 2021). Three frameworks have emerged as likely explanations for the mechanistic basis of life-history traits and trade-offs: the Metabolic Theory of Ecology (Brown et al. 2004, 2024), the Oxidative Stress Theory of Ageing (Monaghan et al. 2009, Costantini 2019), and the Pace-of-Life Syndrome hypothesis (Montiglio et al. 2018, Vasilieva 2022). The Metabolic Theory of Ecology broadly proposes that most life-history traits, such as growth, development, and performance in fitness-associated tasks, are driven by metabolic rate and efficiency. The Oxidative Stress Theory of Ageing is a refined version of the traditional “free-radical theory of ageing” (Harman 1956), which proposes that oxidative damage to DNA, lipids, and proteins caused by reactive oxygen species (ROS) determines an individual’s lifespan and rate of ageing. In the modern era, the oxidative stress and free-radical theories of ageing have been expanded to include mitochondrial dynamics, as mitochondria are a primary source of ROS production [the “mitochondrial free-radical theory of ageing”; Barja (2014); Koch et al. (2021)]. The Pace-of-Life Syndrome hypothesis proposes that an organism’s “pace of life” is determined by the trade-offs between investment into current performance (i.e., growth, metabolism, current survival) versus future reproductive potential (i.e., long-term survival). Together, these frameworks propose that high metabolic rates promote rapid growth and activity but also elevate production of reactive oxygen species (ROS), which damage DNA, lipids, and proteins, reducing longevity and future reproductive potential. Lower metabolic rates may also constrain growth and delay or impair reproduction but enhance maintenance and survival. Thus, mitochondrial function and oxidative stress dynamics represent key physiological mediators of the growth–survival trade-off that underpins variation in life-history strategies (Ricklefs and Wikelski 2002).

Mitochondria sit at the nexus between energy metabolism and oxidative stress. As the primary site of ATP production, they determine both the efficiency of energy use and the rate of ROS generation (Brand 2000, Koch et al. 2021). Developmental programming of mitochondrial density, efficiency, or repair capacity can therefore have lasting effects on organismal performance and longevity (Gyllenhammer et al. 2025) – both incubation temperature and maternal CORT influence mitochondrial physiology and oxidative pathways. Elevated temperatures generally accelerate metabolism during development, increasing energetic demand and potentially elevating ROS production (Nord and Nilsson 2011, Sun et al. 2015). Similarly,

exposure to elevated CORT stimulates gluconeogenesis and energy mobilization, which can induce oxidative damage or alter mitochondrial efficiency (Costantini et al. 2011, Majer et al. 2023, Crino et al. 2024, Haussmann et al. 2024). The combination of these stressors could therefore have additive or compounding effects on mitochondrial function and oxidative balance, shaping growth, survival, and lifespan. Integrating measures of mitochondrial function and oxidative damage alongside changes to key life-history traits offers a mechanistic framework for understanding how developmental environments influence the physiological trade-offs that define an organism's pace of life. However, such studies are largely logistically unfeasible, owing in part to difficulties in tracking the phenotypes (e.g., growth and survival) of free-living animals throughout long lifespans (Speakman et al. 2015). Additionally, high-throughput and affordable methods for quantifying multiple physiological biomarkers expected to be correlated with life-history traits and measures of fitness (e.g., mitochondrial function, oxidative damage) are lacking. The lack of such methods has therefore limited most studies to measuring only a select few physiological markers, despite the complex interplay between physiological pathways. Thus, there is a paucity of studies linking long-term effects of developmental effects to interacting physiological mechanisms, which demands the identification of suitable study systems and the development of novel methodologies for mechanistic life-history studies.

Reptiles are model systems for testing developmental consequences of early life experiences in physiology and fitness

Reptiles provide an excellent model for examining how maternal effects and developmental environments interact to shape offspring phenotypes. Most reptile species are oviparous and lack parental care following oviposition (Shine 2005), meaning that maternal and developmental environments are temporally and spatially separated. This separation disentangles the independent and interactive influences of maternal physiology and incubation conditions on offspring traits. Moreover, in oviparous vertebrates, glucocorticoids, such as corticosterone (CORT), are known to be transferred to eggs via yolk deposition (Hayward and Wingfield 2004) and can be experimentally elevated through CORT treatment (Crino et al. 2024), providing a well-characterized pathway for maternal effects to act during development. Finally, the independent effects of developmental CORT exposure (e.g., Sinervo and DeNardo 1996, Meylan et al. 2002, Meylan and Clobert 2005, Warner et al. 2009, Crino et al. 2024, Recio et al. 2025a, 2025b) and incubation temperature (reviewed in: Deeming 2004, Noble et al. 2017, While et al. 2018) on traits such as hatching success, body size, and post-hatching growth have been extensively studied across reptile taxa, offering a robust foundation for predicting their potential interactions.

Within reptiles, *Lampropholis* skinks make an especially tractable system for experimentally testing these mechanisms. Both *Lampropholis delicata* and *L. guichenoti* are small (typically <2 g), ground-dwelling skinks common throughout southeastern Australia (Cogger 2018). They co-occur across much of their range, occupying similar ecological niches as active foragers of small invertebrates in leaf litter and garden habitats. Both species are oviparous, lack postnatal parental care, and reach sexual maturity within a year of hatching, which allows entire life cycles to be studied within a manageable timeframe (Joss and Minard

1985). They reproduce seasonally, and their moderate clutch sizes enable split-clutch experimental designs that control for familial effects – a key strength in developmental studies (e.g., Shine et al. 1997, Ballen et al. 2013, Sun et al. 2018). These shared features make *Lampropholis* skinks well suited to laboratory or semi-natural experiments that manipulate both maternal and developmental environments.

Despite their ecological and morphological similarity, *L. delicata* and *L. guichenoti* differ subtly in reproductive investment strategies. *Lampropholis delicata* typically lays a single clutch per season consisting of 2–6 relatively small eggs (~0.142 g), whereas *L. guichenoti* often produces 1–2 clutches per season with fewer (1–4 eggs) but larger eggs (~0.18 g). Such differences in per-egg investment could influence how embryos respond to developmental stressors. The greater yolk reserves provided to larger eggs may buffer embryos from high energetic demands, potentially mitigating the costs of elevated CORT or the effects of incubation temperature on metabolism. Thus, the *Lampropholis* system presents an opportunity to test whether closely related species that share similar ecologies but differ slightly in reproductive strategy also differ in their developmental responses to maternal and environmental effects. Using two closely related species also strengthens the broader comparative scope of this research. Multi-species experiments remain relatively rare in developmental physiology, yet they are critical for assessing whether mechanistic frameworks are conserved across closely-related taxa. Together, these species provide ideal models for examining how maternal and developmental environments interact to shape physiological trade-offs across levels of biological organization.

Overview and Scope of Thesis

In this thesis, I aimed to test key questions in developmental physiology that few studies have managed to fully address. I tested the immediate and long-term effects of multiple developmental stressors on life-history traits, growth and survival (Chapter 1, Chapter 3). To understand the mechanisms driving developmental effects, I developed a novel functional assay for simultaneous measurement of several parameters of cellular physiology using flow cytometry (Chapter 2). I then applied these novel methods to assess how developmental conditions affect physiology in tissues associated with growth and survival (Chapter 3, Chapter 4). Finally, I tested how cellular physiology in brains was associated with cognitive processes that may facilitate life-history tradeoffs (Chapter 4). The comprehensive, longitudinal experimental design and the development of new methods were necessary for testing predictions of the oxidative stress theory of ageing from a metabolic ecology and developmental programming perspective. The inclusion of cognition experiments offers insight into alternative mechanisms that could explain patterns of growth and survival.

In Chapter 1, I assessed the short-term, immediate influence of multiple developmental stressors on hatching success and hatchling size. Hatching success and hatchling size are two key early-life determinants of long-term survival and reproductive potential in *L. delicata* and *L. guichenoti*. Additionally, I tested the influence of egg size variation – reflective of maternal investment – as a mediator of developmental effects, both within and between species (published as Leibold et al., 2026). Chapter 1 therefore addressed whether simultaneous exposure to developmental stressors imposes critical fitness consequences early on in an individual's

lifespan, and whether these developmental effects are mediated by variation in maternal investment strategy.

Chapter 2 presents a methodological framework for the assessment of cellular physiology using flow cytometric analysis. For this Chapter, I developed novel functional assay panels that use fluorescent probes to measure parameters of cellular morphology, mitochondrial respiration, and oxidative damage. Chapter 2 provides a methodological pipeline for introducing ecophysiologicals to flow cytometry as a tool for measuring cellular physiology, along with basic guidelines for adapting functional assay panels for diverse systems, and for the extraction and interpretation of fluorescence data. I present data from three tissues (liver, blood, and gonads) of *L. delicata* as a case study, and a validation of the method using fluorescence activated cell sorting and confocal microscopy. The methods developed in Chapter 2 provide a foundation for multiparametric measurement of mitochondrial and oxidative physiology using established methods from biomedical research that are underutilised in ecology. I subsequently used the methods for testing how developmental environments affect physiological pathways associated with life-history trade-offs.

In Chapter 3, I reared hatchlings (*L. delicata* and *L. guichenoti*) from the same cohort of animals treated in Chapter 1 in naturalistic outdoor enclosures. I tracked the growth and survival of individuals in these enclosures from juveniles to adulthood. Thus, Chapter 3 assessed the long-term phenotypic and fitness consequences of developmental CORT and incubation temperature. In Chapter 3, I also tested whether the programming of metabolic and cellular physiology mediates developmental stress effects on fitness-associated phenotypes. To accomplish this, I measured the metabolic rates of juveniles. I also measured mitochondrial function and oxidative damage in two tissues (livers and hearts) of both juveniles and adults using novel flow cytometry methods developed in Chapter 2. This Chapter represents the core aims of this thesis, testing how developmental environments and maternal effects influence life-history trade-offs (between growth and survival) and the physiological mechanisms that drive them.

Chapter 4 was an experimental Chapter which differed in scope from Chapters 1 and 3. In Chapter 4, my colleague and I tested the effects of CORT and incubation temperature on cognitive processes and brain physiology in lab-reared *L. delicata* (published as Recio and Leibold et al., 2025). This was a joint experiment, comprised of cognition experiments and physiological assays utilising the novel functional assays described in Chapter 2. Chapter 4 extends the basic life-history questions of Chapters 1 and 3 to another key fitness trait – cognition – that has received considerable attention in the developmental programming literature (reviewed in Dukas 2004, Buchanan et al. 2013, Snell-Rood 2013). Cognition is relevant to life-history traits like growth and survival, as cognitive processes inform how individuals may effectively exploit resources or habitats. Therefore, Chapter 4 approaches questions of how behavioural mechanisms may underlie shifts in life-history trade-offs. To assess the physiological basis of cognitive ability, I quantified the mitochondrial and oxidative physiology in the medial cortex region of the brain, which is associated with spatial learning.

Overall, my thesis applies new methods and approaches to generate a rich set of new empirical results capturing both short- and long-term effects of interacting developmental conditions on both fundamental life-history traits (size, growth, and survival) and cognitive processes in two species with different life-history strategies. I also provide new insights into the

295 mechanistic role of physiological programming in the development of these traits in reptiles.
296 Such a comprehensive understanding of developmental programming on traits through life (and
297 under natural conditions) is rarely achieved, often due to logistical and methodological
298 constraints. As such, my research fills a significant research gap, particularly outside lab models
299 (mice, zebrafish, etc.), offering potential avenues for future work to build upon.

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Chapter 1: Life-history strategy mediates the effects of multiple developmental stressors on Australian lizards

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Abstract

Maternal stress and the developmental environment jointly affect offspring fitness and phenotype during early life. Mothers transmit stress to offspring through the deposition of hormones and energy into developing embryos, who are themselves subject to environmental stressors. A species' life-history strategy (e.g., size and number of eggs) may further buffer or exacerbate the effects of developmental stress on offspring. We experimentally tested the joint effects of stress hormones (exogenous corticosterone applied directly to eggs) and the developmental environment (incubation temperature) on the hatching success and hatchling size of two lizards (*Lampropholis delicata* and *Lampropholis guichenoti*) that differ in maternal investment strategy. Hatching success was reduced by corticosterone exposure, but only in *L. delicata*, which produces clutches of more numerous but smaller eggs than *L. guichenoti*. Hatchling size in both species, however, was reduced by corticosterone exposure, incubation at high temperatures, or both. In both species, maternal investment (egg size) was the best predictor of hatching success and hatchling size, regardless of treatment. Our findings suggest that, both within- and between-species, greater maternal investment buffers offspring from maternal and developmental stress effects on early-life fitness (survival) but does not prevent their effects on fitness-associated phenotypes (hatchling size).

Introduction

Global climate change increasingly exposes organisms to novel environmental stressors [1,2]. Beyond elevated temperatures, global climate change is predicted to affect organismal physiology and fitness through changes in water availability, salinity or pH, exposure to

pollutants or diseases, or predation pressure and competition, which can act together additively, antagonistically or synergistically [3,4]. Consequently, multiple stressors and how they interact are now recognized as key to understanding how novel environments will impact populations ([5] and citations therein; reviewed in [6,7]; freshwater ecosystems - [8]; marine ecosystems - [3]; amphibians - [9]). The adverse fitness effects of multiple stressors will have important implications for the persistence of populations, particularly at critical life stages [10–12]. Early life-history stages (e.g., embryonic, hatchling) are a critically sensitive period where numerous traits like body plan and size are established, often with lifelong consequences for fitness [13]. Indeed, meta-analyses suggest that the impacts of multiple stressors are more severe when experienced during embryonic life stages (e.g., [4]). However, most multi-stress work considers only the effects of simultaneously occurring stressors that act directly on a single individual, which overlooks how stressors acting on one generation may interact with those acting on another.

Stressors experienced by parental generations can result in dramatic physiological changes that affect offspring. Carry-over effects from parental generations experiencing the same or different stressors result in ‘legacy’ effects that can impact offspring in complex ways (i.e., parental/transgenerational effects; [14–16]). It is essential to consider how intergenerational stressors act together with offspring environmental stress, particularly for egg-laying organisms, for several reasons. First, the timing of multiple stressors is more likely to coincide at early life stages because parents, particularly mothers, establish the developmental environment for their offspring. Second, early embryonic stages are highly sensitive to environmental perturbations [17,18], and there is limited opportunity for embryos of egg-laying organisms to mitigate stress through behavioural plasticity ([19]; but see [20]). Finally, understanding how stressors interact or not in early life stages is crucial for predicting individual survival and population growth [21–24]. Despite the potential importance of intergenerational stressors in influencing offspring stress responses, there is a paucity of studies applying a multi-stressor framework to this problem.

The mechanisms by which parents (specifically, mothers) transmit stress to their offspring are diverse, ranging from shifts in resource investment strategies [25], changes in maternal nesting behaviour [26,27], inheritance of epigenetic marks [28,29], or the deposition of hormones that have direct effects on embryonic development (reviewed in [30]; e.g., [31–33]). In vertebrates, one physiological response to stressors involves activation of the hypothalamic-pituitary-adrenal (HPA) axis that results in the release of glucocorticoid (stress) hormones [34,35]. Glucocorticoids induce physiological changes that help vertebrates cope with stressors but can have cascading effects on traits like reproductive investment and maternal behaviour, which in turn influence offspring size and survival [29,36]. Additionally, glucocorticoids can be directly transferred from mothers to offspring *via* deposition in yolk, which in turn influences offspring physiology and behaviour. Thus, offspring survival and phenotypes are a cumulative result of the complex three-way interactions between the maternal environment, the maternal phenotype, and the offspring environment. Consequently, a major challenge in predicting how maternal and offspring stressors interact is that stress experienced by mothers results in a multitude of physiological, behavioural, and epigenetic changes to the maternal phenotype that affect offspring and their environment both directly and indirectly (reviewed in [37–39]).

Lizards are excellent model systems for testing the effects of developmental stressors (e.g., glucocorticoids, incubation temperature) on offspring phenotype and fitness because embryonic development occurs almost entirely external to the mother. Using two congeneric skinks (*Lampropholis delicata* [de Vis, 1888] and *Lampropholis guichenoti* [Duméril and

Bibron, 1839]) with different life-history strategies (r- and K-selected, e.g., [40]), we tested how prenatal stress (simulated *via* application of glucocorticoids to eggs) and developmental thermal stress interact to affect fitness-associated traits like egg mortality and the size at hatching. We also explore how these developmental conditions are mediated by maternal resource investment (i.e., egg mass). We predicted that prenatal CORT exposure and warm incubation temperatures would independently increase embryonic mortality and reduce body size. Additionally, we predicted that prenatal corticosterone (CORT, the primary glucocorticoid in reptiles) and warm incubation temperatures would have additive effects, thereby further increasing embryonic mortality and reducing body size at hatching as the sum of their independent effects. Maternal investment, both within and between species, should act as a buffer to the stressors, such that larger eggs should be more robust to the effects of developmental stress on embryonic mortality and hatchling size. As such, we predicted that smaller eggs within species would be more affected by developmental conditions than larger eggs, and *L. guichenoti* eggs would be more robust than *L. delicata*.

Methods

Study System and Animal Husbandry

We used two congeneric lizards – the delicate skink (*Lampropholis delicata*) and the garden skink (*Lampropholis guichenoti*) – to test the hypothesis that “maternally-derived” stress, the developmental environment, and maternal investment jointly influence offspring survival and phenotypes. Both species are common in semi-urban habitats (e.g., leaf litter, gardens) throughout their widespread distribution in southeastern Australia, where they occupy near-identical ecological niches as small-bodied (~1-2g adult size), relatively short-lived (~2-3 years), active foragers of small invertebrates [41,42]. However, these two species diverge in their reproductive investment strategy [43]. *Lampropholis delicata* lay more numerous (2-6 eggs), smaller (~0.145g) eggs per clutch (more r-selected), while *L. guichenoti* lay fewer (1-4 eggs per clutch), larger (~0.188g) eggs per clutch (more K-selected). By using two ecologically identical, congeneric lizards with divergent reproductive investment strategies as a model system, we aimed to assess how the joint effects of (simulated) maternal and developmental stress (see *Treatment and Data Collection*) on offspring development are buffered or exacerbated by variation in maternal investment both within and between species.

All eggs used in this study were from a breeding colony established from wild populations of *L. delicata* and *L. guichenoti* caught in 2019 in Sydney, Australia. The parent generation from which we collected eggs in 2022/2023, therefore, primarily consisted of first-generation lizards reared entirely from oviposition under laboratory conditions. Lizards were housed in communal enclosures (41.5 L x 30.5 W x 21 H cm) containing two males and four females of a single species. Lizards were fed three times per week with ~40 crickets (*Acheta domestica*) dusted with reptile calcium and multivitamin supplements and provided water daily. Enclosures were fitted with a cabinet liner, PVC refuges, and eucalyptus bark. A 22-32°C temperature gradient was generated in each enclosure using heat cords, and enclosures were lit with UVA/UVB bulbs on a 12-h light:dark cycle. Throughout the reproductive season (Oct. 2022 – Feb. 2023), a nesting box (12.5 L x 8.3 W x 5 H cm) filled with moist vermiculite was placed

on the warm side of the thermal gradient in each enclosure. We checked nesting boxes for eggs three times per week.

Treatment and Data Collection

Upon finding eggs, we measured the length and width with digital calipers (± 0.01 mm) and the mass (± 0.001 g) on a digital scale (OHAUS, Model spx123). We pseudo-randomly assigned eggs from each clutch to each of four “maternal stress” (CORT or Control) by incubation temperature (23°C or 28°C) treatments, such that we attempted to balance each clutch across treatments (“split-clutch” design; Figure 1). For the “maternal stress” treatment, $5\mu\text{L}$ of either CORT dissolved in 100% ethanol ($10\text{ pg CORT} / \text{mL}$) or a 100% ethanol only control was applied topically *via* pipette to the side of each egg containing the yolk. When topically applied to *L. delicata* eggs, this concentration and amount of CORT solution increases circulating CORT concentrations in the yolk to 2 s.d. above the naturally-occurring mean, and is within the natural range of CORT variation [29]. Following stress treatment, eggs were placed in 80 mL cups containing moist vermiculite (12g water to 4g vermiculite) and covered with plastic wrap, then placed in one of two incubators (LATWIT 2X5D-R1160) programmed to either a ‘cool’ ($23 \pm 3^{\circ}\text{C}$) or ‘warm’ ($28 \pm 3^{\circ}\text{C}$) thermal regime. These thermal regimes represent the lower and upper ends of the natural range of incubation temperatures for these two species [44,45]. Specifically, 23°C approximates the average temperature of shaded nests in natural populations, while 28°C approximates the average temperature of unshaded, heat-exposed nests in open grasslands [46; 47]. Both temperatures are also approximately equal magnitude from the optimal temperature for hatching success (26°C ; at least in *L. delicata*) and within the range of optimal temperatures for development for these species [48]. We checked the incubators three times per week for hatchlings and dead eggs. We determined that eggs were dead if desiccated or covered in mold. We recorded the date dead eggs were found and disposed of them. Upon finding hatchlings, we recorded the hatch date, then measured their mass (± 0.001 g) on a digital scale (OHAUS, Model spx123) and their snout-vent-length (SVL; ± 1.0 mm) and tail length (± 1.0 mm) using a ruler.

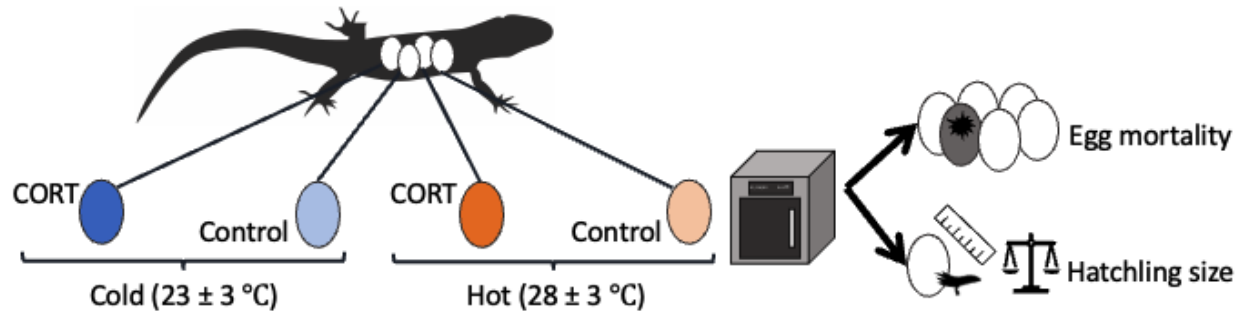


Figure 1. Schematic of experimental design. Eggs from each clutch of lizards (*Lampropholis delicata* and *L. guichenoti*) were divided evenly among four ‘maternal stress’ (CORT or Control) by incubation temperature (23°C or 28°C) treatments using a 2x2 factorial split-clutch design. Treated eggs were incubated until they either died or hatched. Upon hatching, we measured the mass and SVL of hatchlings.

Statistical Analyses

All processing and analyses were performed in R v. 4.2.1 [49]. All data and code are available as a public GitHub repository [50]. Prior to hypothesis testing, we performed a model selection process to simplify models, but always retained our hypothesized main effects. We constructed separate Bayesian generalized linear mixed effects models (GLMM) for egg mortality and hatchling mass for each species. Comparison models included each permutation of the additive, two-way, and three-way interactions between stress treatment (Control or CORT), incubation temperature (23°C or 28°C), and egg mass as fixed effects terms. Species were not included as fixed effects terms in comparison or analysis models. Instead, we modelled the egg mortality and hatchling mass separately for each species to reduce model complexity and because the Bayesian framework allows for comparisons of fixed effects (e.g., CORT, temperature) between species even if species is not included as a term in models (explained below). Clutch identity was included as a random intercept in all models to control for the effects of eggs coming from the same mother. Because egg mortality was a binomial response variable (1 if they died, 0 if they hatched), it was modelled assuming a Bernoulli error distribution (‘logit’ link function).

Hatchling mass is a numeric trait that was modelled assuming a Gaussian error distribution. We fit all models for comparison in rstan (v. 2.32.6; [51]) using the brms (v. 2.18.0; [52-54]) R package. All comparison models were run on 4 parallel MCMC for 2000 iterations with 1000 warmup iterations at an adapt delta value of 0.99. We compared models using the ‘loo’ package in R (v. 2.8.0; [55]) by first fitting expected logwise predicted densities (ELPD) to each model, then comparing the difference in ELPD between each model (‘loo_compare’ function). The ELPD estimates the predictive accuracy of each model using leave-one-out (LOO) cross-validation and returns a score of model fit which can be compared amongst candidate models, similar to AIC [56]. We determined the model of best fit as the model with the lowest ELPD value among the models being compared. If the ELPD values and their error overlapped between multiple models, we considered these models statistically indistinguishable from each other. If multiple models were indistinguishable, we defaulted to the simplest model with the fewest interaction terms among those models.

For both egg mortality and hatchling mass, the best fitting models for both species included the additive effects of stress treatment, incubation temperature treatment, and starting egg mass as fixed effects with no interaction terms, and clutch identity was included as a random intercept. When the best fitting model structures were determined, we refit the best models, running them on 4 MCMC chains for 5000 iterations with 2500 warmup iterations at an adapt delta value of 0.99. We inspected summaries of the model outputs for chain convergence ($R_{hat} < 1.02$) and sufficient effective and bulk sample sizes (both >1000) prior to further analyses.

For hypothesis testing, we tested the independent effects of temperature, CORT treatment, and their interaction by constructing relevant posterior distributions for comparisons of interest and evaluating significance using pMCMC value ($pMCMC < 0.05$). The pMCMC value tests the hypothesis that the difference between predicted posterior distributions differs from zero.

We also tested the independent effect of egg mass on embryonic mortality, because we predicted that egg mass, regardless of treatment, would be the greatest predictor of embryonic mortality. Again, to compare the effects of CORT and temperature between species, we extracted the posterior distributions generated for each main effect (i.e., Stress = CORT – Control; Temperature = 28°C – 23°C) for each species. Then we subtracted the “effect distribution” of *L. guichenoti* from that of *L. delicata* (i.e., Stress = Stress_{delicata} – Stress_{guichenoti}; Temperature = Temperature_{delicata} – Temperature_{guichenoti}) to make a distribution of differences in effect sizes. From the resulting species comparison distribution, we tested for differences in effects between species using the pMCMC value of the distribution.

As an indicator of effect sizes, we also estimated the mean (Estimate) and standard error (Estimate Error) of each resulting distribution. For comparisons of treatment levels, species, or effect sizes, the Estimate and Estimate Error are reported as the mean and standard error of the difference between distributions (denoted with Δ). For the main effect of egg size, or for individual treatment levels, the Estimate and Estimate Error are the mean and standard error of the predicted posterior distribution generated by the model (denoted with β).

Results

We treated a total of 684 *L. delicata* eggs from 160 unique clutches sourced from 44 communal enclosures, and 568 *L. guichenoti* eggs from 197 unique clutches from 30 communal enclosures. Due to the split-clutch design we employed, the eggs from each clutch were roughly evenly divided among each of the four temperature-by-CORT treatment combinations. For *L. delicata*, 168 eggs from 140 clutches were assigned to the “Control, 23°C” treatment, 172 eggs from 141 clutches to “Control, 28°C”, 168 eggs from 144 clutches to “CORT, 23°C”, and 176 eggs from 144 clutches to “CORT, 28°C”. For *L. guichenoti*, 143 eggs from 136 clutches were assigned to the “Control, 23°C” treatment, 140 eggs from 133 clutches to “Control, 28°C”, 138 eggs from 134 clutches to “CORT, 23°C”, and 147 eggs from 142 clutches to “CORT, 28°C”.

Do maternal and developmental stress jointly influence survival?

Stress treatment affected *L. delicata* egg mortality, with CORT-treated eggs more likely to die than Control eggs (Δ CORT – Control; Est. $\pm$ Est. Err. = 0.713 ± 0.300 ; $pMCMC = 0.015$). However, there was no effect of CORT on the survival of *L. guichenoti* eggs (Δ CORT –

Control; Est. $\pm$ Est. Err. = 0.466 ± 0.397 ; pMCMC = 0.229). Incubation temperature did not affect the egg mortality of *L. delicata* ($\Delta 28^\circ\text{C} - 23^\circ\text{C}$; Est. $\pm$ Est. Err. = 0.71 ± 0.295 ; pMCMC = 0.208) nor *L. guichenoti* ($\Delta 28^\circ\text{C} - 23^\circ\text{C}$; Est. $\pm$ Est. Err. = 0.218 ± 0.397 ; pMCMC = 0.579). Egg mass alone affected the mortality of *L. guichenoti*, such that smaller eggs were more likely to die than larger eggs (β Egg Mass; Est. $\pm$ Est. Err. = -19.015 ± 7.483 ; pMCMC = 0.009). Egg mass similarly influenced the mortality of *L. delicata*, but this effect was non-significant (β Egg Mass; Est. $\pm$ Est. Err. = -15.350 ± 8.265 ; pMCMC = 0.059). Posterior distributions derived from Bayesian GLMMs of embryonic mortality and proportions of hatching success from raw data are provided as figures in Figure 2. Summary statistics on embryonic mortality are provided in Table 1. Model outputs of CORT, incubation temperature, and maternal investment effects on embryonic mortality are provided in Table 2.

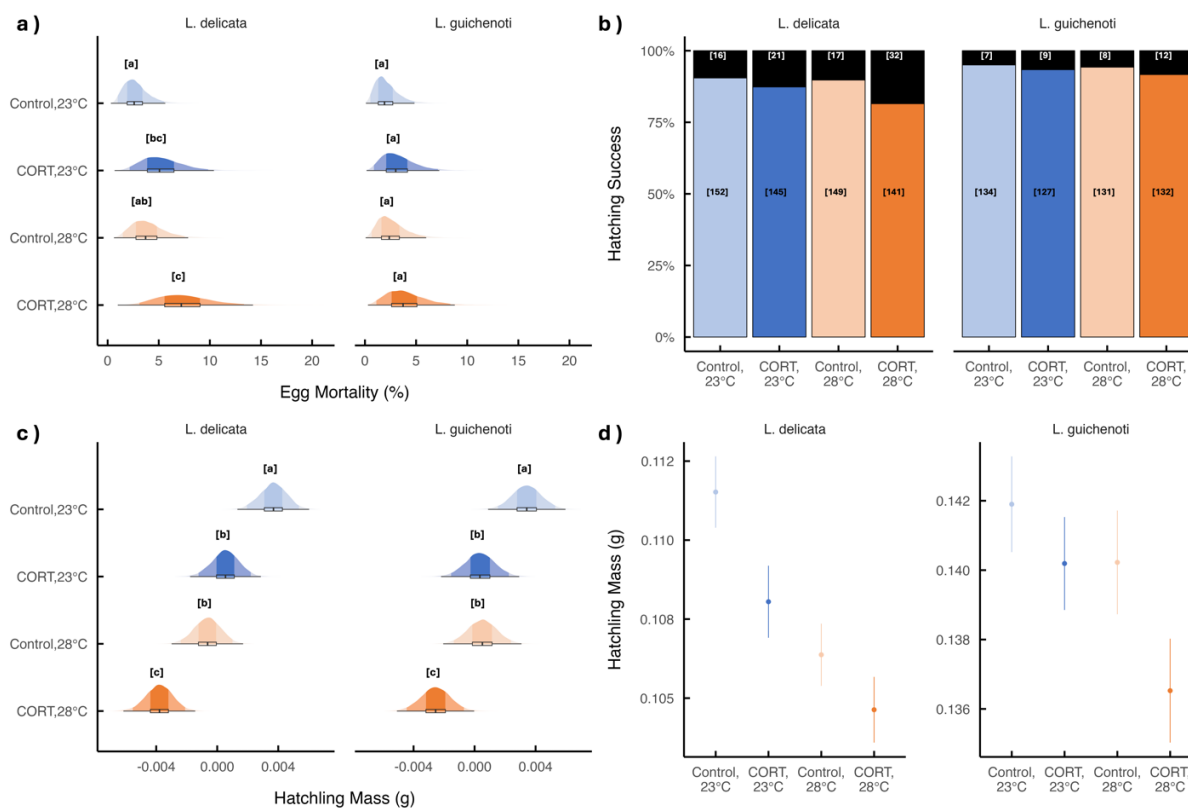


Figure 2. Effects of prenatal stress (CORT or Control) and incubation temperature (23°C or 28°C) on embryonic mortality and hatchling mass in *Lampropholis delicata* and *L. guichenoti*. Plots a) and c) are density plots overlaid with box-and-whisker plots derived from the predicted posterior distributions of each treatment level generated by Bayesian generalized linear mixed-effects models (GLMM) of embryonic mortality and hatchling mass, respectively. Shaded regions represent the 50%, 80%, and 95% confidence intervals of each distribution, while the center of the box-and-whisker plot is the mean. Letters in brackets indicate similarities among treatment levels within each species. Embryonic mortality is the percentage of mortality explained by the treatment level. Plot b) is a bar chart derived from raw data. Colored regions are the proportion of eggs that survived to hatching in each treatment level within each species. Black regions are the proportion of eggs that died. Numbers in brackets are the total number of eggs that survived or died. Plot d) is a summary statistic plot derived from raw data. Dots are the mean hatchling mass in each treatment group, and lines are the standard error.

Does developmental stress affect fitness-related phenotypes?

Hatchling mass in both species was independently affected by stress treatment, incubation temperature, and starting egg mass in the mass of both *L. delicata* and *L. guichenoti* hatchlings (all pMCMC's < 0.001). Starting egg mass was the strongest predictor of hatchling mass in both *L. delicata* (β Egg Mass; Est. $\pm$ Est. Err. = 0.310 ± 0.022) and *L. guichenoti* (β Egg Mass; Est. $\pm$ Est. Err. = 0.296 ± 0.021). In both species, larger eggs yielded larger hatchlings, regardless of treatment group. Treatment with CORT affected hatchling mass in both *L. delicata* (Δ CORT – Control; Est. $\pm$ Est. Err. = -0.003 ± 0.001) and *L. guichenoti* (Δ CORT – Control; Est. $\pm$ Est. Err. = -0.003 ± 0.001), such that CORT-treated eggs yielded smaller hatchlings than control eggs. Incubation temperature similarly affected hatchling mass in both *L. delicata* (Δ 28°C – 23°C; Est. $\pm$ Est. Err. = -0.004 ± 0.001 ; pMCMC < 0.001) and *L. guichenoti* (Δ 28°C – 23°C; Est. $\pm$ Est. Err. = -0.003 ± 0.001 ; pMCMC = 0.001), with eggs incubated at 28°C yielding smaller hatchlings than eggs incubated at 23°C. The effects of CORT-treatment and incubation temperature on hatchling mass were additive in both species. Compared to control eggs incubated at 23°C (*L. delicata*: $N_{\text{Control},23^\circ\text{C}} = 152$, Mass = 0.112 ± 0.013 g; *L. guichenoti*: $N_{\text{Control},23^\circ\text{C}} = 134$, Mass = 0.142 ± 0.016 g), treatment with CORT (*L. delicata*: $N_{\text{CORT},23^\circ\text{C}} = 145$, Mass = 0.108 ± 0.013 g; *L. guichenoti*: $N_{\text{CORT},23^\circ\text{C}} = 127$, Mass = 0.140 ± 0.015 g) or incubation at 28°C (*L. delicata*: $N_{\text{Control},28^\circ\text{C}} = 149$, Mass = 0.106 ± 0.012 g; *L. guichenoti*: $N_{\text{Control},28^\circ\text{C}} = 131$, Mass = 0.140 ± 0.017 g) resulted in smaller hatchlings. Simultaneous treatment with CORT and incubation at 28°C resulted in the smallest hatchlings among treatment groups (*L. delicata*: $N_{\text{CORT},28^\circ\text{C}} = 141$, Mass = 0.105 ± 0.012 g; *L. guichenoti*: $N_{\text{CORT},28^\circ\text{C}} = 132$, Mass = 0.137 ± 0.017 g). Posterior distributions derived from Bayesian GLMMs of embryonic mortality and stat summary plots of hatchling mass from raw data are provided in Figure 2. Summary statistics on hatchling mass are provided in Table 1. Model outputs of CORT, incubation temperature, and maternal investment effects on hatchling mass are provided in Table 2.

Table 1. Summary statistics of the raw data for each treatment group within each species. Eggs (Died) and Eggs (Hatched) are counts of eggs that either died or hatched, respectively. Egg Mass (Died) and Egg Mass (Hatched) are the initial mass of eggs that either died or hatched, respectively. Hatchling Mass and is the size of hatchlings from eggs that successfully hatched. All mass values are given as Mean $\pm$ S.D.

Species	Trait	Control,23°C	CORT,23°C	Control,28°C	CORT,28°C
L. delicata	Eggs (Died)	16	21	17	32
	Eggs (Hatched)	152	145	149	141
	Egg Mortality	9.5%	12.7%	10.2%	18.5%
	Egg Mass (Died)	0.126 ± 0.032	0.138 ± 0.024	0.142 ± 0.039	0.137 ± 0.03
	Egg Mass (Hatched)	0.145 ± 0.027	0.146 ± 0.026	0.146 ± 0.024	0.147 ± 0.022
	Hatchling Mass (g)	0.112 ± 0.013	0.108 ± 0.013	0.106 ± 0.012	0.105 ± 0.012
L. guichenoti	Eggs (Died)	7	9	8	12
	Eggs (Hatched)	134	127	131	132
	Egg Mortality	5%	6.6%	5.8%	8.3%
	Egg Mass (Died)	0.132 ± 0.026	0.182 ± 0.059	0.176 ± 0.046	0.169 ± 0.043
	Egg Mass (Hatched)	0.184 ± 0.027	0.186 ± 0.029	0.185 ± 0.029	0.188 ± 0.035
	Hatchling Mass (g)	0.142 ± 0.016	0.14 ± 0.015	0.14 ± 0.017	0.137 ± 0.017

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Table 2. Summary of main effects of prenatal stress (CORT or Control), incubation temperature (23°C or 28°C), and maternal investment (egg mass) on embryonic mortality and hatchling size of *L. delicata* and *L. guichenoti*. All values are derived from Bayesian generalized linear mixed-effects models (GLMM). For each response variable and each species, the independent effects of Stress (CORT - Control), Temperature (28°C - 23°C), and Egg Mass are summarized. The Estimate is the mean of the difference between predicted posterior distributions (Stress and Temperature), Est.Error is the error about the resultant distribution, and l-95% CI and u-95% CI are the lower and upper 95% confidence intervals of the distribution, respectively. The pMCMC value is a test statistic suitable for testing whether two or more predicted posterior distributions overlap, with pMCMC < 0.05 indicating that the difference between distributions is not equal to zero.

Trait	Species	Contrast	Estimate	Est.Error	l-95% CI	u-95% CI	pMCMC
Egg Mortality	<i>L. delicata</i>	Stress [CORT - Control]	0.696	0.309	0.109	1.328	0.020
		Temperature [28°C - 23°C]	0.366	0.300	-0.228	0.946	0.220
		Egg Mass	-16.062	8.457	-32.985	0.637	0.057
	<i>L. guichenoti</i>	Stress [CORT - Control]	0.463	0.404	-0.323	1.273	0.250
		Temperature [28°C - 23°C]	0.220	0.397	-0.561	1.004	0.578
		Egg Mass	-19.081	7.645	-34.265	-4.326	0.011
Hatchling Mass	<i>L. delicata</i>	Stress [CORT - Control]	-0.003	0.001	-0.004	-0.002	0.000
		Temperature [28°C - 23°C]	-0.004	0.001	-0.005	-0.003	0.000
		Egg Mass	0.310	0.022	0.267	0.353	0.000
	<i>L. guichenoti</i>	Stress [CORT - Control]	-0.003	0.001	-0.005	-0.001	0.000
		Temperature [28°C - 23°C]	-0.003	0.001	-0.005	-0.001	0.001
		Egg Mass	0.297	0.020	0.257	0.337	0.000

Does life-history strategy buffer offspring from developmental stress?

Despite CORT treatment having a direct effect on the embryonic mortality of *L. delicata*, but no direct effect on *L. guichenoti*, there were no detectable differences in the effects of CORT on mortality between species ($\Delta \text{Stress}_{\text{delicata}} - \text{Stress}_{\text{guichenoti}}$; Est. $\pm$ Est.Err. = 0.244 ± 0.044 ; pMCMC = 0.622). There were no direct effects of incubation temperature on the mortality of either *L. delicata* nor *L. guichenoti*, and the effects of incubation temperature on mortality did not differ between species ($\Delta \text{Temperature}_{\text{delicata}} - \text{Temperature}_{\text{guichenoti}}$; Est. $\pm$ Est.Err. = 0.154 ± 0.003 ; pMCMC = 0.758). Similarly, neither the effects of CORT ($\Delta \text{Stress}_{\text{delicata}} - \text{Stress}_{\text{guichenoti}}$; Est. $\pm$ Est.Err. = 0.000 ± 0.000 ; pMCMC = 0.943) nor incubation temperature ($\Delta \text{Temperature}_{\text{delicata}} - \text{Temperature}_{\text{guichenoti}}$; Est. $\pm$ Est.Err. = -0.001 ± 0.000 ; pMCMC = 0.153) on

hatchling mass differed between *L. delicata* and *L. guichenoti*. Results of between-species comparisons of the main effects of prenatal CORT and incubation temperature are provided in Table 3.

Table 3. Comparison of main effects between *L. delicata* and *L. guichenoti*. Using the predicted posterior distributions from Bayesian GLMMs, we calculated the distribution of each main effect (prenatal CORT or incubation temperature) on egg mortality and hatchling mass in each species (see Table 2). Then, we compared the distributions of each resultant main effect distribution between species to test the hypothesis that prenatal CORT and incubation temperature have different effects on *L. delicata* and *L. guichenoti*. The Estimate is the mean of the difference between predicted posterior distributions (*L. delicata* – *L. guichenoti*), Est.Error is the error about the resultant distribution, and l-95% CI and u-95% CI are the lower and upper 95% confidence intervals of the distribution, respectively. The pMCMC value is a test statistic suitable for testing whether two or more predicted posterior distributions overlap, with pMCMC < 0.05 indicating that the difference between distributions is not equal to zero.

Trait	Effect	Contrast	Estimate	Est.Error	l-95% CI	u-95% CI	pMCMC
Egg Mortality	Stress [CORT - Control]	<i>L. delicata</i> - <i>L. guichenoti</i>	0.233	0.004	-0.771	1.242	0.643
	Temperature [28°C - 23°C]	<i>L. delicata</i> - <i>L. guichenoti</i>	0.146	0.004	-0.829	1.112	0.768
Hatchling Mass	Stress [CORT - Control]	<i>L. delicata</i> - <i>L. guichenoti</i>	0.000	0.000	-0.002	0.002	0.943
	Temperature [28°C - 23°C]	<i>L. delicata</i> - <i>L. guichenoti</i>	-0.001	0.000	-0.003	0.001	0.153

Discussion

We found that prenatal CORT induced greater embryonic mortality compared to the control treatment, but only significantly in *L. delicata*. Rather, in both *L. delicata* and *L. guichenoti*, maternal investment (egg size) was the strongest predictor of survival to hatching, with “more stressed” (CORT-treated or warm-incubated) eggs requiring greater maternal investment to hatch than “less stressed” (Control-treated or cool-incubated) eggs. In contrast, while egg size positively influenced hatchling size, treating eggs with CORT and incubating eggs at high temperatures resulted in smaller hatchlings of both *L. delicata* and *L. guichenoti*. Our findings suggest: 1) Maternal stress (simulated *via* CORT application) induces embryonic mortality, but variation in incubation temperature does not; 2) Variation in maternal investment both within- and among-species buffers developing offspring from the lethal effects of developmental stressors; and 3) Exposure to multiple developmental stressors affects post-embryonic fitness-related traits like body size regardless of maternal investment strategy.

Maternally-derived stress is lethal to embryos

Treatment with prenatal CORT alone increased embryonic mortality in *L. delicata*, while incubation temperature had weak or no influence on mortality in either *L. delicata* or *L. guichenoti*. This finding suggests that treating eggs with exogenous CORT, which mimics an

increase in maternally-derived stress hormones, challenges successful development, but warm incubation temperatures representing heat-exposed nests did not. The lack of thermal effects on hatching success is perhaps unsurprising, as the incubation temperatures we used were designed to be similar to natural nest temperatures rather than thermal extremes, which indicates that lizard embryos are relatively robust to thermal stress across at least a narrow range of incubation regimes. However, CORT reduced hatching success in *L. delicata* but not *L. guichenoti*, raising the question of why hatching success is affected by maternally derived stress in some species but not others. We propose that glucocorticoids (e.g., CORT, cortisol) modulate the balance between maternal investment (energy availability) and the cost of development (energy demand).

As the primary stress hormone in reptiles, CORT makes energy available for mounting the energetically demanding physiological stress response [36,57-58]. Prenatal CORT is metabolized by embryos, thereby increasing baseline metabolic rates. As such, prenatal CORT functionally increases developmental costs and can rapidly consume resources (e.g., yolk) that would be otherwise available for successful development (e.g., [59]). Additionally, energy is made available for the stress response by shunting energy away from other biological processes (e.g., immune function), thereby potentiating embryonic sensitivity to lethal stressors (e.g., infection). Similarly, incubation at high temperatures is frequently associated with elevated metabolic rates, reduced yolk incorporation, and greater total energy requirements for development [60,61]. Consequently, exposure to CORT, high incubation temperatures, or both can induce developmental costs to exceed the energy available for maintaining development and responding to additional challenges, resulting in hatching failure. If energetic mismatches underlie the effects of CORT or poor thermal environments on embryonic mortality, then it follows that species or individuals with more energy for development (i.e., those receiving greater maternal investment) would be more robust to developmental stress. Indeed, this is precisely the pattern we observed between and within species in the present study. Future studies should explore this possibility by investigating how CORT directly affects energy expenditure (e.g., metabolic rate) in eggs or through experiments manipulating both CORT and the amount of energy (yolk) available to eggs.

Maternal investment buffers embryos from lethal stress effects

Consistent with general predictions of life-history theory [62], embryonic mortality was higher in the more r-selected (more numerous, smaller eggs) *L. delicata* than the more K-selected (fewer, larger eggs) *L. guichenoti*, regardless of CORT treatment or incubation temperature. Additionally, the greater per-offspring investment provided to *L. guichenoti* eggs relative to *L. delicata* eggs [63] may also have buffered the increased developmental costs associated with elevated CORT or high incubation temperatures, explaining why there was no effect of CORT on *L. guichenoti* mortality, but also no difference in the effect of CORT on mortality between species. In other words, the patterns of mortality associated with CORT were similar between species, just weaker in *L. guichenoti*. Furthermore, the strongest predictor of hatching success for both *L. delicata* and *L. guichenoti* was the natural variation in egg mass, which is representative of maternal investment. Within each species, larger eggs were more likely to survive than smaller eggs, regardless of CORT treatment or incubation temperature. Interestingly, however, the average size of hatched eggs versus those that died varied among treatment groups within each species. Eggs that died in the CORT-treated, hot-incubated, and CORT-treated and hot-incubated groups were larger on average than control eggs that died. However, the average size

of the eggs that hatched was similar among all experimental groups. We interpret this as a threshold minimum amount of energy (i.e., yolk or egg mass) required for eggs to develop, and exposure to one or more physiological stressors (e.g., CORT, incubation temperature) raises that minimum. Our results suggest that mothers may improve the hatching success of their eggs under stressful environmental conditions by increasing resource investment. If stressful conditions persist across multiple generations, such as we would expect under current climate change projections, the “filtering” of small eggs could induce more r-selected populations or species (like *L. delicata*) to shift to more K-selected life-history strategies that are more robust to developmental stress (like *L. guichenoti*).

Although increasing egg size may increase fitness in stressful environments, species may be limited in their ability to shift towards greater investment due to physical or physiological constraints on the size or number of eggs produced in each clutch [64–66]. Additionally, the stressful environments in which adopting a greater per-offspring investment strategy would benefit hatching success will likely limit the resources available to mothers for reproduction. Furthermore, producing or carrying larger eggs in such environments may impose fitness costs on the mother that outweigh the benefits of improving offspring survival or maximizing offspring size or quality (reviewed in [39,67]; e.g., [68–70]). Indeed, a more realistic scenario – relative to this experiment where resources available to mothers for reproduction were virtually unlimited – is that not only are eggs subjected to multiple stressors like maternally-derived CORT and high incubation temperatures, but also that the amount of energy available to eggs for coping with these stressors and completing development is reduced. Therefore, the interactions between maternally-derived stress, thermal environments, and maternal investment are likely to be a strong determinant of hatching success in natural environments, with potential consequences for fitness-associated traits post-hatching.

Developmental stressors have lingering effects on fitness-related traits

Although *Lampropholis* embryonic mortality was relatively robust to the combined effects of CORT treatment and incubation temperature, and evidence suggests that both within- and between-species variation in reproductive investment strategy acts as a buffer to these effects, the same could not be said for hatchling size in either *L. delicata* or *L. guichenoti*. Unsurprisingly, larger eggs generally resulted in larger hatchlings regardless of CORT or incubation temperature in both species. However, treatment with CORT or high temperature alone resulted in smaller hatchlings, and treatment with both CORT and high temperature resulted in even smaller hatchlings relative to untreated control eggs incubated at cool temperatures in both species. Size at hatching is a strong determinant of survival to adulthood in many reptiles, as well as growth and adult size, which in turn influences reproductive output [71, 72; e.g., 73–75; but see 76]. Thus, while embryonic mortality may be relatively robust to multiple stressors like CORT and incubation temperature, these developmental stressors induce phenotypic effects that will likely persist into adulthood and the next generation.

We note, however, that the effects of CORT and high temperatures on hatchling body size were relatively small – CORT-treatment or warm-incubation induced an ~0.003g reduction in body size on average, while treatment with both CORT and warm incubation temperatures resulted in an ~0.006g reduction in hatchling size. Even so, these are small-bodied lizards, especially *L. delicata*, which hatched at 0.108g on average. Therefore, the cumulative effects of CORT and warm incubation temperature resulted in an ~6% reduction in *L. delicata* body mass

(~3.5% for *L. guichenoti*). Although it seems unlikely these mild reductions in hatchling body mass will carry strong fitness consequences under laboratory conditions, even small reductions in body size may be significant in natural environments when environmental conditions are more extreme, resources are limited, competition is high, or juveniles are under predation pressure [e.g., 77, 78]. This may be especially true if reduced body size limits an individual's ability to acquire food that would allow them to compensate for their smaller size at hatching, thereby leading to a pattern of reduced body size or body condition throughout life compared to their larger-bodied peers [79-81].

Beyond their effects on early-life size, stressors experienced during development can have long-lasting mechanistic effects on multiple biological systems throughout an organism's life ([13]; reviewed in [29,82,83]). Indeed, developmental CORT exposure and incubation temperature can permanently alter immune function [84], HPA axis reactivity [85-86], growth and metabolism [87-91], ageing and lifespan [85,91], reproduction [92], cognition [93-96], and behavior [97,98]. If stressors like increased temperature persist across multiple generations, as we would expect in the face of global climate change, even mild developmental challenges acting on one generation may have far-reaching consequences for the health, persistence, and evolution of populations into future generations.

Ethics Statement

All experimental procedures were approved by the Australian National University Animal Experimentation Ethics Committee (Protocol Number A2021/56).

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Author Contributions

DCL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing.

PR: Conceptualization, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing

AYP: Investigation, Methodology, Writing - review & editing.

OLC: Conceptualization, Methodology, Resources, Validation, Writing - review & editing.

CRF: Conceptualization, Funding acquisition, Methodology, Project administration, Writing - review & editing.

DWAN: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing - review & editing.

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Chapter 2: Go with the flow: Flow cytometry applications in ecology and physiology

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Abstract

Physiological ecology is a rapidly growing field with ever-changing needs, which requires innovative techniques to overcome the limitations of current methodologies. Flow cytometry, an established technique in biomedical research, is a potentially valuable tool for ecophysiolgists. Flow cytometry offers a cheap and high-throughput method that allows for the simultaneous measurement of multiple physiological biomarkers from a single tissue sample and is highly flexible in its applications due to the wide range of available fluorescent probes. Here, we present a practical workflow describing how we designed and implemented functional assay panels that simultaneously measure cellular biomarkers of mitochondrial function and oxidative damage. Specifically, we designed a functional assay panel to quantify mitochondrial mass, membrane potential, and superoxide, and a separate panel to quantify lipid peroxidation and oxidative DNA damage (8-OHdG). This workflow includes the selection of fluorescent probes for compatibility in a functional assay panel, the preparation of tissue samples, the acquisition of data via flow cytometry, and the post-processing of fluorescence data into biologically-meaningful values. As a case study, we present data quantifying mitochondrial function and oxidative damage in the livers, blood, and gonads of a model lizard species, *Lampropholis delicata*. We further validate the efficacy of these methods via fluorescence-activated cell sorting and confocal microscopy of livers and blood from *L. delicata*. Our case study finds that the functional assay panels presented here are highly repeatable, demonstrate tissue-specific relationships between physiological parameters, and that relative levels of physiological parameters are moderately correlated among tissues. Flow cytometry methods are, therefore, a promising technique for quantifying complex physiological relationships in diverse experimental contexts, which may be expanded upon for additional utility in answering fundamental questions in ecophysiology.

Introduction

A mechanistic understanding of physiological function underpins diverse questions and theory in ecology and evolution, from patterns of senescence to fertility and disease dynamics (Ricklefs & Wikelski, 2002; Metcalfe & Alonso-Alvarez, 2010; Costantini, 2024). Life-history trade-offs are central to explaining many such patterns, as individuals have a finite amount of energy to allocate towards competing fitness traits like current survival versus future reproductive potential (Stearns, 1989). Life-history trade-offs are, therefore, thought to be mediated by variation in the metabolic allocation of energy (Glazier, 2014; Brown, Hou, Hall, & Burger, 2024). At the cellular level, trade-offs may be driven by the balance between mitochondrial respiration (Flatt & Heyland, 2011; Koch et al., 2024) and oxidative stress dynamics (Monaghan, Metcalfe, & Torres, 2008; Costantini, 2019).

Mitochondrial respiration produces most of the energy required for key fitness traits like growth, reproduction, and performance. However, mitochondrial respiration also inevitably produces reactive oxygen species (ROS) as a byproduct. At low levels, ROS are involved in intracellular signaling, cell proliferation and repair, and immune function (Ansari et al., 2025). At high levels, ROS can damage lipids, proteins, DNA, and telomeres associated with ageing and senescence (Speakman & Garratt, 2013; Salin et al., 2015; Koch et al., 2021; Monaghan, 2024). ROS production is dependent on both the rate and efficiency of mitochondrial respiration, with greater ROS produced as respiration rates and efficiency (proportion of ATP produced to oxygen consumed) increases. Therefore, investment into energetically demanding fitness traits like growth or reproduction are expected to require higher mitochondrial efficiency or respiration rates, thereby increasing the production of ROS that impairs longevity. However, ROS production may be ameliorated through mitochondrial uncoupling, which reduces mitochondrial efficiency to dissipate ROS, thereby increasing longevity (Brand, 2000). ROS concentrations may also be quenched through antioxidant defenses (Catoni, Peters, & Schaeffer, 2008), and the accumulation of oxidative damage may be limited by cellular repair mechanisms (). Thus, life-history is expected to be underpinned by the interplay between an organism's mitochondrial metabolism, ROS production, and mechanisms to quench ROS or repair oxidative damage (Gyllenhammer, Entringer, Buss, & Wadhwa, 2025).

There is a strong theoretical foundation linking oxidative stress, specifically by ROS, to patterns of ageing and senescence ("free radical theory of ageing" – Harman, 1956; Beckman & Ames, 1998; Ziada, Smith, & Côté, 2020). More modernly, the free radical theory of ageing has been expanded to incorporate mitochondrial metabolism as a source of ROS and oxidative damage that contributes to longevity ("mitochondrial free radical theory of ageing" – Barja, 2014; Koch et al., 2021). Additionally, telomere dynamics that are dependent on oxidative stress are intimately tied to lifespan and ageing (Monaghan, 2010; Olsson, Wapstra, & Friesen, 2018b), and oxidative stress has been broadly associated with life-history traits and trade-offs beyond longevity alone (Metcalfe & Alonso-Alvarez, 2010; Costantini, 2019). Thus, life-history traits are driven by the interactions between multiple related physiological systems, not even accounting for the contributions of oxidative damage from sources beyond mitochondria (i.e., other organelles, toxins or metal ions – Ansari et al., 2025). The relationships between physiological systems and life-history traits may also vary by taxonomic group, sex, or the tissue in which oxidative stress is being measured (Costantini, 2024). Comprehensive experimental designs (e.g., multi-factorial, multi-species, or longitudinal studies) also compound this

complexity, requiring these measurements to be repeated for individuals from multiple treatment levels, multiple time points, or in multiple species. Thus, there is a growing need for low-cost, high-throughput methods that allow for the rapid simultaneous measurement of multiple physiological biomarkers.

There exist several valuable established methods in ecophysiology for the quantification of physiological biomarkers of mitochondrial function and oxidative stress. Commercially available assay kits (colourimetric or fluoremetric) are a relatively high-throughput and easily applied method for quantifying single physiological biomarkers. Such assay kits have been frequently used to quantify traits like antioxidant activity, telomerase activity, mitochondrial density, and markers of oxidative damage in life-history studies of birds, fish, and reptiles (e.g., Montgomery, Buttemer, & Hulbert, 2012; Han et al., 2020; Costantini, 2025). However, they require substantial tissue volumes per biomarker measured. Thus, measuring multiple biomarkers from the same sample may be unfeasible if tissue samples are limited. Furthermore, kits are costly per sample, reducing their feasibility for multi-factorial experimental designs or limiting sample sizes.

High-resolution fluo-respirometry (*via* Clark electrode systems) is a powerful method for real-time measurements of several parameters of mitochondrial function, including respiration rates, mitochondrial efficiency, proton leak, and sometimes ROS production (Gnaiger, 2020). Fluo-respirometry is frequently used to quantify mitochondrial dynamics across taxa, including in birds, fish, and reptiles (e.g., Stier et al., 2014; Telemeco et al., 2017; Voituron et al., 2017; Salin et al., 2019). Fluo-respirometry offers low per-sample costs, rich data on mitochondrial respiration, and is compatible with live cell homogenates, permeabilized cells, or isolated mitochondria. However, this method is low-throughput and relies on highly specialised equipment, limiting the accessibility of fluo-respirometry and its feasibility in large-scale studies.

Extracellular flux analysis *via* the Seahorse XF analyser offers similarly high-quality data on cellular energetics, providing real-time measurements of glycolysis and mitochondrial respiration. Extracellular flux is also high-throughput and requires low sample volumes, making this method ideal for large-scale or multifactorial studies of cellular energetics. However, the preparation of samples for extracellular flux requires substantial *ex vivo* handling and processing under standardised laboratory conditions and is most compatible with adherent cell cultures. Extracellular flux analysis is less readily applied to freshly collected tissue samples from wild or experimentally manipulated individuals. This method is therefore limited in its flexibility for addressing metabolic responses to rapid environmental or experimental effects.

Telomere length, another key biomarker of life-history, is measured using several methods, including Southern blot analysis, quantitative PCR, or fluorescence *in situ* hybridization (Nussey et al., 2014). For ecological applications, however, qPCR amplification is the typical method for quantifying telomere length, owing to its low cost and high throughput sampling (Nussey et al., 2014; Zhang et al., 2018; Lin et al., 2019). Notably, however, some reptile studies have used Flow-FISH, a high accuracy measure of telomere length using flow cytometric analysis, in their measures of physiology (Ballen et al., 2012; Olsson et al., 2025). Beyond its applications in quantifying telomere length, flow cytometry is a potentially valuable tool for quantifying other physiological biomarkers of mitochondrial function and oxidative damage.

Flow cytometry is a well-established technique commonly employed in biomedical research and medical diagnostics (see McKinnon, 2018 for an overview). Flow cytometry uses fluorescent probes, often conjugated to antibodies, designed to bind to or gather in subcellular targets like surface proteins, antigens, nuclei, or the mitochondrial intermembrane space. Single cell suspensions are then flowed single file through the cytometer, where they are exposed to a series of lasers that excite the fluorescent probes in each cell. Detectors then measure the intensity of different wavelengths of light emitted by the excited cell. The fluorescent intensity of each wavelength corresponds to the spectra of light emitted by each fluorescent probe, and thus fluorescent intensity values provide multi-parametric quantitative data on each cell. This method enables researchers to classify cell populations based on the fluorescence of surface proteins or antigens, quantify protein expression levels, and isolate targeted subsets of cells for downstream analyses *via* fluorescence-activated cell sorting (FACS). Less commonly employed, and perhaps of more relevance to ecophysiologicals, are functional fluorescence probes.

Despite its ubiquity in biomedical research, flow cytometric analysis has seen only limited use in the ecophysiology space. However, the growing suite of “functional” fluorescent probes and recent advances in cytometer capabilities are increasing the utility of flow cytometry beyond biomedical applications. Functional fluorescent probes emit fluorescent signals based on biochemical processes, often with variation in fluorescent intensity reflecting variation in the physiological state of a cell. For example, the MitoSOX series of functional fluorescent probes gather in the mitochondrial intermembrane space and emit a fluorescent signal when they have been oxidized by superoxide ions – a ROS produced as a byproduct of mitochondrial respiration. Variation in the fluorescent intensity of the MitoSOX probe therefore reflects the concentration of superoxide ions within the mitochondria. Functional fluorescent probes, including MitoSOX, have been applied in several reptile studies to quantify ROS levels (Olsson et al., 2008a; 2008b; 2008c; 2009; 2012; Olsson, Wapstra, & Friesen, 2018a; Fitzpatrick et al., 2019; 2025; Lindsay et al., 2020; Friesen et al., 2021; Giraudeau et al., 2025; Tobler et al., 2025). However, most of these applications have yet to capitalise on the full diversity of functional fluorescent probes or the capacity of modern flow cytometers for multi-parametric quantification of multiple physiological traits. A notable exception is the work of Olsson et al. (2018), which used a multi-parameter functional assay panel of MitoSOX Red, dihydrorhodamine 123, and MitoTracker Deep Red FM to simultaneously measure superoxide concentrations, total ROS, and mitochondrial content in lizard blood (*Ctenophorus pictus*). As new functional fluorescent probes become available and advances in cytometry technology support greater numbers of lasers and detectors (Liu, Wang, & Guan, 2025), multi-parametric functional assay panels may prove a powerful tool for quantifying complex physiological relationships. Paired with the high-throughput, low per-sample costs, and flexibility in application for multiple tissue types and preparations, flow cytometry is a promising and accessible technique for ecophysiologicals.

We designed two functional assay panels for flow cytometry, which simultaneously quantify multiple physiological biomarkers relevant to ecology. The first of these functional assay panels characterizes parameters of mitochondrial function – mitochondrial mass, mitochondrial membrane potential, and mitochondrial superoxide concentration. The second functional assay panel quantifies two indicators of oxidative damage – lipid peroxidation and oxidative DNA damage (8-OHdG). These functional assay panels leverage traditional cytometry instruments and diverse fluorescent probes to generate high-resolution, high-throughput, and low-cost data on cellular physiology. Here, we present a practical workflow outlining the

development and implementation of multi-parametric functional assay panels. This workflow covers the selection of fluorescent probes, sample preparation, cytometer setup, and data extraction and processing. As a case study, we provide fluorescence data on three relevant tissues (blood, liver, gonads) from a model lizard (*Lampropholis delicata*). Our goal is to highlight the flexibility of flow cytometry as a tool for rapid quantification of cellular physiology, and provide a foundation for building upon these methods for broader applications in ecophysiology.

Methods

Designing Probe Panels

We developed two multi-parametric functional assay panels for use in flow cytometry: one quantifying mitochondrial function and the other quantifying oxidative damage. For each assay panel, we selected fluorescent probes that (1) were compatible with the same sample preparation methods and (2) which minimized overlap in spectral properties.

To accomplish (1), we checked the manufacturer datasheets of each probe for whether the probe is cell-permeant or cell-impermeant, relies on live cells for staining or fluorescence, and is retained following aldehyde fixation. If a probe is cell-permeant, it can pass through intact cell membranes. If a probe is cell-impermeant, the cell membrane must be made permeable through treatment with detergents like digitonin or Triton X-100 (permeabilisation). Some functional probes rely on physiological processes in live cells for proper binding and fluorescence, while other probes may be added to dead or aldehyde-fixed cells. Finally, many fluorescent probes are retained in the cell following aldehyde fixation, regardless of whether staining must first be performed on live cells. We therefore selected fluorescent probes for each functional assay panel which operated on shared physiological mechanisms and were retained following aldehyde-fixation.

To accomplish (2), we used the ThermoFisher spectral viewer to guide probe selection, aiming primarily to minimize overlap in excitation spectra (Figure 1). The excitation spectrum of a fluorescent probe is the wavelength of light which excites the probe to its maximum fluorescence, while the emission spectrum is the wavelength of light emitted by the probe following excitation by a laser. We aimed to minimize the overlap in excitation spectra to ensure each probe was excited by different lasers in the cytometer, thereby reducing the overlap in emission spectra detected by the fluorescent detectors associated with each laser. Because we developed functional assay panels for a 5-laser cytometer (Becton Dickson LSRFortessa X-20), we selected four fluorescent probes for the mitochondrial function assay panel, and three probes for the oxidative damage assay.

To design a functional assay panel for measuring mitochondrial function, we used the fluorescent probes: MitoTracker Deep Red FM (MTDR), MitoTracker Orange CMTMRos (MTO), and MitoSOX Red (MTSOX). All three probes are cell-permeant, gather in the mitochondria of live cells, and are retained in the mitochondria following subsequent aldehyde-fixation (but see note in Table 1). MTDR is a dim fluorescent probe that is relatively insensitive to variation in mitochondrial membrane potential, thereby gathering equally in all mitochondria. Thus, variation in the fluorescent intensity of MTDR is indicative of the presence of mitochondria or total mitochondrial content in the cell. MTO is sensitive to variation in variation

in mitochondrial membrane potential, with more of the probe gathering in mitochondria with greater membrane potential. Therefore, variation in the fluorescent intensity of MTO reflects the total mitochondrial membrane potential of the cell, which can be generally interpreted as a measure of mitochondrial respiration. MTSOX also gathers in the mitochondrial matrix and fluoresces upon being oxidized by superoxide radicals. Thus, variation in MTSOX fluorescence is indicative of the concentration of superoxide in the mitochondria. Additionally, we included the nuclear viability probe Hoechst 33342, which binds to intact nuclei, to help distinguish intact cells from cellular debris.

To design a function assay panel for quantifying parameters of oxidative damage, we used the fluorescent probes: BODIPY 665/676 Lipid Peroxidation Sensor (LPX) and an 8-OHdG antibody conjugated to Alexa Fluor 488 (collectively, OXO). LPX is cell-permeant, but can be added before or after fixation and permeabilization, and is retained following aldehyde-fixation (but see note in Table 1). LPX initially emits fluorescence at 676 nm, but its fluorescent signal shifts to 665 nm upon oxidation by peroxyl radicals responsible for lipid peroxidation. Thus, an increase in fluorescent intensity (or decrease, depending on the detector used) caused by this shift indicates greater levels of lipid peroxidation. The 8-OHdG polyclonal antibody is cell-impermeant, requiring fixation and permeabilization to enter the cell and bind to 8-OHdG (or 8-oxo-2'-deoxyguanosine), a marker of oxidative damage to nuclear DNA. The 8-OHdG antibody does not fluoresce on its own, so we counterstained samples with a conjugate antibody containing the Alex Fluor 488 fluorescent probe. Like the mitochondrial function panel, we also included Hoechst 33342 in the oxidative damage panel to help distinguish intact cells from debris. A summary of all probes used in functional assay panels, including excitation and emission spectra, is provided in Table 1.

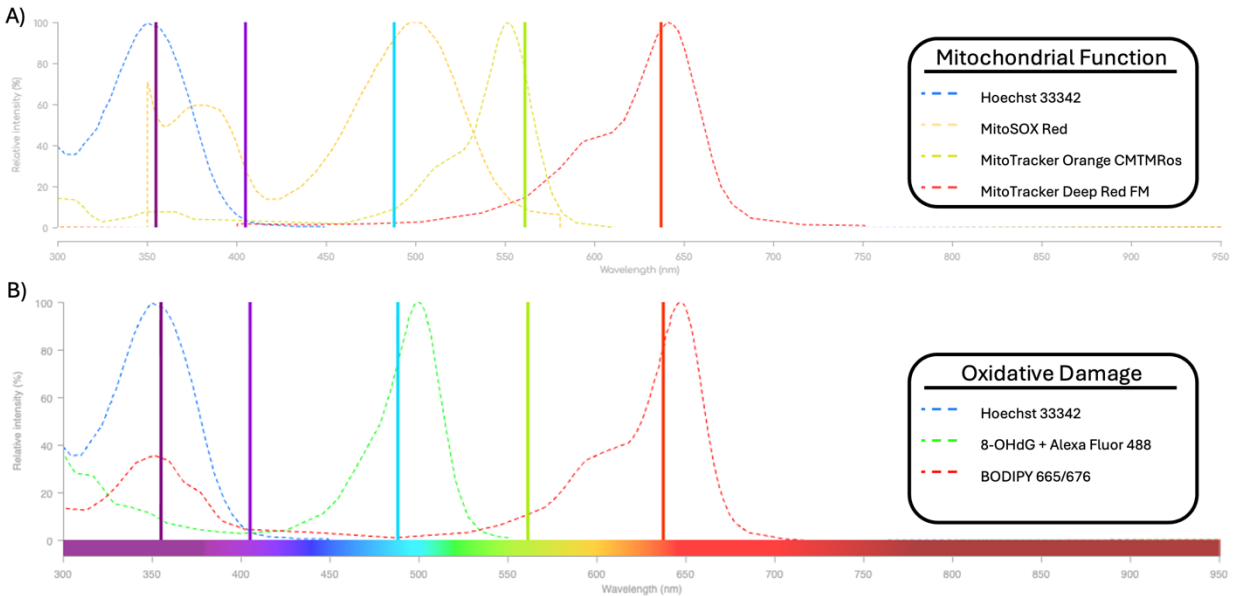


Figure 1. Fluorescent probe panel design using a spectral viewer (ThermoFisher). We depict the excitation spectra (dashed lines) for each fluorescent probe used in mitochondrial function (A) and oxidative damage (B) panels, as well as the wavelengths of the 5 lasers (solid vertical lines) in the BD LSRFortessa X-20 flow cytometer. Our goal in panel design was to select probes with distinct excitation spectra and which were maximally excited using different lasers. For the mitochondrial function panel (A), we selected Hoechst 33342 (UV laser), MitoSOX Red (blue laser), MitoTracker Orange CMTMRos (yellow-green laser), and MitoTracker Deep Red FM (red laser). For the oxidative damage panel (B) we selected Hoechst 33342 (UV laser), Alexa Fluor 488 (blue laser), and BODIPY 665/676 (red laser). Because BODIPY 665/676 is used infrequently, the ThermoFisher spectral viewer did not include it as an option for plotting excitation spectra, so we used another BODIPY probe with similar excitation spectra (BODIPY 650/665) as a stand in for the purposes of this figure. Additional probes may be included in each panel to capitalize on unused lasers (violet in both, yellow-green in only the oxidative damage panel), but this was beyond the current scope of our applications. When including additional or alternative probes to a panel, we must also consider compatibility of sample preparation methods (e.g., whether the probes require live cells, or are retained following fixation or permeabilisation).

1564 **Table 1. Descriptions of fluorescent probes and parameters.** We provide information on each fluorescent parameter on which we collected data.
1565 For each parameter or probe, we give the name and an acronym (in brackets) of the parameter, its excitation and emission spectra (in nm), and the
1566 laser used to excite the fluorescent probe (with its wavelength in brackets). Because we selected detector channels for data acquisition based on
1567 similarities in excitation spectra between the probe we used and the probe for which the detector channel is named, we also give the name of the
1568 corresponding detector channel and the peak excitation spectra of that probe. Finally, we give a general description of each probe or parameter,
1569 which describe the cellular target of the probe, the conditions under which it fluoresces, the interpretation of its fluorescent signal, and notes on
1570 best practices for using that probe.

Fluorescent Parameter	Excitation/Emission	Laser	Detector Channel	Description
Forward-Angle Scatter (FSC)	-	0°	FSC	The scatter of light emitted by the cell when hit by a frontward facing (0°) photomultiplier tube. Forward-angle scatter can be interpreted as the size of a cell.
Side-Angle Scatter (SSC)	-	90°	SSC	The scatter of light emitted by the cell when hit by a laser from the side (90°). Side-angle scatter can be interpreted as the complexity of a cell (e.g., granularity, blebbing or membrane distortion).
Hoechst 33342 (H42)	355/461nm	UV (355nm)	BUV-496 (350nm)	A bright cell-permeant probe which binds to intact nuclei. A positive Hoechst 33342 signal can be used to distinguish among populations of cells. Retained following fixation and permeabilization.
MitoTracker Deep Red FM (MTDR)	644/665nm	Red (640nm)	APC (650nm)	A dim, cell permeant probe that is passively drawn into mitochondrial membranes from the cytosol. Variation in the fluorescent intensity of MTDR is indicative of total mitochondrial content in the cell. Relies on cells being live to gather in mitochondria but is retained following aldehyde fixation.
MitoTracker Orange CMTMRos (MTO)	554/576nm	Yellow-Green (561nm)	PE (566nm)	A cell-permeant probe that is drawn into mitochondrial membranes from the cytosol. The binding of MTO is sensitive to variation in mitochondrial membrane potential, with brighter fluorescent signals indicating greater protonmotive force. MTO relies on live cells for binding but is retained following aldehyde fixation.

MitoSOX Red (MTSOX)	510/610nm	Blue (488nm)	PerCP-Cy5.5 (482nm)	A cell-permeant probe that is drawn into the mitochondrial membrane and fluoresces upon oxidation by superoxide free radicals – a byproduct of cellular respiration. Variation in MTSOX fluorescence is indicative of the total amount of superoxide produced by mitochondrial respiration. MTSOX is retained following aldehyde fixation, but unbound probes may be oxidized by other free radicals over time. Extra washing steps, storing sample in cool and dark conditions, or running samples within a few days of staining are recommended.
BODIPY 665/676 (LPX)	665/676nm	Red (640nm)	APC (650nm)	A cell-permeant probe that gathers in the cytosol, which shifts in fluorescent signal when oxidized by peroxyl radicals. Variation in fluorescent intensity in either of these wavelengths is indicative of lipid peroxidation. BODIPY probes can be added to samples both before and after fixation, but unbound probes can become oxidised by free radicals over time. Extra washing steps, storing samples in cool and dark conditions, or running samples within a few days of staining are recommended.
8-OHdG Antibody + Alexa Fluor 488 (OXO)	488/510nm	Blue (488nm)	Alexa Fluor 488 (488nm)	An antibody which binds to molecules of 8-OHdG that are a common byproduct of oxidative damage to DNA. Requires cells to be fixed and permeabilised for the antibody to pass through the cell membrane. Does not fluoresce on its own, so requires a conjugate fluorescent probe. We used a conjugate containing Alexa Fluor 488.

Sample Preparation

To prepare samples for flow cytometry, tissues must be made into a homogenous single-cell suspension (homogenate) in a neutral buffer (e.g., PBS, Tris-EDTA). Homogenisation methods vary by tissue type. Some tissues require relatively little processing to prepare a homogenate, such as whole blood which needs to only be diluted to a suitable concentration (typically 10^5 to 10^7 cells per mL of homogenate). Other tissues, like livers, require physical homogenisation like slicing, crushing, or grinding the tissue. More robust tissues, like hearts or skeletal muscle, may also require enzymatic digestion (i.e., with collagenase) prior to physical dissociation. Additionally, homogenates may be further processed with steps to remove debris or connective tissues, such as treating the homogenate with DNase. Several methods for homogenisation may be viable for a given tissue type and the efficacy of competing methods, as well as whether to include enzymatic digestion steps, should be confirmed through preliminary validation tests. In these validations, the aim would be to test whether each method yields suitable numbers of intact single cells for further analyses (ideally >2000 cells) and to ensure the concentration of homogenate accounts for the rate at which the cytometer can record data (<10% observations aborted; see section '*Flow Cytometry*'). We depict a hypothetical homogenisation process incorporating enzymatic digestion and physical dissociation steps in Figure 2A.

Following from homogenisation, fluorescent probes are added to the sample in conjunction with their described chemistry. For cell-permeant probes relying on intact live cells, these probes should be added immediately after homogenisation, while cell-impermeant probes should be added only after the cell has been fixed and permeabilised. Samples should always be washed – centrifuged at a low speed and supernatant removed – following staining to remove unbound fluorescent probes. For multi-parametric assay panels, a separate sample or pooled homogenate must be split among different aliquots which are left unstained to serve as a negative control, or each stained with only one fluorescent probe to serve as single-color controls. Negative and single-color controls should be otherwise treated identically to fully stained samples. The inclusion of negative and single-color controls is necessary in downstream analyses to account for any spillover in fluorescent signal among different fluorescent probes or for the autofluorescence of cells.

Both the concentration of fluorescent probes, and the staining conditions, must be optimised for each study system. Probe manufacturers often suggest a range of probe concentrations to be added to samples for various fluorescence-based assays. The concentration of each probe should be determined in line with these recommendations and fine-tuned through preliminary titration experiments. In titration experiments, the aim is to identify the approximate minimum concentration of probe required to distinguish a positive fluorescent signal from the fluorescent signal of an unstained sample. This approach maximises the resolution of fluorescent signals while minimising noise caused by unbound probes present in the sample.

Most fluorescent probes are designed with mammalian cells in mind, and the staining of live cells is often carried out at 37°C to be in line with the average mammalian body temperature. For functional probes, the expected influence of temperature and time exposure on cellular physiology should be considered when determining staining conditions. In our applications with ectotherms, we incubated live cells at 32°C to match the preferred body temperature of our study species and extended the incubation period to ensure complete binding of fluorescent probes.

1615 However, additional applications of our functional assay panels suggested that performing
1616 staining at 37°C would have no negative effects on cell counts or cellular physiology.

1617 To stain homogenates with cell-impermeant fluorescent probes, homogenates must be
1618 fixed and subsequently permeabilised. Fixation is a commonly employed sample preparation
1619 method which involves the addition of fixative agents like aldehydes or ethanol to halt biological
1620 processes and preserve cellular integrity. Many fluorescent probes are compatible with aldehyde-
1621 fixation (see Table 1), which functions to preserve cellular structure through the cross-linkage of
1622 proteins. Following aldehyde fixation, cell membranes may be made permeable through treating
1623 samples with detergents like digitonin or Triton X-100. Permeabilisation allows large antibodies
1624 or cell-impermeant fluorescent probes to pass into the cell. For our consensus panels, only the 8-
1625 OHdG antibody required cells to be permeabilised following aldehyde-fixation, but all
1626 fluorescent probes were compatible with aldehyde-based fixation and permeabilisation.

1627 Fewer functional assay probes are known to be compatible with ethanol-based fixation,
1628 which functions by progressively dehydrating cells and denaturing proteins. Ethanol fixation can
1629 also be harsh on cells and is performed at near-freezing temperatures, potentially resulting in
1630 reduced cell counts or inadvertently impairing the efficacy of functional probes. However,
1631 ethanol fixation is safer and more accessible in some laboratory settings than aldehyde fixation.
1632 Additionally, ethanol fixation simultaneously permeabilises cells through disrupting cell
1633 membranes, making this a potentially valuable alternative to aldehyde fixation if functional
1634 probes prove to be compatible with the method.

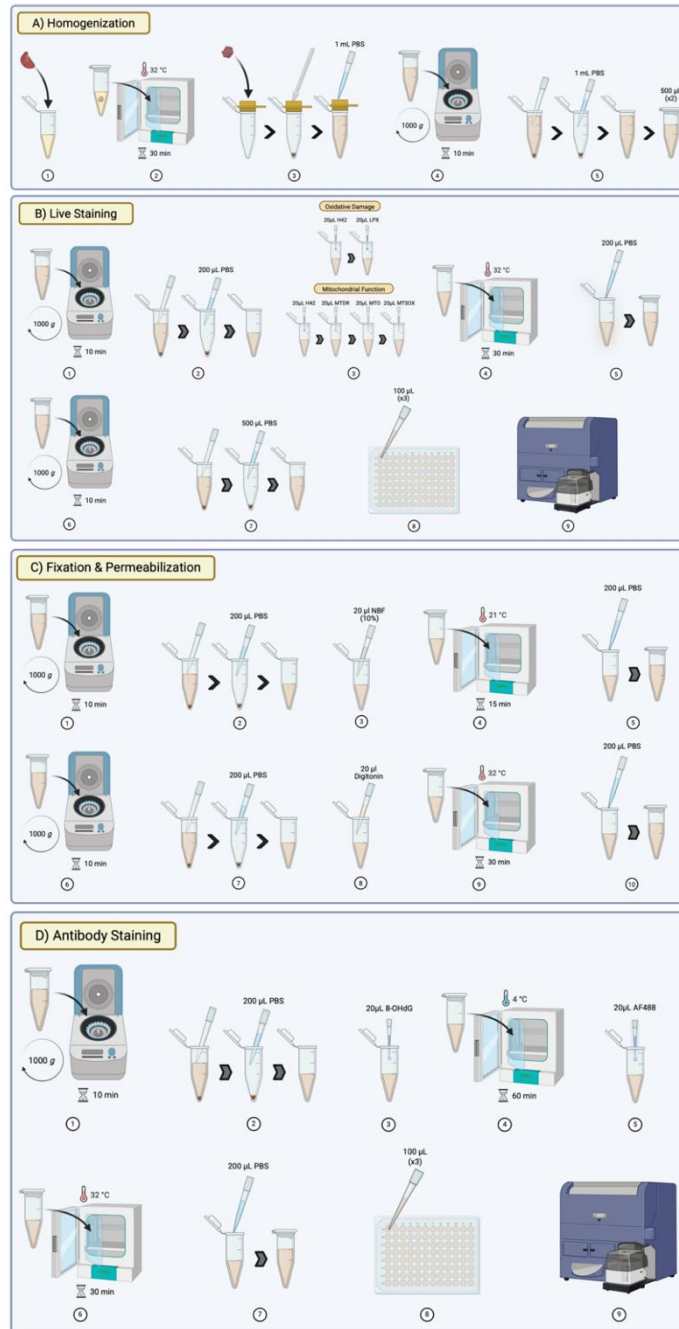


Figure 2. A schematic depicting the different aspects of sample preparation. A) homogenisation of tissue into single-cell suspensions; B) staining of intact live cells; C) aldehyde fixation and permeabilisation of the cell membrane; and D) the staining of fixed and permeabilised cells with antibodies and their conjugates. For homogenisation (A), we depict the homogenisation of a heart, wherein the tissue is first digested in collagenase (125 U/mL) prior to mechanical homogenisation. For live staining (B), we depict plating samples immediately after staining. This is because samples can hypothetically be plated and measured at this stage, prior to additional fixation and permeabilisation and staining with antibodies. However, in practice, we fixed mitochondrial function samples prior to plating, and oxidative damage samples were both fixed and permeabilised (C). Antibody staining (D) was only applied to oxidative damage samples, as the mitochondrial function samples did not include antibodies requiring permeabilisation.

Flow Cytometry

Flow cytometry, briefly, functions by siphoning cells from a homogenate in a single-file line, subjecting cells to a series of lasers at different wavelengths, and then detecting the fluorescent signals emitted by cells on various fluorescent detectors. Fluorescent detectors detect the fluorescent intensity emitted by cells within a range of wavelengths. This range of wavelengths can be narrowed through the inclusion of bandpass filters, thereby reducing noise from fluorescent signals outside of the filtered range. Fluorescent signals are converted to fluorescence intensity values based on the voltage or gain settings for the associated detector. Fluorescent intensity is quantified as the height, width, or area of the fluorescent pulse emitted by a cell. The height of a fluorescent signal represents the maximum fluorescent intensity value emitted during the pulse, while the width of the pulse is indicative of the amount of time the particle spent in the path of the laser. The height and width of the fluorescent pulse are largely used in distinguishing between single cells and doublets, but height can also be used as an indicator of fluorescent intensity. The area of a fluorescent pulse is the integral of the pulse and is representative of the total fluorescent signal emitted by the cell, thus is typically used as a measure of fluorescent intensity. For our purposes, we used the area of the fluorescent pulse to represent the fluorescent intensity.

We initially developed functional assay panels for compatibility with a traditional flow cytometer. Traditional cytometers are commonly available in biomedical laboratories, and typically are available as three-laser or five-laser systems, with some also being capable of high-throughput sensing. Specifically, we used a Becton-Dickson LSRFortessa X-20 with five lasers and a high-throughput plate reader. The lasers on the X-20 and their associated wavelengths are ultraviolet (355 nm), violet (405), blue (488 nm), yellow-green (561 nm), and red (640 nm). Flow cytometers also contain a photomultiplier tube that estimates the forward scatter (FSC) of light emitted by each particle, which is generally used as an indicator of cell size. The amount of light detected at a 90° angle, known as the side scatter (SSC), is generally used as an indicator of cellular complexity. The X-20 has 20 fluorescent detectors each capturing a wavelength range that corresponds with commonly used fluorescent probes. When selecting which fluorescent detectors from which to record data, we chose for each probe the detector with the closest similarity in excitation spectra. For example, the fluorescent signal of Hoechst 33342 was recorded using the BUV-496 detector, because both probes have a peak excitation spectrum of 350 nm. The detector channels we used are summarised in Table 1 along with the corresponding fluorescent probes used in each functional assay panel.

Before a cytometer can be used on any given experimental day, the cytometer must be calibrated to account for any drift in laser delays. This is accomplished by using calibration beads that contain a range of fluorescent probes and using on-board dialogue options for calibrations. For Becton-Dickson systems like the X-20, this calibration process is called the Cytometer Setup & Tracking (CS&T) and uses BD CS&T Beads (Cat. No. 656504) diluted in 300 µL of 1X PBS for manually loading tubes, or 150 µL when using a plate reader. We ran CS&T on the X-20 immediately prior to setting voltages, thresholds, and data acquisition.

Prior to data collection, the voltage or gain settings for each detector channel must be optimised to maximise the resolution between negative and positive fluorescent signals. Voltage determines the conversion of fluorescent signals to measures of fluorescent intensity. Too high of voltage settings amplifies negative signals, causing high background noise, and potentially

increasing positive signals beyond the detectable range of raw fluorescence data (>250000 MFI). Too low of voltage settings dampens positive signals, reducing resolution between negative and positive signals, and potentially decreasing positive signals below the detectable range (<0 MFI). We optimised voltage settings by increasing the minimum voltage in each detector until the fluorescent signal in a single-color control was distinct from the fluorescent signal of an unstained sample. As a baseline, we aimed to centre the distribution of fluorescence in a negative control about 10^0 MFI and the distribution of fluorescence in a single-color control about 10^3 MFI. Once voltage settings for each single-color control were determined, we checked whether these settings maintained the fluorescence of a fully stained sample within the detectable range ($0\text{--}250000$ MFI). For the data presented here, all data was acquired on a single experimental day, using voltage settings determined immediately prior to data acquisition. For experiments spanning multiple trial dates, voltage settings should be kept identical throughout the experiment to allow for more direct comparisons of fluorescence values across experimental days (Maecker & Trotter, 2006).

Also prior to data acquisition, we set threshold filters to minimise the acquisition of data on cellular debris. Thresholds determine whether fluorescence data below certain values are measured and stored. Setting thresholds reduces the size of data files and reduces noise in the resulting data. Thresholds also reduce the number of aborted observations caused by the cytometer being unable to record data as fast as cells pass through the lasers, thereby allowing for more rapid data acquisition. We set thresholds based on the cell size (forward scatter, FSC) and complexity (side scatter, SSC) of intact cells. To determine thresholds, we identified the FSC and SSC ranges of cells that also had intact nuclei, as evidenced by a positive signal for Hoechst 33342. We the FSC and SSC thresholds to discard any observations that were smaller and less complex than the intact, nucleated cells.

With voltage settings and thresholds determined, we then proceeded to data acquisition. In line with general practices in flow cytometry, and to allow for estimating repeatability between technical replicates, we ran each tissue of each lizard in triplicate $100\text{ }\mu\text{L}$ samples. Negative and single-color controls were run as single samples, rather than as triplicates, as these were necessary only for data processing. We sampled $70\text{ }\mu\text{L}$ of each $100\text{ }\mu\text{L}$ at a rate of $3\text{ }\mu\text{L} \cdot \text{sec}^{-1}$. We left $30\text{ }\mu\text{L}$ of each sample as a buffer to prevent the flow cytometer from drawing air. We used a high-throughput plate reader to rapidly and automatically cycle through all samples in a 96-well flat-bottom plate. Bleach and water were run between each single-color control to clear the flow cytometer line of any lingering fluorescent probes, thereby improving the reliability of single-color controls. We recorded all data using the Becton-Dickson FACSDiva (v.8.1) software that is compatible with the X-20 and exported data as flow cytometry standard (*.fcs) files for downstream processing. Regardless of the software used to record data, flow cytometry data is commonly recorded as flow cytometry standard files, which also contain metadata on the cytometer used and the associated settings.

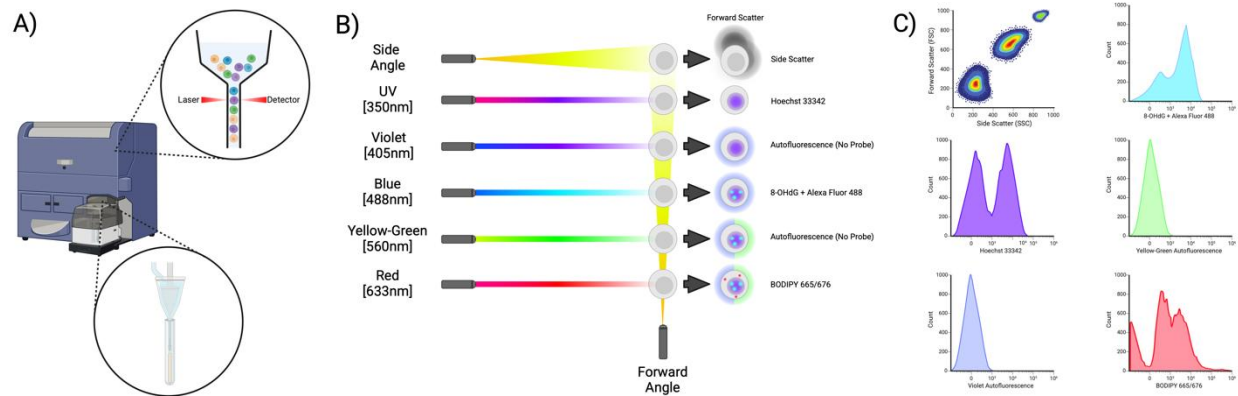


Figure 3. Schematic of flow cytometry data acquisition. A) depicts how cell homogenates are drawn single file through the flow cytometer line, where each cell is subjected to lasers, and their fluorescence subsequently measured by detectors. B) depicts a parallel laser system, in which cells are subject to separate lasers of different wavelengths. Laser beams are absorbed by the cell and excite different fluorescent probes. Here, we depict how lasers may elicit fluorescent signals from a cell stained with the oxidative damage panel. Hoechst 33342 binds to the nucleus and is excited by the UV laser; the 8-OHdG antibody and its conjugate bind to damaged DNA and are excited by the blue laser; BODIPY 665/676 enters the cytoplasm and is excited by the red laser, with its fluorescent signal shifting when it is oxidized by peroxy radicals. Because there are no probes in this panel that are excited by violet and yellow-green lasers, any fluorescent signal emitted in response to these lasers is driven by autofluorescence – the inherent property of cells to reflect light. Additionally, the scatter of light from a front angle laser and side angle laser are measured in each cell and are interpreted respectively as cell size and cellular complexity (e.g., distortion of the cell membrane or granularity). C) depicts what data from a full sample may look like. Forward and side scatter are displayed on two axes to demonstrate how different populations of cells may appear based on cellular size and complexity, whereas the fluorescence values of each detector are displayed as density plots to demonstrate the distribution of fluorescent signals in a sample.

Data Processing and Extraction

Flow cytometry data must be processed and extracted prior to formal analyses. There are many options for processing flow cytometry data, including licensed software like Becton-Dickson's FlowJo and Cytex's SpectroFlo programs, and the CytoExploreR R package (Hamill, 2021). FlowJo is most often used for traditional cytometry data, while SpectroFlo is more suited for spectral cytometry data. CytoExploreR is an R-based front end for cytometry analysis with many of the same utilities as FlowJo. For our purposes, however, we performed all data processing and extraction using the FlowJo (v.10) software. We processed and extracted data for each tissue separately for ease of applying gates and compensation matrices.

Before any downstream analyses, we first added threshold gates to filter all data to only intact single cells, using a similar process as described for setting thresholds for data acquisition. We gated to only observations that were positive for Hoechst 33342, indicating the cells had intact nuclei. We then gated around the FSC and SSC values of these Hoechst 33342 positive cells to determine the size and complexity of intact cells. Finally, we moved the FSC by SSC gate to the top gating level to account for negative- and single-color controls not having Hoechst 33342. This gating process was done to confirm that we did not have data outside of the thresholds set during data acquisition, and that all observations were intact, nucleated, single cells.

From the subpopulation of intact, nucleated, single cells, we then compensated for fluorescent spillover. Each fluorescent probe emits a fluorescent signal over a range of wavelengths. Consequently, there is some spillover between the signals of each probe, where some portion of fluorescence from one probe may be detected when measuring the fluorescence of another probe. Additionally, cells may emit autofluorescence – fluorescence not attributed to any fluorescent probe which is caused by light reflecting off the cell. Compensation accounts for spillover and autofluorescence by calculating the relative contributions of each fluorescent probe to the fluorescent signal in each detector, based on the signals in each single-color and negative control. Compensated fluorescent values are, therefore, the fluorescent intensity of each probe after subtracting out the contributions of each other probe. This improves the resolution of true positive signals from negative signals in each detector and allows for accurate biological interpretation of fluorescent signals.

We compensated for spillover using FlowJo's built-in compensation matrix calculator. We first identified "positive" and "negative" signals for each fluorescent signal. A positive signal was the brightest 2.5% of the distribution of fluorescent values in each single-color control for its respective detector. A negative signal was the total distribution of the fluorescent signal for each detector in the unstained negative control. Using these values, we calculated a traditional compensation matrix and applied it to fluorescence data from fully stained samples. Compensation matrices were generated for each tissue type and for each of the mitochondrial function and oxidative damage panels. A depiction of compensation principles is provided in Figure 4A.

We repeated the gating process on compensated fluorescence values to confirm the FSC and SSC thresholds of intact single cells. We then set gates on each detector channel to subset our observations to a physiologically active population of cells. For the mitochondrial function panel, we were interested in assessing only single cells (predetermined FSC and SSC gates),

which contained both nuclei and mitochondria (positive for both Hoechst 33342 and MTDR), and were actively respiring (positive for both MTO and MTSOX). For the oxidative damage panel, we were interested in only single cells (predetermined FSC and SSC) gates, which contained nuclei (positive for Hoechst 33342), and exhibited both lipid peroxidation and oxidative DNA damage (positive for both LPX and 8-OHdG). A depiction of the gating process is provided in Figure 4B. We used these fully gated populations to calculate summary statistics for each sample, which we then exported for downstream analyses.

Using on-board summary statistic calculators in FlowJo, we calculated the geometric mean of each fluorescent signal in each sample. The geometric mean is a more robust estimate of the central tendency of data than the arithmetic mean, and which is less sensitive to the influence of outliers. As fluorescence values are on a non-linear scale, the geometric mean and median are more appropriate estimates of the central tendency of fluorescent intensity than the arithmetic mean and are reported as the mean or median fluorescence intensity (MFI). FlowJo also contains options for calculating cell counts or proportions of the parent population, as well as robust estimates of standard deviation (rSD) and confidence values (% CV). These options may be of interest in applications such as comparing proportions of live cells to dead cells in a population of cells, or for comparing variation in cellular function among samples. We exported the MFI values for each fluorescent probe and for each sample replicate (as a *.csv) to serve as response variables in downstream analyses. FlowJo exports the linear geometric mean values, but flow cytometry data is often viewed and interpreted on a logicle or biexponential scale. The logicle scale is a variation of the logarithmic scale, which allows for very low, zero, or negative values that appear in compensated fluorescence values (Parks, Roederer, and Moore, 2006). All depictions of flow cytometry data shown here (Figure 3C, Figure 4) are based on their appearance on the logicle scale.

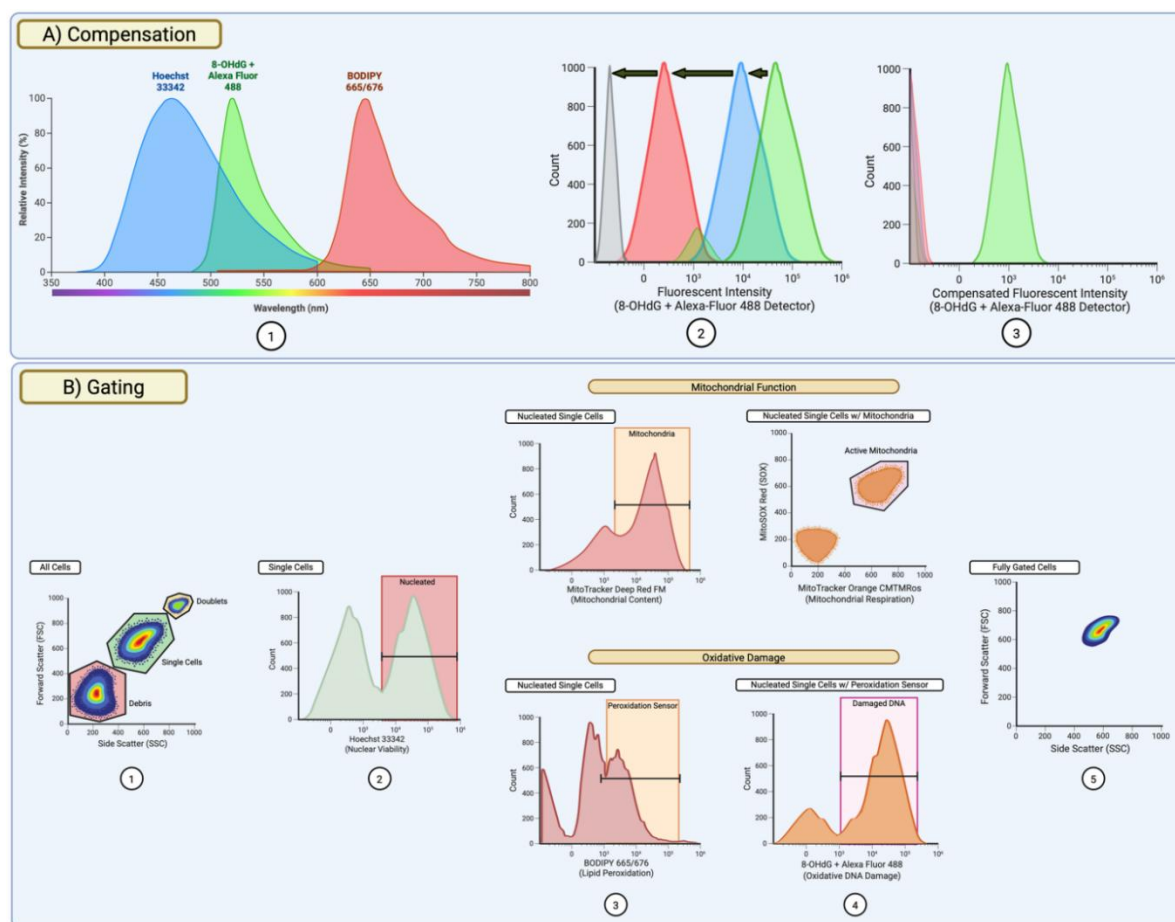


Figure 4. Schematic of data processing. A) depicts the concept of compensating for spillover in fluorescent signal among different fluorescent probes. (1) Each probe fluoresces across a range of wavelengths, some of which may overlap with the emission of other fluorescent probes. Here, we depict the overlap between the emission spectra of Hoechst 33342, Alexa Fluor 488, and BODIPY 665/676. (2) For each of these individual probes, the fluorescent signal emitted by each other probe in the same detector is subtracted from the fluorescent signal. Here, we depict compensation for the Alexa Fluor 488 probe (green), accounting for the relative fluorescent signals of Hoechst 33342 (blue), BODIPY 665/676 (red), and autofluorescence (gray). (3) The resulting compensated fluorescence centers fluorescent spillover about 0 and aids in resolving positive from negative fluorescent signals. B) Following compensation, we depict a gating process - filtering data down to a target population of cells. (1) We first gated to single cells based on the size (forward scatter) and complexity (side scatter) of cells. (2) From the single cells, we gated to only those cells with a positive signal for Hoechst 33342, thereby indicating they have intact nuclei. (3 and 4) For each panel, we gate to only those cells which have positive signals (fluorescent intensity $>10^3$ is a common heuristic for a “positive” signal) for all fluorescent probes in the panel. For the mitochondrial function panel, we first gate to only those cells with a positive MitoTracker Deep Red FM signal, indicating the presence of mitochondria (3). From these mitochondria-positive cells we then gate to cells which both have a positive signal for MitoTracker Orange CMTMROS and MitoSOX Red, indicating that cells are actively respiring (4). For the oxidative damage panel, we gate to populations with positive signals for both 8-OHdG (3) and BODIPY 665/676 (4), therefore exhibit both oxidative DNA and lipid damage (all active cells should have some degree of both damage metrics). The resulting gated population of cells is depicted in (5) on the forward scatter and side scatter axes and is notably a tighter population than the initial “single cell” population identified in (1).

Validating application of flow cytometric methods within and across tissues of a lizard

To demonstrate the flexibility of our functional assay panels, we quantified mitochondrial function and oxidative damage in three tissues – blood, liver, and gonads – taken from 12 adult male lizards (*Lampropholis delicata*). Briefly, lizards were anaesthetized with 10 μ L Alfaxan (10 mg/mL) and euthanized by decapitation. Blood (≤ 20 μ L) was collected from the trunk into heparinized microcapillary tubes, centrifuged at 7000g for 7 min, and plasma removed. Red blood cells were resuspended in 1 mL PBS ($\sim 50\times$ dilution) following established protocols (Olsson et al., 2025). Livers and gonads were homogenised via physical dissociation as depicted in Fig. 2A (no enzymatic digestion step). Because *L. delicata* is small (< 2 g), we used whole tissues in our experiments. Once homogenised, blood, liver, and gonads were treated identically and prepared as depicted in Fig. 2B-D. All temperature-dependent incubation steps were carried out at 32°C, which is just above the preferred body temperature of *L. delicata* ($31.0^\circ\text{C} \pm 0.47$; Zhang et al., 2023). Final concentrations of fluorescent probes in the mitochondrial function panel were: MitoTracker Deep Red FM (0.5 μ M), MitoTracker Orange CMTMRos (0.25 μ M), MitoSOX Red (5 μ M), and Hoechst 33342 (10 μ g/mL). Final concentrations of fluorescent probes in the oxidative damage panel were: BODIPY 665/676 Lipid Peroxidation Sensor (10 μ M), 8-OHdG (7 μ M), Alexa-Fluor 488 (10 μ g/mL), and Hoechst 33342 (10 μ g/mL).

Fluorescence data was acquired and processed as described above for each of blood, livers, and gonads. Using the extracted MFI values of each fluorescent probe, we then estimated: (1) the repeatability of measurements among technical replicates, (2) correlations among physiological parameters within each tissue, (3) correlations among tissues for each physiological parameter, and (4) between- and within-individual correlations among measurements. All analyses were performed in R (v.4.2.1; R Core Team, 2022). Mean fluorescence intensity (MFI) values for each measurement (MTDR, MTO, MTSOX, LPX, OXO) were normalized by dividing by cell size (FSC), log₁₀-transformed, and centred about tissue-level means prior to analyses.

We first assessed the repeatability of measurements among technical replicates of each specimen using the *rpt* function (`rptR` package - Stoffel, Nakagawa, & Schielzeth, 2017), which estimates repeatability from linear mixed-effects models based on log-likelihood ratios. We estimated repeatability separately for each measure (MTDR, MTO, MTSOX, LPX, and OXO) for each tissue (blood, liver, gonads). Each measure was modelled assuming a Gaussian distribution and using an intercept-only model, including individual specimen as a random effect. From each model, we extracted the repeatability estimate, the 95% confidence interval about the repeatability estimate, and the p-value as a test statistic of whether observations were indeed repeatable ($\alpha < 0.05$).

We then modelled all measures together to estimate between-and within-individual correlations among measurements using a multi-response Bayesian generalized linear mixed effects models (GLMMs) with the *brms* package (Bürkner, 2021; O’Dea, Noble, & Nakagawa, 2021). For each tissue, we modelled each physiological measure (MTDR, MTO, MTSOX, LPX, and OXO) assuming a Gaussian distribution and using an intercept-only model (no fixed-effects). We included individual specimen as a random effect. Correlated random intercepts (between-individual correlations) were estimated using the `|p|` syntax within the specimen

random effect term. We also estimated within-individual (residual) level correlations. Each multi-response model was run with four parallel MCMC chains (10000 iterations, 2000 warm-up) at an adapt-delta level of 0.99.

Secondary validation of fluorescent probe localisation and variation in cellular condition

We used fluorescence-activated cell sorting (FACS) couple with fluorescent confocal microscopy as a secondary validation of the mitochondrial function and oxidative damage panels. The aims of this secondary validation were to demonstrate (1) proper localisation of fluorescent probes to the purported cellular targets and (2) that fluorescent intensity values accurately reflect variation in physiological condition among cells in a population. To accomplish these aims, we first sorted cells in each functional assay panel into subpopulations of cells in “good” physiological condition and cells in “bad” physiological condition. Then, we used fluorescent confocal microscopy to image the morphology and fluorescent profile of cells in each subpopulation. This validation was qualitative in nature; thus, we present images captured during microscopy as evidence of proper localisation and discrimination among cell condition.

To validate localisation of and discrimination between signals of fluorescent probes, we prepared blood and liver samples from two adult *L. delicata* for mitochondrial function and oxidative damage assay panels identically as described for the main case study. However, we also included the fluorescent probe Ghost Red 780 into the oxidative damage panel. Ghost Red 780 binds to amines associated with compromised cell membranes, thereby fluorescing in dead cells. Ghost Red 780 was added to the oxidative damage panel to aid in identifying populations of cells in different physiological states. We added Ghost Red 780 to homogenates prior to fixation and permeabilisation and at the same time as BODIPY 665/676.

All cell sorting was conducted on a Becton-Dickson FACS Aria, with an identical 5-laser and 20-detector configuration as the previously described LSRFortessa X-20. As such, we used identical voltage and threshold settings for FACS as we did for flow cytometric analysis in the main case study. Using the FACS Aria therefore allowed us to sort cells based on directly comparable fluorescence intensity (FI) values to those we would record during flow cytometric analysis. Briefly, cell sorting functions by setting gates on different fluorescent parameters, then the cell sorter separates the homogenate into aliquots of each defined subpopulation.

For the mitochondrial function panel, we sorted blood and liver cells by first gating to a main population of cells based on cell size (FSC = 2000–250000 FI) and cellular complexity (SSC = 100–100000 FI). This step excluded any observations that were off-scale or clearly cellular debris. Then, we gated the main population to only observations with an intact nucleus (Hoechst 33342 = 10000–30000 FI). From the population of intact, nucleated single cells, we then gated to observations containing detectable mitochondria (MTDR = 4000–20000 FI). Within the nucleated, mitochondria-containing cells, there were two distinct subpopulations which differed in cell size – “small” cells (FSC = 3000–30000 FI) and “big” cells (FSC = 40000–200000 FI). From within each of the “small” and “big” cell populations, we further identified two subpopulations of cells in different physiological states. Cells in “happy” physiological condition were the subpopulation of cells with relatively lower mitochondrial membrane potential (lower MTO FI) and mitochondrial superoxide (lower MTSOX FI). Cells in “sad” physiological condition were the subpopulation of cells with relatively higher

1924 mitochondrial membrane potential (higher MTO FI) and mitochondrial superoxide (higher MTO
1925 FI). Notably, the “happy” subpopulation was more clearly defined in “small” cells, while the
1926 “sad” population was more clearly defined in “big” cells.

1927 For the oxidative damage panel, we similarly initiated the sorting of blood and liver cells
1928 by gating to a main population of cells based on cell size (FSC = 2000–100000 FI) and cell
1929 complexity (SSC = 100–4000 FI). We then gated to only cells containing nuclei (Hoechst 33342
1930 = 10000–30000 FI). From the main population of intact, nucleated cells, we identified
1931 subpopulations of “live” cells (Ghost Red 780 < 1000 FI) and “dead” cells (Ghost Red 780 >
1932 1000 FI). Within the “live” subpopulation, we defined “damaged” cells as those with high levels
1933 of oxidative DNA damage (8-OHdG > 10000 FI) and “undamaged” cells as those with low
1934 levels of oxidative DNA damage (8-OHdG < 10000 FI). We note that “undamaged” does not
1935 actually mean the absence of oxidative DNA damage in this case, as “damaged” cells were
1936 relatively rare and were the cells with only the highest levels of 8-OHdG. Within the
1937 “undamaged” population, we further subset into “peroxidised” cells as cells with the highest
1938 degree of lipid peroxidation (BODIPY 665/676 > 2000 FI). We defined “healthy” cells as the
1939 subpopulation of “undamaged” cells that also had low levels of lipid peroxidation (BODIPY
1940 665/676 < 2000 FI).

1941 To prepare sorted cell populations for fluorescent confocal microscopy, we used
1942 cytopinning – a specialised form of centrifugation designed to sediment cells onto a microscope
1943 slide in a monolayer. We generally followed procedures for cytopinning as described by Koh
1944 (2013). Briefly, we cytopinned 100 µL of each sorted sample onto a microscopy slide at 1000 g for
1945 5 min. We gently shook excess buffer from the slide, then applied 8 µL of VECTASHIELD
1946 Antifade Mounting Medium to the adhered cells to preserve fluorescent signal. We sealed the
1947 slide with a glass coverslip and clear acrylic nail polish. To further preserve fluorescence, all
1948 prepared slides were stored in slide boxes and kept in the dark at 4°C until imaging. All
1949 fluorescent imaging was completed within 3 days of preparing the slides.

1950 Fluorescent imaging was performed using a Leica SP5 confocal microscope with 7 laser
1951 lines: 405 nm, 458 nm, 476 nm, 488 nm, 514 nm, and 633nm. The Leica SP5 contains 4
1952 photomultiplier detectors capable of imaging four spectral channels, as well as brightfield
1953 imaging for capturing cellular morphology. For each of the mitochondrial function and oxidative
1954 damage panels, we imaged single-color controls for each probe in the panel, as well as fully
1955 stained samples that had been sorted *via* FACS. Imaging was performed separately for each of
1956 blood and liver cells.

1957 We imaged the “happy” and “sad” subpopulations from each of the “small” and “big”
1958 populations in the mitochondrial function panel. Inspection of cellular morphology *via*
1959 brightfield imaging suggested that “small” and “big” cells were the same cell type and not
1960 clearly distinct morphologically, thus we do not distinguish among them in our presentation of
1961 fluorescent images. Lasers used to excite each probe in the mitochondrial function panel were:
1962 Hoechst 33342 (405 nm), MitoTracker Orange CMTMRos (561 nm), MitoSOX Red (514 nm),
1963 and MitoTracker Deep Red FM (633 nm). We also imaged the “damaged” and “healthy”
1964 subpopulations of the oxidative damage panel. Lasers used to excite each probe in the oxidative
1965 damage panel were: Hoechst 33342 (405 nm), 8-OHdG + Alexa Fluor 488 (488 nm), BODIPY
1966 665/676 (633 nm), and Ghost Red 780 (633 nm). However, we note that the emission spectrum
1967 of Ghost Red 780 is largely beyond the detectable range of the Leica SP5, thus the signals of this

1968 dye are very weak or absent in imaging. Images depicting the localisation of each individual
1969 probe in the mitochondrial function assay panel are given in Figure 5, while comparison images
1970 of blood cells sorted by physiological condition are in Figure 6 and liver cells in Figure 7.
1971 Images depicting the localisation of each individual probe in the oxidative damage assay panel
1972 are given in Figure 8, while comparison images of blood cells sorted by physiological condition
1973 are in Figure 9 and liver cells in Figure 10.

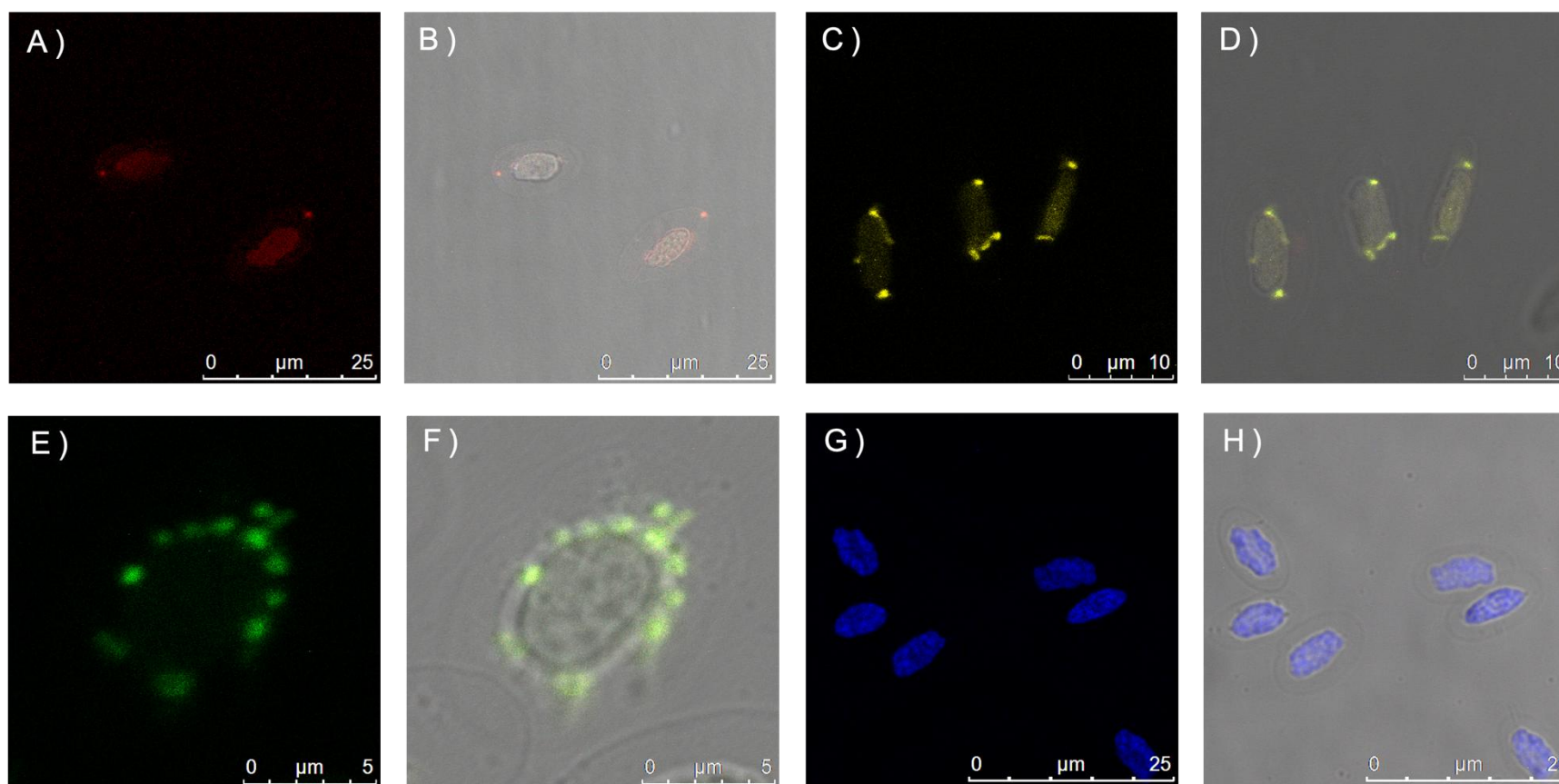


Figure 5. Confocal microscopy images of single-colour controls from each fluorescent probe in a mitochondrial function panel. Cells are *Lampropholis delicata* nucleated red blood cells that have been stained with probes at 32°C and fixed in 1% formalin. Cells were adhered to slides via cytospinning and preserved with VECTASHIELD mounting medium. Images were taken on a Leica SP5 confocal microscope. A) and B) are cells stained with MitoTracker Deep Red FM – a dim probe which localises in mitochondria. C) and D) are cells stained with MitoTracker Orange CMTMRos – a probe which localises in mitochondria and varies in fluorescence with mitochondrial membrane potential. E) and F) are cells stained with MitoSOX Red – a probe that localises in mitochondria and fluoresces when oxidised by superoxide radicals. G) and H) are cells stained with Hoechst 33342 – a probe that localises in intact nuclei. A), C), E), and G) are images of fluorescence from only the detector channel associated with the probe. B), D), F), and H) are full transmission spectra with fluorescence signals of all detector channels and overlaid with brightfield images to show cellular morphology.

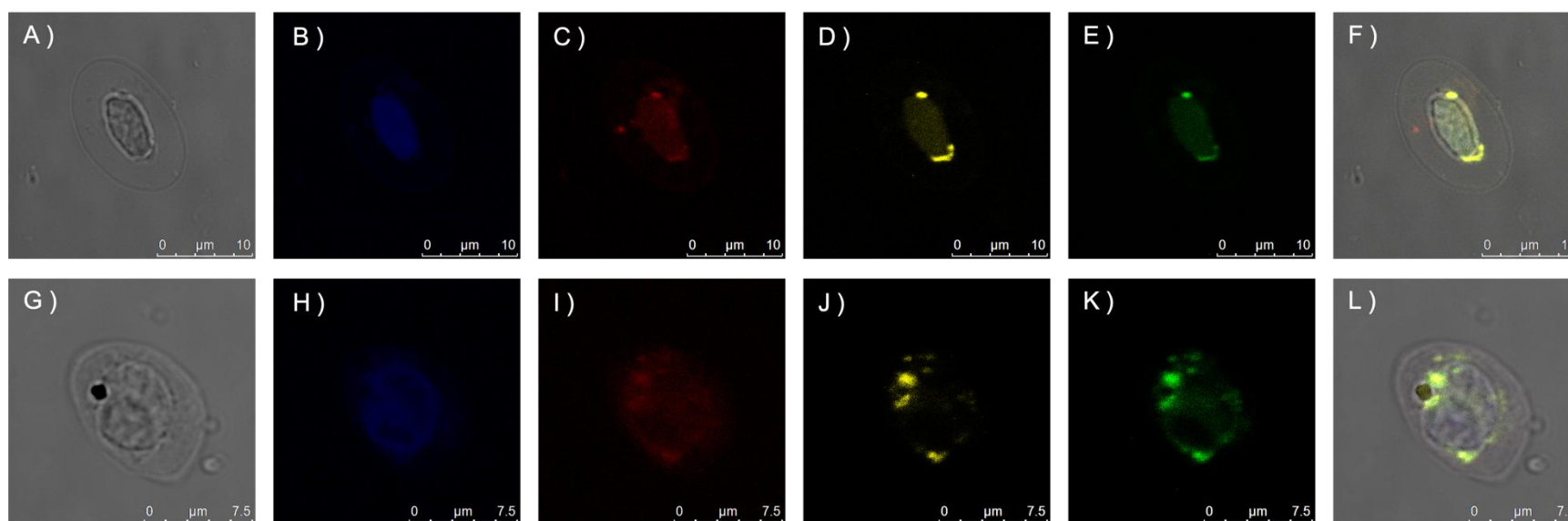
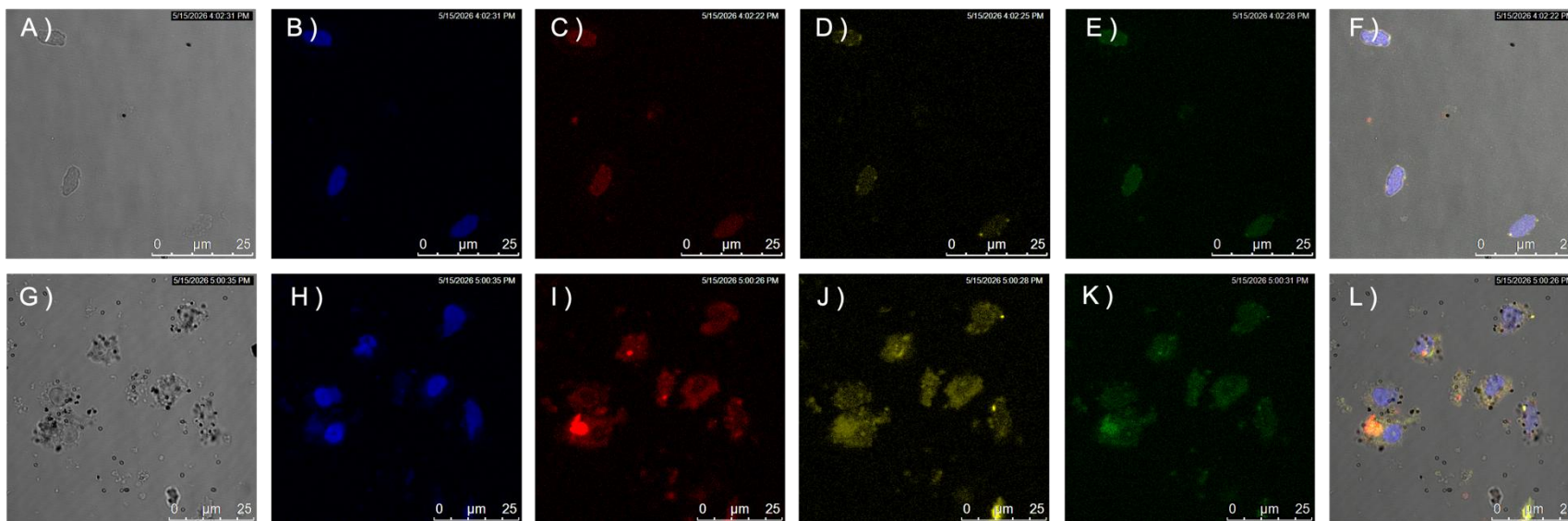


Figure 6. Confocal images of cells in different physiological conditions stained with a mitochondrial function assay panel. Cells are nucleated red blood cells from *Lampropholis delicata*. Cells have been stained at 32°C and fixed in 1% formalin. Cells were sorted by physiological condition using fluorescence-activated cell sorting prior to imaging. Images were taken on a Leica SP5 confocal microscope. Panels A) through F) are a cell in good physiological condition – the cell is oblong and symmetrical, contains an intact nucleus, and has tight clustering of mitochondrial probes in the mitochondria around the nucleus. Panels G) through H) are a cell in poor physiological condition – the cell membrane and nucleus are misshapen, mitochondrial probes are more dispersed. Images in each row, from left to right, show: brightfield, Hoechst 33342, MitoTracker Deep Red FM, MitoTracker Orange CMTMRos, MitoSOX Red, and a full transmission spectrum overlaid with the brightfield.



1993

1994 **Figure 7. Confocal images of cells in different physiological conditions stained with a mitochondrial function assay panel. Cells are nucleated red**
 1995 **liver cells from *Lampropholis delicata*.** Cells have been stained at 32°C and fixed in 1% formalin. Cells were sorted by physiological condition using
 1996 fluorescence-activated cell sorting prior to imaging. Images were taken on a Leica SP5 confocal microscope. Panels A) through F) are a cell in good
 1997 physiological condition – the cells are oblong and symmetrical, contains an intact nucleus, and has tight clustering of mitochondrial probes in the
 1998 mitochondria around the nucleus. Panels G) through H) are a cell in poor physiological condition – the cell membranes are misshapen and show signs of
 1999 membrane degradation (G), mitochondrial probes are more dispersed (I-K), and autofluorescence is high across fluorescent detectors (I-K). Images in
 2000 each row, from left to right, show: brightfield, Hoechst 33342, MitoTracker Deep Red FM, MitoTracker Orange CMTMRos, MitoSOX Red, and a full
 2001 transmission spectrum overlaid with the brightfield.

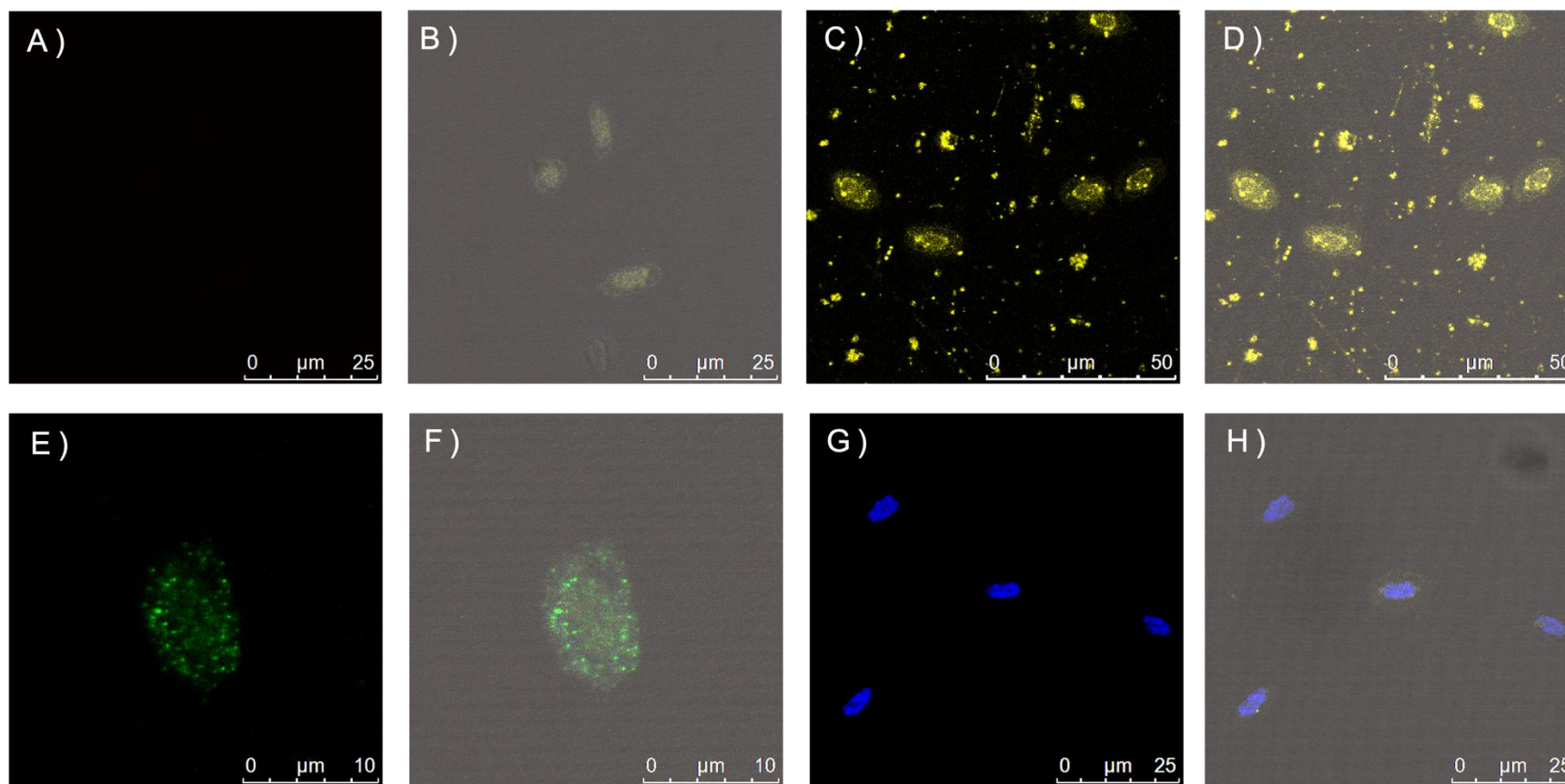
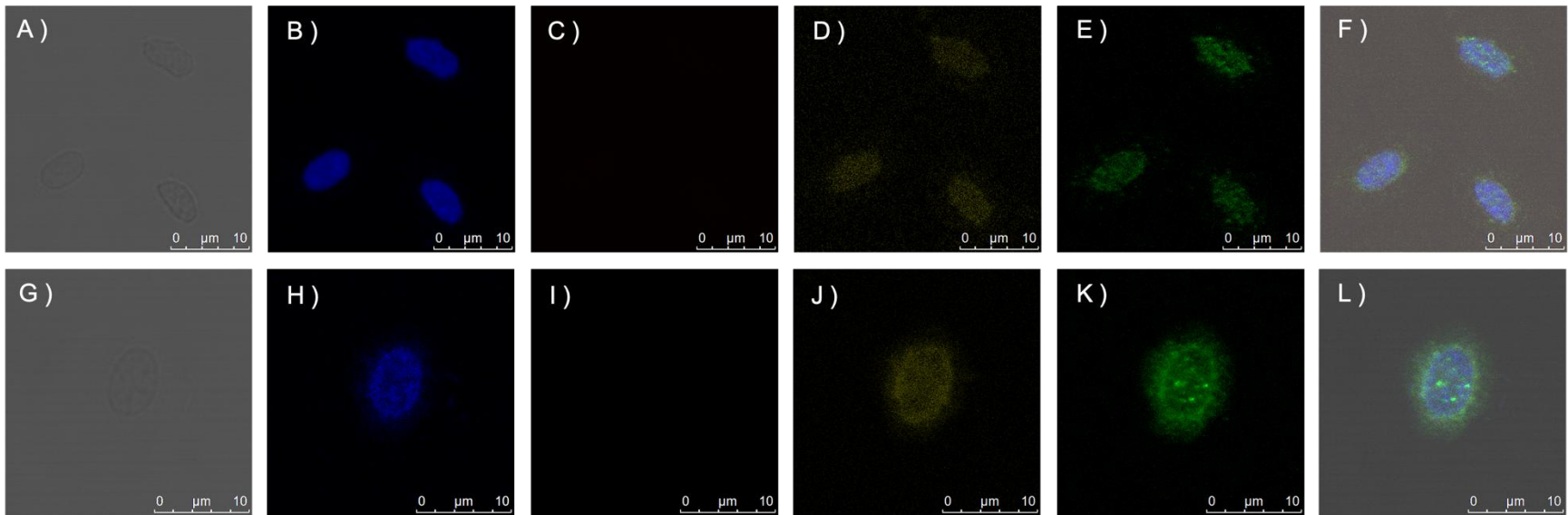


Figure 8. Confocal microscopy images of single-color controls from each fluorescent probe in an oxidative damage functional panel. Cells are *Lampropholis delicata* nucleated red blood cells that have been stained with probes at 32°C, fixed in 1% formalin, and permeabilised with digitonin. Cells were adhered to slides *via* cytopinning and preserved with VECTASHIELD mounting medium. Images were taken on a Leica SP5 confocal microscope. A) and B) are cells stained with Ghost Red 780 – a probe that binds to amines and fluoresces in dead cells. Ghost Red 780 emits fluorescence at the edge of the detectable range of the Leica SP5 microscope, therefore the signal is weak or absent. C) and D) are cells stained with BODIPY 665/676 Lipid Peroxidation Sensor – a probe that fluoresces when oxidised by peroxyl radicals. Unbound BODIPY 665/676 was detected in single-color controls and can be distinguished from probes bound to cells in D). E) and F) are a cell stained with 8-OHdG antibody and an Alexa Fluor 488 conjugate – an antibody that binds to the 8-OHdG molecule that is a result of oxidative DNA damage. G) and H) are cells stained with Hoechst 33342 – a probe that binds to intact nuclei. A), C), E), and G) are images of fluorescence from only the detector channel associated with the probe. B), D), F), and H) are full transmission spectra with fluorescence signals of all detector channels and overlaid with brightfield images to show cellular morphology.



2014

2015 **Figure 9. Confocal images of cells in different physiological conditions stained with an oxidative damage assay panel. Cells are nucleated red**
 2016 **blood cells from *Lampropholis delicata*.** Cells have been stained at 32°C, fixed in 1% formalin, and permeabilised with digitonin. Cells were sorted by
 2017 physiological condition using fluorescence-activated cell sorting prior to imaging. Images were taken on a Leica SP5 confocal microscope. Panels A)
 2018 through F) are a cell with low oxidative damage – the signal for both lipid peroxidation (D) and oxidative DNA damage (E) are weak. Panels G) through
 2019 H) are a cell in poor physiological condition – the signal for lipid peroxidation (J) is also weak, but oxidative DNA damage (K) is more pronounced.
 2020 Images in each row, from left to right, show: brightfield, Hoechst 33342, Ghost Red 780, BODIPY 665/676 Lipid Peroxidation Sensor, 8-OHdG antibody
 2021 with an Alexa Fluor 488 conjugate, and a full transmission spectrum overlaid with the brightfield. Note that Ghost Red 780 emits fluorescent signal at the
 2022 end of the detectable range of the Leica SP5, thus the signal is weak or absent.

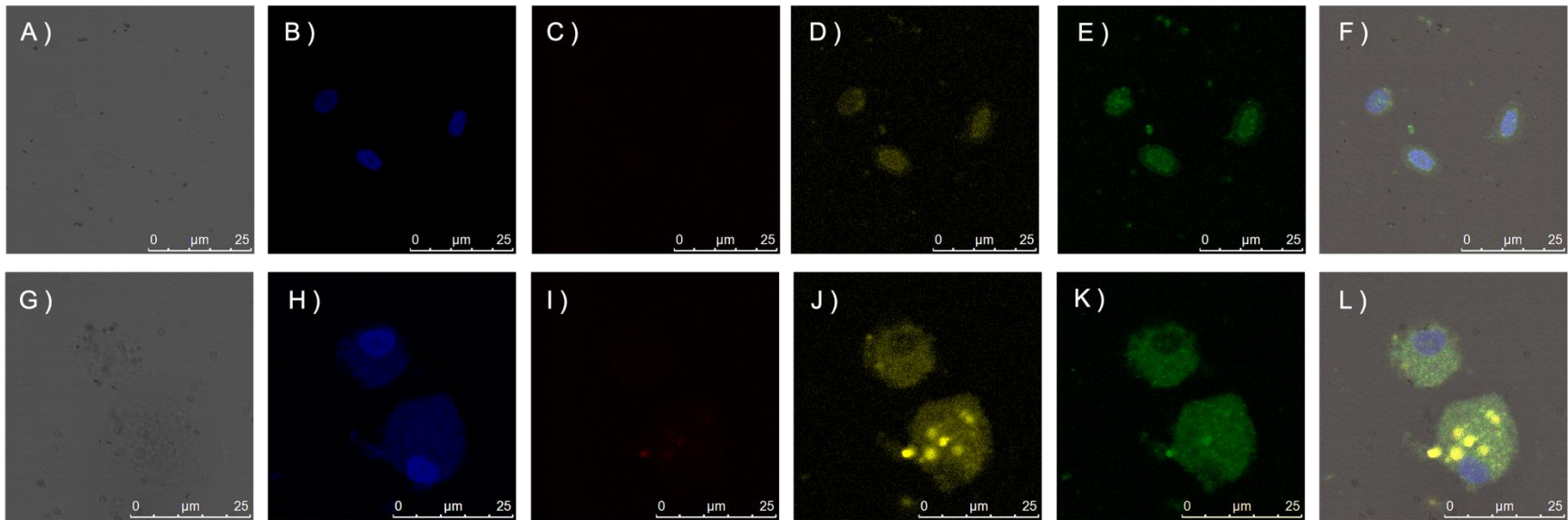


Figure 10. Confocal images of cells in different physiological conditions stained with an oxidative damage assay panel. Cells are homogenised liver cells from *Lampropholis delicata*. Cells have been stained at 32°C, fixed in 1% formalin, and permeabilised with digitonin. Cells were sorted by physiological condition using fluorescence-activated cell sorting prior to imaging. Images were taken on a Leica SP5 confocal microscope. Panels A) through F) are a cell with low oxidative damage – cells are oblong and symmetrical, and the signal for both lipid peroxidation (D) and oxidative DNA damage (E) are weak. Panels G) through H) are a cell in poor physiological condition – the cell is misshapen and shows signs of membrane disruption (G), has bright spots consistent with marks of lipid peroxidation (J), and has a pronounced signal for oxidative DNA damage throughout the cell (K). Images in each row, from left to right, show: brightfield, Hoechst 33342, Ghost Red 780, BODIPY 665/676 Lipid Peroxidation Sensor, 8-OHdG antibody with an Alexa Fluor 488 conjugate, and a full transmission spectrum overlaid with the brightfield. Note that Ghost Red 780 emits fluorescent signal at the end of the detectable range of the Leica SP5, thus the signal is weak or absent.

Results

All measurements were highly repeatable across technical replicates ($ICC > 0.85$, $p < 0.001$; Table 2), except for OXO in gonads ($ICC = 0.41$, $p = 0.02$). The lower repeatability of OXO in gonads may reflect greater variability in oxidative DNA repair rates, but even this indicated moderate repeatability, confirming minimal measurement error (Table 2).

Within (r_w) and between individual-level correlations (r_b) from multi-response models showed moderate correlations between physiological measures, with the strength of the correlation varying across tissues (Table 3). As expected, samples with cells having high mitochondrial density had high mitochondrial respiration (MTDR:MTO: $r_w = 0.45$ - 0.60) and ROS production (MTDR:MTSOX: $r_w = 0.60$ - 0.68) while samples with high lipid damage also had high DNA damage ($r_w = 0.43$ - 0.54). In addition, there was a moderate between-individual correlation between ROS production and DNA damage in gonads ($r_b = 0.4$), but correlations were weak in blood and liver ($r_b < 0.15$), possibly because of antioxidant capacity varying across individuals and tissues.

In contrast, and possibly unsurprisingly, correlations between measurements across tissues were generally weak (Fig. 5). Physiological parameters were most consistent between blood and liver, whereas gonads exhibited several opposing relationships. MTDR was modestly associated between blood and liver ($R = 0.15$) but uncorrelated with gonads ($R = 0.03$, -0.06). MTO showed moderate consistency among all tissues ($R = 0.16$ - 0.20). MTSOX was linked between blood and liver ($R = 0.40$) and between liver and gonads ($R = 0.26$) but not between blood and gonads ($R = 0.01$). LPX was negatively associated between blood and liver ($R = -0.15$) and blood and gonads ($R = -0.35$), yet positively associated between liver and gonads ($R = 0.37$). OXO showed only weak consistency among tissues ($R = 0.06$ - 0.08). These patterns indicate that while some parameters (notably MTO) are broadly consistent, others show pronounced tissue-specific variation.

Table 2. Estimates of repeatability among replicates for each physiological parameter within each tissue. Repeatability values are intraclass correlation coefficients (ICC) derived from generalized linear mixed effects models (GLMM) fitted with restricted maximum likelihood (REML) using the *rpt* function (*rptR* package; Stoffel, Nakagawa, & Schielzeth, 2017). Also provided are the lower and upper 95% CI of the repeatability estimates. A p-value ($\alpha < 0.05$) testing whether repeatability differs from zero (no consistency among measurements within individual) is also given.

Tissue	Parameter	Repeatability	L95% CI	U95% CI	p-value
Blood	MTDR	0.980	0.942	0.992	< 0.0001
	MTO	0.990	0.970	0.996	< 0.0001
	MTSOX	0.982	0.942	0.993	< 0.0001
	LPX	0.980	0.944	0.992	< 0.0001
	OXO	0.989	0.968	0.996	< 0.0001
Liver	MTDR	0.931	0.803	0.972	< 0.0001
	MTO	0.996	0.987	0.998	< 0.0001
	MTSOX	0.952	0.865	0.981	< 0.0001
	LPX	0.890	0.685	0.957	< 0.0001
	OXO	0.976	0.922	0.990	< 0.0001
Gonads	MTDR	0.955	0.870	0.982	< 0.0001
	MTO	0.946	0.837	0.979	< 0.0001
	MTSOX	0.954	0.868	0.981	< 0.0001
	LPX	0.864	0.658	0.947	< 0.0001
	OXO	0.408	0.000	0.691	0.02

Table 3. Intercept-level and residual correlations among physiological parameters (MTDR, MTO, MTSOX, LPX, and OXO) estimated separately for blood, liver, and gonads using multi-response Bayesian GLMMs. Models included specimen as a random effect with correlated intercepts (ρ) and estimated residual correlations among responses. Intercept-level correlations indicate between-individual covariation (e.g., whether individuals with higher MTDR also have higher MTO), and residual correlations indicate within-individual co-fluctuations (e.g., whether variation in MTDR is associated with variation in MTO). Positive values denote parameters that vary in the same direction, negative values denote opposing trends, and larger magnitudes indicate stronger associations.

Correlation	Tissue	Between-individual level Correlation	Within-individual level Correlation
MTDR:MTO	Blood	0.024	0.589
	Liver	0.043	0.450
	Gonads	-0.092	0.603
MTDR:MTSOX	Blood	0.172	0.680
	Liver	0.409	0.684
	Gonads	0.171	0.607
MTDR:LPX	Blood	0.089	0.118
	Liver	0.329	0.091
	Gonads	-0.150	0.029
MTDR:OXO	Blood	0.002	-0.024
	Liver	0.240	-0.154
	Gonads	-0.101	-0.021
MTO:MTSOX	Blood	0.155	0.463
	Liver	0.284	0.287
	Gonads	0.076	0.375
MTO:LPX	Blood	0.074	-0.124
	Liver	0.053	-0.031
	Gonads	0.123	-0.060
MTO:OXO	Blood	-0.118	0.025
	Liver	-0.149	-0.168
	Gonads	-0.026	-0.007
MTSOX:LPX	Blood	0.153	0.169

MTSOX:OXO	Liver	0.092	-0.026
	Gonads	-0.330	-0.035
	Blood	0.119	0.288
	Liver	0.184	-0.140
	Gonads	0.402	0.028
LPX:OXO	Blood	0.551	0.434
	Liver	0.104	0.464
	Gonads	-0.230	0.536

2075

2076

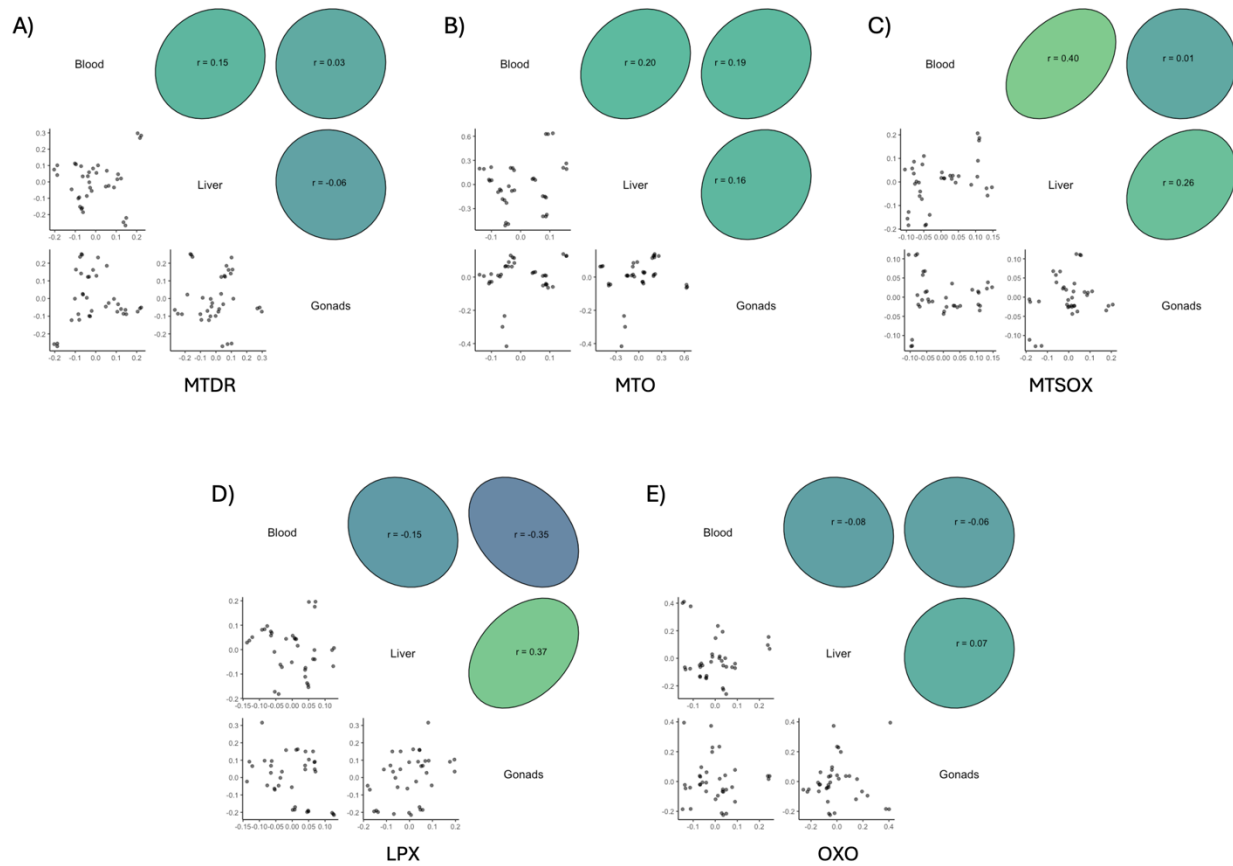


Figure 11. Correlation matrices of individual physiological parameters measured among three different tissues – blood, liver, and gonads – taken from the same individuals. Correlation matrices are derived from raw fluorescent intensity data, shown as scatterplots in the bottom left half of each panel, and Pearson correlation coefficients are given in the top right half of each panel. Panels are split among each individual physiological parameter – (A) mitochondrial density, MTDR; (B) mitochondrial membrane potential, MTO; (C) mitochondrial superoxide, MTSOX; (D) lipid peroxidation, LPX; (E) oxidative DNA damage, OXO.

Discussion

We developed and applied two multi-parametric functional assay panels for flow cytometric quantification of mitochondrial function and oxidative damage. Our functional assay panels resulted in highly repeatable measures among technical replicates within each of three tissues – blood, liver, and gonads – in a model lizard, *Lampropholis delicata*. Patterns of association among parameters of mitochondrial function and oxidative damage also aligned with physiological expectations in many cases, supporting the overall validity of the approach. For example, mitochondrial membrane potential correlated with higher levels of mitochondrial superoxide, and cells that exhibited greater levels of oxidative damage to lipids also exhibited greater levels of damage to DNA. However, correlations of physiological parameters among tissues were weak, suggesting tissue-specific physiological profiles. Even so, blood and liver exhibited comparable correlation structures to each other, while gonadal tissue exhibited a markedly different correlation structure.

The comparable correlational structures among blood and liver suggests that blood could potentially serve as a proxy for other somatic tissues in studies of oxidative physiology. Such insight has important practical implications because sampling blood (a non-invasively collected tissue) enables repeated sampling of the same individuals, thereby facilitating longitudinal experimental designs (Speakman et al., 2015). That the physiological profile of gonadal tissue notably differed from that of liver and blood is perhaps reflective of the distinct physiological state of gametic cells compared to somatic cells. As gonadal tissues in this study came from males, it is likely that the cells we observed were predominantly sperm cells, which rapidly accumulate ROS and oxidative damage, but also have highly active repair systems. Our findings from gonadal tissue therefore indicate potential for applying these methods to sperm to address the links between oxidative stress with reproductive performance and the transmission of oxidative damage across generations (Wang, Fu, & Li, 2025). Indeed, we expect that the flexibility of flow cytometry will allow our functional assay panels to be applied across many tissues, including critical organs such as heart, brain, or skeletal muscle. Optimising the methods described here for these tissues would open up scope to address a multitude of research questions linking cellular physiology to aerobic respiration, cognition, or locomotor performance.

Our simple case study demonstrates that flow cytometry provides a flexible, high-throughput platform for multi-measure physiological assessment across tissues and individuals. While we focused on mitochondrial function and oxidative damage, the panels can be expanded to include additional physiological parameters of interest (e.g., cell death, telomere dynamics, additional ROS or reactive nitrogen species) as new fluorescent probes and increasingly powerful flow cytometers become available (Marrocco, Altieri, & Peluso, 2017; Liu et al., 2025). Additionally, existing fluorescent probes for measuring oxidative stress markers (e.g., DNPH probe that measures protein carbonylation - Alomari et al., 2018; Dilek, 2022) through other fluorescence-based methods (i.e., fluorescence microscopy, ELISA) can potentially be adapted for use in flow cytometry applications. Indeed, this is what we did for the 8-OHdG antibody used in the oxidative damage consensus panel described here. Emerging spectral cytometry technologies are particularly promising, as they enable the simultaneous measurement of fluorescent signals from as many as 35 antigens or fluorescent probes on a single sample (Nolan, 2022; Brestoff, 2023; Astakhova et al., 2025). Spectral cytometry would allow for large multiparametric fluorescence panels while minimizing the need to split samples across separate panels as we did here. However, these spectral technologies are relatively new (Nolan & Condello, 2013) and likely not accessible

to many researchers. Even using traditional flow cytometry, these methods offer a broadly applicable framework for quantifying physiology, enabling experimental manipulations at the cellular level, cross-tissue comparisons, repeated sampling, and the generation of rich datasets.

A valuable next step in defining the role of flow cytometry in the ecophysiology space would be to confirm its measures with similar measures acquired using other techniques on tissues from the same individuals. Specifically, it would be worthwhile to test how mitochondrial membrane potential and superoxide values acquired via flow cytometry compare to high-resolution measures of mitochondrial respiration acquired via oximetry (*via* Oxygraph O2k) or extracellular flux (*via* SeaHorse XF). Likewise, mitochondrial content values estimated by the fluorescence of MitoTracker Deep Red FM could be compared to measures of mitochondrial mass estimated via Bradford protein assays. Or oxidative damage markers like 8-OHdG may be compared against commercially available colorimetry assay kits. While each of these established ecophysiological methods offer robust data on key aspects of mitochondrial and oxidative physiology, flow cytometry offers an efficient way to rapidly quantify multiple measures typically acquired only *via* employing several distinct techniques. The adoption of flow cytometric analysis into the already existing repertoire of powerful physiological techniques can, therefore, aid in meeting the needs of the rapidly advancing field of ecophysiology.

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Ethical statement

All experimental procedures and laboratory housing complied with Australian law and were approved by the Australian National University Animal Experimentation Ethics Committee (A2021/56).

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Author Contributions

DCL: Conceptualization, methodology, data collection, data curation, formal analysis, visualization, writing – original draft, writing, review and editing

MD: Conceptualization, methodology

CRF: Conceptualization, methodology, funding acquisition, writing, review and editing

DWAN: Conceptualization, methodology, funding acquisition, formal analysis, writing, review and editing.

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2345

Chapter 3: Developmental temperatures and stress hormones influence growth but not survival or physiology in two Australian lizards

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Abstract

Climate change is creating multigenerational stressors that interact to affect development. Temperature and early developmental stress hormones each induce plastic responses in offspring, but we know little about how they interact to drive long-term effects on phenotypes and fitness. Developmental responses are predicted to be driven by underlying changes in physiology, particularly mitochondrial dynamics and oxidative stress. However, few studies have followed animals across life to understand the long-term dynamics of physiology, life history and fitness. Using two congeneric lizards, *Lampropholis delicata* and *Lampropholis guichenoti*, we tested the effects of embryonic corticosterone exposure and incubation temperature on growth and survival throughout life (~760 days, 75% of their expected lifespan) in naturalistic conditions. As a test of the mechanisms underlying developmental effects, we quantified metabolism and cellular physiology in juveniles and adults, using new flow cytometric techniques. Embryonic corticosterone and warm incubation temperatures induced lizards to hatch at smaller body size, grow rapidly, but ultimately remain smaller in adulthood – a pattern of incomplete compensatory growth. Despite inducing faster growth, developmental treatments did not simultaneously impact survival. Furthermore, the effects of corticosterone and incubation temperature on phenotypes were not facilitated by changes in juvenile metabolism, or by long-term changes in cellular physiology. Our findings suggest that developmental conditions can simultaneously affect key life-history traits like growth and body size, but do not support that these effects are accompanied by trade-offs against survival or longevity, nor by persistent changes in mitochondrial or oxidative physiology as proposed by the ‘oxidative stress theory of life-history’.

Introduction

Developmental conditions influence an individual’s phenotype which can have cascading effects on fitness (“developmental plasticity” *in sensu* West-Eberhard, 2003). Developmental

plasticity may match an individual's phenotype to the prevailing conditions resulting in adaptive responses ("predictive adaptive response", Bateson et al., 2004). However, environmental conditions are also likely to change across an individual's life. In these scenarios, developmental effects can create mismatches between phenotypes and the environment ("developmental mismatch", Gluckman et al., 2019). Developmental mismatches that result in maladaptive phenotypes may, over time, select for compensatory responses that alter growth, reproduction, and longevity to alleviate the costs of any mismatch or result in canalization of developmentally plastic responses (Metcalf and Monaghan, 2001; West-Eberhard, 2003; Forsman, 2015). Regardless, it is expected that developmental plasticity will shape an individual's life-history trajectory and responses will depend on interacting biotic and abiotic processes that affect offspring development.

Importantly, developmental plasticity does not only depend on the environment directly impacting offspring but also the environment created by mothers (i.e., 'maternal effects', Mousseau and Fox, 1998). For example, incubation temperature is a well-established driver of developmental plasticity in oviparous animals with implications for growth, performance, and survival (Goodman, 2008; Siviter et al., 2019, Andrews et al., 2000; Van Damme et al., 1992). Oviparous animals that lack parental care, such as many species of reptiles, are especially sensitive to ambient developmental conditions because embryos in these taxa cannot behaviourally respond to environments in a comparable way to other life stages (Booth, 2018; Noble et al., 2018, but see Du and Shine, 2022). However, even in taxa that lack parental care, mothers can influence their developing embryos through maternal effects such as nest site selection, egg size, and the constituents of eggs including yolk proteins and hormones such as glucocorticoids (Resetarits, 1996; Sinervo, 1999; Iglesias-Carrasco et al., 2024; Sheriff et al., 2017). Glucocorticoids can become elevated in response to environmental stressors experienced by the mother and can be transferred from mothers to developing embryos via placental transfer (viviparous taxa, e.g., Itonaga et al., 2011) or *via* deposition in the yolk (oviparous taxa, e.g., Hayward and Wingfield, 2004; Pitk et al., 2012; Ensminger et al., 2018). Corticosterone (hereafter, 'CORT') is a primary glucocorticoid in vertebrates, and maternally derived CORT influences body size, growth, and survival and may be integral for driving population level responses to environmental changes (Crino et al., 2023; Schoech et al., 2011; Meylan et al., 2012).

In egg-laying species, there is substantial evidence that incubation temperature and maternal stress act as independent mediators of developmental plasticity (Noble et al., 2018; While et al., 2018; Raynal et al., 2022; Sheriff and Love, 2013; Crino and Breuner, 2015; Sopinka et al., 2017). Yet in natural settings, embryos are simultaneously exposed to both thermal variation and maternal stress (Marshall and Uller, 2007; Burgess and Marshall, 2014). Even so, relatively few studies have empirically tested how maternal effects and the developmental environment interact (but see Crino et al., 2024; Recio et al., 2025a; 2025b), and these have focused mainly on impacts on lab-reared animals. Furthermore, the independent effects of glucocorticoids and incubation temperature can be context-dependent, varying among species, sexes, or life stages (Warner et al., 2009; Uller et al., 2009; de Jong et al., 2023). Longitudinal studies incorporating multiple species, and potentially interacting developmental conditions, are therefore necessary for elucidating the long-term effects of developmental plasticity on key life-history traits like growth and survival.

Identifying the mechanisms underlying developmental plasticity has the potential to improve our capacity for predicting plasticity across diverse ecological contexts (Speakman et al., 2015). Furthermore, whether the same mechanisms are conserved among species can also provide important insights into common regulatory pathways and mechanisms governing developmentally plastic responses, yet such comparisons are rare. Developmental conditions that alter mitochondrial and oxidative stress dynamics can induce trade-offs in key life-history traits (the 'oxidative stress theory of life-history'; Monaghan, Metcalfe, & Torres, 2008; Costantini, 2024). Metabolism, mitochondrial function, and oxidative stress are sensitive to the environment, can be developmentally programmed, and provide a mechanistic foundation for understanding changes in life-history traits such as growth and survival (Speakman et al., 2015; Koch et al., 2024; Brown et al. 2024; Monaghan, Metcalfe, & Torres, 2008; Costantini, 2019). Metabolism and mitochondrial respiration are the primary source of cellular energy needed for growth and reproduction, yet produce reactive oxygen species (ROS) which damage DNA, lipids, and proteins compromising longevity (Speakman et al., 2015). Incubation

temperature is known to affect post-hatching metabolism and mitochondrial efficiency in birds and lizards (Nord and Nilsson, 2011; Ritchie and Friesen 2022; Friesen et al., 2022; de Jong et al., 2023; Crino et al., 2024), while developmental CORT exposure is frequently associated with oxidative stress and damage in birds (Haussman et al., 2011; Marasco et al., 2012; Gil et al., 2018). These early environmental stressors are therefore important in shaping the mechanism underlying key life history traits, yet how they interact to affect physiology and fitness remains elusive.

Here, we tested the joint influence of developmental CORT exposure (simulating maternal hormone transfer) and incubation temperature (23°C or 28°C) on long-term patterns of growth and survival across life for two congeneric lizards (*Lampropholis delicata* and *Lampropholis guichenoti*) under semi-natural conditions. In addition, we assessed physiological biomarkers in a subset of juvenile and adult lizards to test predictions about the physiological drivers underlying changes in life-history brought about by developmental environments. More specifically, we address the following questions: 1) Do maternal effects and the developmental environment interact to affect patterns of growth and survival throughout the lifespan?; 2) Is developmental plasticity in life-history traits mediated by underlying changes in mitochondrial physiology or oxidative damage that persist into adulthood?; and 3) Are developmentally plastic responses similar across closely-related species and driven by common physiological mechanisms? Our study offers rare empirical insights into interacting environmental stressors on both fitness associated phenotypes and physiological mechanisms across multiple species in naturalistic conditions.

Methods

Study System

We used two congeneric skinks (*Lampropholis delicata* and *Lampropholis guichenoti*), which co-occur throughout southeastern Australia for our experiments. Both species are oviparous, capital income breeders, and exhibit no postnatal parental care. *Lampropholis delicata* lays more, smaller eggs (~0.142g per egg) than *L. guichenoti* (~0.188g per egg). Accordingly, *L. delicata* hatches at relatively smaller sizes than *L. guichenoti*, and *L. guichenoti* tends to remain larger throughout life (Joss and Minard, 1985; but see Cogger, 2019; Wilson and Swan, 2021). Both species are expected to reach sexual maturity within 1 year of hatching and live about 2-3 years under natural conditions (Joss and Minard, 1985).

Animal Husbandry

All animals used in this study were reared from eggs sourced from a laboratory breeding colony established in 2019 from natural populations in Sydney, Australia and housed at the Australian National University. Breeding enclosures were comprised of 4 females and 2 males of a single species, and have been previously described (Crino et al., 2024; Recio et al., 2025a; 2025b; 2025c; Leibold et al., 2026). Each breeding enclosure included a plastic nest box filled with moist vermiculite to provide suitable substrate for oviposition. We checked nest boxes three times per week for freshly-laid clutches of eggs. Individual clutches were distinguished by eggs being connected or in proximity within the nest box. We measured the length and width of each egg using digital calipers (+0.01mm) and weighed each egg on a digital scale (OHAUS; +0.001g). Eggs from each clutch were distributed evenly among four ‘stress’ by incubation temperature treatments following a split-clutch design.

Experimental Treatments

To simulate elevated maternal CORT transfer, 10µL of either CORT dissolved in 100% ethanol (10pg/mL) or a 100% ethanol control was topically applied to each egg. This concentration of CORT was previously found to increase circulating amounts of yolk corticosterone to 2 SDs above the naturally-occurring mean (but within the range of variation) when applied topically to *L. delicata* eggs (Crino et al., 2024). We applied a standardized volume of CORT to each egg, regardless of mass. This approach allowed us to test for the independent influence of variation in maternal investment, both within- and between- species, as a mediator of developmental effects. Eggs were held individually in

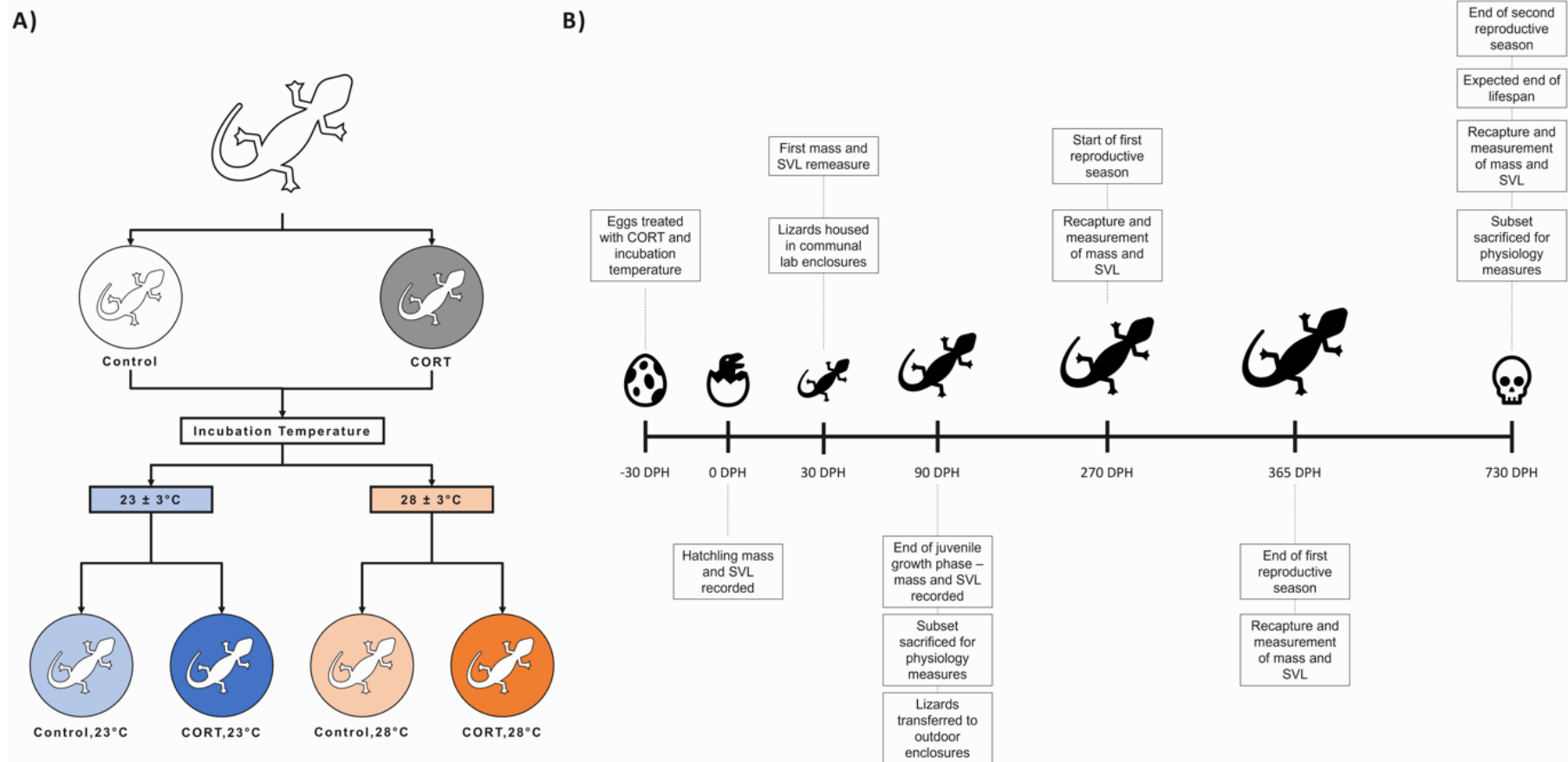
plastic cups containing moist vermiculite (12g water to 4g vermiculite), suitable for these species preference for humid nests (Cheetham et al., 2011). Cups containing eggs were covered with plastic wrap and placed in incubators (LABWIT) programmed to either 23°C or 28°C (both $\pm 3^\circ\text{C}$ simulating daily thermal cycles). Temperatures were chosen based on mean natural nest temperatures (26°C; Qualls and Shine, 2000; Bilcke et al., 2006) and represent the mean temperatures in shaded nests or heat-exposed nests (Cheetham et al., 2011; Chapple et al., 2014). A schematic depicting the experimental treatments is given in Figure 1A.

Growth and Survival

We checked for hatchlings three times per week, whereupon we measured their snout-vent-lengths (SVL) with a ruler (± 1 mm) and weighed them on a digital scale (OHAUS; ± 0.001 g). Hatchlings were housed individually in plastic bins for ~30 days post-hatching, after which we remeasured their mass and SVL, toe-clipped them for permanent identification, and transferred them to mixed-species communal enclosures for an additional 60 days. Mixed-species enclosures contained equal numbers of *L. delicata* and *L. guichenoti* at either a low (16 total animals; $N_{\text{LOW}} = 11$) or high (32 total animals; $N_{\text{HIGH}} = 12$) density. At ~90 days post-hatching, lizards were remeasured, sexed via hemipene eversion, and released into naturalistic outdoor mesocosms. A subset of experimental lizards was not released into mesocosms but instead retained for measurements of juvenile metabolic rate and cellular physiology (described later).

Outdoor mesocosms were 3 m diameter plastic tubs containing gravel and mulch substrate, three ceramic tile hides, a large plastic water dish, and were partially covered by shade cloth. Water was provided to each mesocosm twice daily using an automated irrigation system. Naturally occurring invertebrates were available in mesocosms as a food source, but we also supplemented each mesocosm with 160 crickets (*Acheta domesticus*) twice per week. We recaptured, sexed, and remeasured (mass, SVL) lizards at the beginning (~270 days post-hatching) and end (~365 days post-hatching) of the first reproductive season and at the end of the second reproductive season (~760 days post-hatching). We recaptured animals by digging around the perimeter of each enclosure and checking under each hide for three consecutive days, until we no longer saw active animals. Animals were considered as having died if they were not found in either the end of the first (~365 days post-hatching) or second (~760 days-post hatching) reproductive season. At the end of the last recapture, a subset of animals was retained for measurements of adult cellular physiology, and all remaining animals were released back into mesocosms for other long-term experiments. A schematic of the experimental timeline is given in Figure 1B.

Population density manipulations in mesocosms were intended for further studies of population dynamics across multiple generations, rather than a focus of the present study. Our expectation was that population density would not influence patterns of growth and survival, given the relatively low total numbers of individuals per enclosure, and the abundance of resources available to lizards in the mesocosms. Instead, we expected any effects of population density to manifest only after the current generation had reproduced and population size grew. Even so, all enclosures were supplemented with equivalent numbers of *Acheta domesticus* so that population density would potentially reflect differences in competition for food. As such, we considered population density as a potential influence on life-history traits in analyses.



2526

2527 **Figure 1. Methods schematic and experimental timeline.** A) depicts the experimental design used, wherein eggs from a clutch were split among two
 2528 developmental stress hormone treatments (elevated CORT or an ethanol Control), and then split among either a cool (23°C) or warm (28°C) incubation
 2529 temperature regime (both $\pm 3^\circ\text{C}$). B) describes the experimental timeline, whereby animals were raised from oviposition to 730 days post-hatching (~75% of
 2530 the total expected lifespan). Animals were transferred to naturalistic outdoor enclosures and remeasured throughout their lifespan. At 90 days post-hatching
 2531 (juvenile) and 730 days post-hatching (adult), subsets of animals were measured for whole-organismal metabolism and subsequently sacrificed for measures
 2532 of cellular physiology.

Whole Organism Metabolism

We measured the resting metabolic rates (RMR) of a subset of juvenile lizards from each of the four treatments via push-mode flow respirometry (Field Metabolic System, 'FMS', Sable Systems Inc.) through a multiplexer (MUX, Sable Systems Inc.) following standard protocols (Lighton, 2009) adjusted for our system. We recorded metabolism data from two separate FMS during each trial, allowing for the simultaneous measurement of up to 14 lizards. Each MUX was connected to eight channels – seven channels containing lizards and one baseline channel. Lizards were held individually in 60 mL plastic respirometry chambers, leaving one chamber empty to serve as a baseline, and housed within incubators (LABWIT) programmed to 32°C. The temperature for metabolism trials was selected to approximate the preferred body temperature of these species (31°C ± 0.47; Zhang et al., 2023) and is a temperature at which metabolic rates are highly repeatable (Kar, Nakagawa, & Noble, 2022). All metabolism trials were performed overnight (between 1900-0900 hours) when these species are inactive. The first two hours of data collection during each trial was discarded, allowing lizards time to acclimate to respirometry conditions before we recorded their metabolic rates.

Incurrent air was scrubbed of CO₂ using Soda Lime and subsequently pumped to the active channel of the multiplexer at a rate of 180 mL · min⁻¹. The multiplexer was programmed to pump air through each respirometry chamber for 300 seconds at a time, switching back to the baseline for 300 seconds, then switching to the next respirometry chamber sequentially. A full cycle between measurements for each chamber took 3900 seconds (65 min). This cycle was repeated for the entire duration of the trial, providing repeated measurements for each lizard. Air returning to the FMS from the respirometry chamber was first scrubbed of water vapor using Drierite, measured for CO₂ concentration, scrubbed of CO₂ using Soda Lime, and measured for O₂ concentration. Gas concentrations were measured in ExpeData (v.1.9.2), then processed into flux measurements of oxygen consumption ($\dot{V}O_2 = \text{mL O}_2 \cdot \text{min}^{-1}$) and carbon dioxide production ($\dot{V}CO_2 = \text{mL CO}_2 \cdot \text{min}^{-1}$) per minute using the MetabR package (Ernest et al., 2012) in R. Briefly, the MetabR functions draw a spline which estimates baseline gas concentrations throughout a trial. Then, the difference between the baseline concentration and the peak gas concentration of each adjacent chamber containing a lizard is calculated. The difference in gas concentrations is transformed into units of volume by multiplying by the volume of the respirometry chamber (60 mL). Finally, rates of gas exchange are calculated by dividing the change in gas volume by the time between each remeasure (65 min).

Cellular Physiology

To test whether developmental CORT and incubation temperature have persistent influence on cellular physiology, we used flow cytometry to measure parameters of mitochondrial function and oxidative damage in the livers and hearts of juvenile (~90 days post-hatch; same animals from RMR measures) and adult lizards (~760 days post-hatch; separate subset of animals from mesocosms). Livers and hearts were used for cellular physiology assays due to their relevance to growth, metabolism, and longevity. Hepatocytes (liver cells) are the site of glucose metabolism for glycolysis and gluconeogenesis, therefore are responsible for the conversion of energy into growth and metabolic activity (Han et al., 2016). Cardiomyocytes (heart cells) are involved in cardiovascular respiration, expected to have high mitochondrial densities, and are major sites of ROS production that can cause oxidative damage associated with longevity (Peoples et al., 2019). We humanely killed lizards via intraperitoneal injection of anaesthetic (10 mg/kg of 10 mg/mL Alfaxan) followed by decapitation. After decapitation, the liver and heart were removed from the body cavity, then homogenised into single-cell suspensions in a 1000µL 1X PBS solution, which were then split into two 500µL aliquots. One aliquot was prepared for quantification of mitochondrial function and the other for quantification of two markers of oxidative damage.

For mitochondrial function assays, we stained one 500µL aliquot of each tissue with 5µM MitoTracker Deep Red FM, 2.5µM MitoTracker Orange CMTMRos, and 50µM MitoSOX Red fluorescent probes. These probes all gather in active mitochondria and their fluorescent intensities represent, respectively, mitochondrial mass, mitochondrial membrane potential, and mitochondrial superoxide. For oxidative damage assays, we stained one 500µL aliquot of each tissue with 10µM

BODIPY 665/676 Lipid Peroxidation Sensor and a 7 μ M 8-OHdG antibody with a 10 μ g/mL Alexa-Fluor 488 conjugate. BODIPY 665/676 binds to lipid membranes and shifts in fluorescent signal when oxidized by peroxide radicals, thereby representing lipid peroxidation, while the 8-OHdG antibody binds to 8-OHdG – a common biomarker of oxidative DNA damage. For both assays, we stained all samples with 100 μ g/mL Hoechst 33342 Nuclear Viability Dye, which allows for distinction between intact, nucleated cells and cellular debris. All staining was performed at 32°C for 30 minutes to match the temperatures used for whole-organism metabolism trials. The exception was tagging 8-OHdG with antibodies, which was done overnight (~12 hours) at 4°C according to manufacturer recommendations. For juveniles, the mitochondrial function aliquots were run on fresh cells within 2 hours of staining, while the oxidative damage aliquots were formalin-fixed and cryopreserved following staining and run within 6 months of cryopreservation. For adults, both aliquots were formalin-fixed following staining and run within 2 days without cryopreservation.

All flow cytometry assays were run on a 5-laser (blue, red, yellow-green, violet, ultraviolet) flow cytometer (Becton-Dickson LSRFortessa X-20) with 20 fluorescence detectors and a high-throughput plate reader, using the default configuration of bandpass filters. We measured fluorescent intensity emitted by each probe in the detector most closely overlapping the probe's emission spectra. Additionally, we recorded the forward-angle (FSC) and side-angle scatter (SSC) of each cell, which are indicative of cell size and complexity, respectively. Fluorescence data was recorded in BD FACSDiva (v. 8.0.1) then imported into FlowJo (v. 10.1) for initial processing. In FlowJo, we compensated for spillover in fluorescent signal among probes using a traditional compensation matrix algorithm provided in FlowJo and following standard compensation procedures (BD Biosciences, 2009). We then applied a basic gating structure that filtered our observations to only intact, nucleated cells that were positive for all the stains used in the assay (all parameters >10³ units of fluorescent intensity). If there were fewer than 200 cells in the resultant population for a replicate, we considered this replicate as missing data. For each fluorescent probe, we calculated the geometric mean fluorescent intensity (MFI) from the compensated, gated population of cells, which we then exported for additional processing and analyses in R.

In R (v. 4.2.1, R Core Team, 2022), we log-transformed (base 10) MFI values for each fluorescent probe to account for the inherent non-linearity of fluorescence intensity. We centered the resultant log-transformed, averaged, MFI values about the population-level mean (by species, age class, and tissue) for ease of modelling and interpretation of main effects. Centering fluorescent intensity values is appropriate for these analyses because fluorescent intensity is inherently a relative measurement of marker expression in a cell. While these are not absolute values, thus cannot be interpreted as a direct equivalent to alternative measures of physiology (e.g., mitochondrial respiration rates as quantified *via* high-resolution respirometry), they are valuable in testing the relative expression of physiological biomarkers among experimental groups.

Modelling and Analyses

All analyses were performed in R (v.4.2.1; R Core Team, 2022) using Bayesian generalised linear mixed-effects models (GLMM) from the *brms* (v.2.23.0) and *rstan* (v.2.32.6) packages (Bürkner, 2017; 2018; 2021; Stan Development Team, 2024). All models followed a generalised structure in which each model was run on four parallel MCMC chains for 10000 iterations with a warmup period of 2000 iterations at an adapt delta value of 0.99, thinning of 1, and maximum tree depth of 15. For all response variables, we modelled *L. delicata* and *L. guichenoti* separately to reduce model complexity and because the Bayesian framework allows for comparisons of effect sizes among species without necessarily including species as a covariate in models.

To determine the inclusion of main effects, covariates, and interaction terms in each model, we used a hypothesis-driven model selection approach, which is described in supplemental materials (see *Supplementary Document – Model Comparisons*). All models supported the inclusion of CORT treatment and incubation temperature as independent main effects, but did not support the inclusion of any interactions between them. The inclusion of additional main effects depended on the response variable, but model selection did not support any interactions between CORT or incubation

temperature with any other variables (i.e., initial egg mass, sex, or population density). Random effects were included where relevant to account for repeated measurements of the same individual or for unexplained sources of variation among trial dates. Below, we describe the specific model structure used for each response variable. Following modelling, we extracted the predicted posterior distributions of each response variable for each developmental CORT by incubation temperature combination, which we used for hypothesis testing.

Development Length and Hatchling Body Size

Models of development length (time-to-hatch), hatchling mass, and hatchling SVL included the independent effects of CORT treatment and incubation temperature, with no interactions. The initial mass of each egg was included as an independent covariate to test for the effects of variation in maternal investment on hatchling traits. To account for similarities among siblings, clutch was included as a random effect in models of development length and hatchling size. We modelled hatchling mass and SVL as a multi-response model, with residual correlations included, to account for the positive relationship between body length and mass. We can model these jointly to prevent spurious effects of experimental treatments on each trait that are driven indirectly by effects on the other trait. Development length was modelled separately.

Growth

To test the effects of developmental treatments on patterns of growth, we modelled growth using the von Bertalanffy growth model, for which we modelled the mass or SVL (collectively, ‘size’) as a function of age. We modelled growth for mass and SVL separately, rather than as a multi-response model, because a multi-response model with residual correlations included prevented the model from converging. The von Bertalanffy growth model estimates size at any given age as follows:

$$Size = L_{inf} \cdot (1 - e^{-K \cdot (age - t_0)})$$

Where L_{inf} is the asymptotic “maximum” size of the animal (in g for mass; in mm for SVL), K is the growth rate coefficient (in days⁻¹), and t_0 is a hypothetical age at which size equals zero (in days). The asymptotic size, L_{inf} , is a predicted maximum body size at which animals cease growing given that they continue to grow as predicted by the model. The growth rate coefficient, K , approximates the proportion of L_{inf} the animal gains per day. Inverted, K approximates the length of time for an animal to reach L_{inf} . The hypothetical age at size equals zero, t_0 , is largely an artefact of the model used to set the intercept of the growth curve. As such, t_0 was modelled as an intercept with no main or random effects, while L_{inf} and K were modelled including the specified main and random effects.

Model structures differed between growth in mass and growth in SVL, as well as varying by species. In all models, CORT treatment and incubation temperature were included as independent main effects with no interactions, and individual as a random effect to account for repeated measurements. Population density was included as an independent effect only in the growth model of mass for *L. delicata*, but not for *L. guichenoti*, nor for SVL of either species. However, exploration of raw data suggested that both sex and population density affected the growth in mass of *L. guichenoti*, so we performed a secondary analysis including only sex and population density as main effects. Population density was not supported as an independent effect in growth in SVL of either species, but sex was included as an independent effect for *L. guichenoti* only.

Juvenile Whole-Organism Metabolism

We jointly modelled the resting metabolic rate of oxygen exchange (RMR $\dot{V}O_2$; mL O₂ · min⁻¹) and carbon dioxide production (RMR $\dot{V}CO_2$; mL O₂ · min⁻¹) of juvenile lizards. The multi-response model included residual correlations to account for the relationship between $\dot{V}O_2$ and $\dot{V}CO_2$. Metabolism was modelled as a function of CORT treatment and incubation temperature, with no interactions. We included mass as an independent covariate to account for the mass-specificity of metabolic rate. Trial was included as a random intercept to account for variation in ambient conditions among trial days.

Cellular Physiology

Cellular physiology comprised of six interrelated response variables: 1) cell size, 2) mitochondrial mass, 3) mitochondrial membrane potential, 4) mitochondrial superoxide, 5) lipid peroxidation, and 6) oxidative DNA damage. We modelled all six response variables in a single multi-response model assuming residual correlations, thereby accounting for the effects of CORT and incubation temperature on one variable affecting another variable. All six responses included CORT treatment and incubation temperature as independent main effects, with no interactions. Additionally, all responses included trial as a random effect to account for any unexplained variation in flow cytometer settings among trial days. Cell size was included as an independent covariate in the model of mitochondrial mass, thereby allowing us to approximate the effect of CORT and temperature on mitochondrial density. In a similar manner, we included mitochondrial membrane potential as a covariate in the model of mitochondrial superoxide. This accounts for the expectation of more active mitochondria generally producing more superoxide, thereby allowing us to approximate the effects of CORT and temperature on mitochondrial efficiency.

We imputed missing data in models of cellular physiology to account for technical errors that rendered some data unusable, using the *mi()* function of *brms* and in accordance with suggestions on missing data imputation (Noble and Nakagawa, 2021; Nakagawa et al., 2023). Missing data imputation was primarily used to account for missing observations on oxidative damage from the hearts of juvenile lizards, for which cell counts were frequently below 200 cells (29 observations in *L. delicata*, 37 for *L. guichenoti*). Data imputation estimated parameters of oxidative damage based on non-missing data from the mitochondrial function assay of the same individual.

For multi-response models of cellular physiology, we modelled the effects of CORT and incubation temperature separately for juveniles and adults, for *L. delicata* and *L. guichenoti*, and for livers and hearts (eight total models). We modelled each of these groups separately to reduce model complexity and because the Bayesian framework allows for comparisons of effect sizes among species, age classes, or tissues without necessarily including these traits as main effects.

Hypothesis Testing

To test the hypotheses that CORT treatment and incubation temperature affect hatchling size, growth, metabolism, and cellular physiology in *L. delicata* and *L. guichenoti*, we extracted the posterior distributions for parameters of each response variable for each treatment combination. Using the posterior distributions, we derived the overall (main) effect of CORT treatment as the difference between the distributions of CORT-treated animals (pooling posteriors for [CORT,23°C] and [CORT,28°C]) and control animals (pooling posteriors for [Control,23°C] and [Control,28°C]) and subtracting these providing a CORT effect that is an average of both temperature treatments (Δ [CORT - Control] = ([CORT,23°C], [CORT,28°C]) - ([Control,23°C], [Control,28°C])). Similarly, we estimated the effect of incubation temperature as the difference between the distributions of 28°C-treated and 23°C-treated animals (Δ [28°C-23°C] = ([Control,28°C], [CORT,28°C]) - ([Control,23°C], [CORT,28°C])). We also tested for differences in effect sizes between species as the marginalised differences in the main effect of CORT-treatment (Δ [CORT - Control]_{delicata} - Δ [CORT - Control]_{guichenoti}) and incubation temperature (Δ [28°C - 23°C]_{delicata} - Δ [28°C - 23°C]_{guichenoti}). For models of cellular physiology, we also tested for differences in effect sizes between livers and hearts and between juveniles and adults within each species using. We estimated the mean, the standard error about the mean, and the lower and upper 95% credible intervals of each resulting contrast. As a test of significance, we calculate the pMCMC ($\alpha \leq 0.05$), which tests whether the difference between two distributions differs significantly from zero. This was a two-tailed test.

Results

We found evidence for developmental effects on life-history variation in *L. delicata* and *L. guichenoti*, with direct effects of developmental CORT and incubation temperature on patterns of growth and body size in both *L. delicata* and *L. guichenoti*. Lizards treated with CORT or incubated at

28°C hatched at reduced mass (Figure 2), grew rapidly early in life (Figure 3), but ultimately remained smaller at adulthood (Figure 4). However, our results do not support that those developmental effects induced a trade-off between growth and survival. Survival to late adulthood was not dependent on CORT or incubation temperature, and survival was high overall in our study (Figure 4).

Rapid juvenile growth was not facilitated by elevated metabolic rates. Neither CORT nor incubation temperature significantly affected juvenile resting metabolic rates (Figure 6). However, there was some non-significant evidence that CORT depressed metabolism in *L. delicata* and warm incubation temperatures depressed metabolism in *L. guichenoti*. Developmental effects on life-history traits were also likely not driven by their sustained effects on cellular physiology. Cellular morphology (Figures 7 & 8), mitochondrial function (Figures 9 & 10), and oxidative damage (Figures 11 & 12) markers in both livers and hearts were largely unaffected by CORT and incubation temperature, both early in life and in late adulthood.

Our results demonstrate a role for developmental CORT and incubation temperature in driving important life-history traits like growth, but do not support that they induce a trade-off between growth and longevity. Furthermore, our findings do not support physiology at the organismal or cellular level as the main mechanism underlying developmental effects. Here, we report on the main effects of CORT and incubation temperature on the mass of hatchling and adult lizards, growth curves for mass, survival, metabolic rate, and cellular physiology. For additional reporting on measures of body length, and for the independent effects of sex and population density on life-history traits, see *Supplemental Results* in Appendix III. For an exhaustive list of tables depicting raw summary statistics and model outputs for main effects, pairwise comparisons among treatment groups, and species-, age-, and tissue- comparisons of effect sizes, see *Supplemental Tables* in Appendix III.

CORT and warm-incubation induce reduced hatchling body size

We predicted that developmental CORT exposure and incubation at warm temperatures would induce *L. delicata* and *L. guichenoti* to hatch at reduced body sizes. Consistent with our predictions, and with previous work on this system (Leibold et al., 2026; *L. delicata* only – Crino et al., 2024), CORT-exposed lizards weighed less at hatching than those treated with an ethanol control (*L. delicata*: Δ [CORT – Control] = $-0.002 \text{ g} \pm <0.001$, pMCMC = 0.011; *L. guichenoti*: Δ [CORT – Control] = $-0.002 \text{ g} \pm <0.001$, pMCMC = 0.019). Likewise, lizards incubated at 28°C were lighter at hatching than lizards incubated at 23°C (*L. delicata*: Δ [28°C – 23°C] = $-0.004 \text{ g} \pm <0.001$, pMCMC < 0.001; *L. guichenoti*: Δ [28°C – 23°C] = $-0.003 \text{ g} \pm <0.001$, pMCMC = 0.001) (Figure 2C, Supplemental Table 6).

The effects of developmental CORT and incubation temperature on hatchling size were additive (Figure 2D, Supplemental Table 1). Lizards that were simultaneously exposed to elevated CORT and incubated at 28°C (*L. delicata*: $N_{\text{CORT},28^\circ\text{C}} = 58$, Mean $\pm$ SD = $0.108 \text{ g} \pm 0.011$; *L. guichenoti*: $N_{\text{CORT},28^\circ\text{C}} = 66$, Mean $\pm$ SD = $0.136 \text{ g} \pm 0.015$) were even lighter at hatching than lizards treated with CORT and incubated at 23°C (*L. delicata*: $N_{\text{CORT},23^\circ\text{C}} = 50$, Mean $\pm$ SD = $0.112 \text{ g} \pm 0.012$; *L. guichenoti*: $N_{\text{CORT},23^\circ\text{C}} = 59$, Mean $\pm$ SD = $0.142 \text{ g} \pm 0.015$) or lizards receiving the Control treatment and incubated at 28°C (*L. delicata*: $N_{\text{Control},28^\circ\text{C}} = 57$, Mean $\pm$ SD = $0.109 \text{ g} \pm 0.011$; *L. guichenoti*: $N_{\text{Control},28^\circ\text{C}} = 63$, Mean $\pm$ SD = $0.140 \text{ g} \pm 0.017$). Control lizards incubated at 23°C hatched at the largest body sizes (*L. delicata*: $N_{\text{Control},23^\circ\text{C}} = 51$, Mean $\pm$ SD = $0.114 \text{ g} \pm 0.011$; *L. guichenoti*: $N_{\text{Control},23^\circ\text{C}} = 64$, Mean $\pm$ SD = $0.142 \text{ g} \pm 0.015$).

Developmental effects on hatchling mass were the same in *L. delicata* and *L. guichenoti*, suggesting conserved responses to developmental conditions among these species despite their ecological differences (Stress: Δ [*L. delicata* – *L. guichenoti*] = $-0.001 \pm <0.001$, pMCMC = 0.669; Temperature: Δ [*L. delicata* – *L. guichenoti*] = $-0.001 \pm <0.001$, pMCMC = 0.753) (Supplemental Table 10). However, the effects of CORT and incubation temperature were relatively small in both species, only accounting for a reduction in body mass of up to 0.007g (~6.2% of total body mass) in

L. delicata and an even smaller reduction of 0.005g (~3.5% of total body mass) in *L. guichenoti* (Supplemental Table 8).

CORT-treated and warm-incubated lizards grow at accelerated rates

Although CORT-treatment and warm incubation temperatures induced *L. delicata* and *L. guichenoti* to hatch at reduced body mass, developmental treatments also induced these lizards to gain mass more rapidly, at least early in life (Figure 3, Supplemental Table 11). Von Bertalanffy growth models predicted that lizards treated with CORT had higher growth rate coefficients (K , days⁻¹) than Control-treated lizards (*L. delicata*: Δ [CORT – Control] = $1.5e^{-4} \pm <1e^{-5}$ days⁻¹; pMCMC < 0.001; *L. guichenoti*: Δ [CORT – Control] = $4.6e^{-4} \pm <1e^{-5}$ days⁻¹; pMCMC < 0.001). Likewise, lizards incubated at 28°C had higher growth rate coefficients than lizards incubated at 23°C (*L. delicata*: Δ [28°C – 23°C] = $1.2e^{-4}$ days⁻¹ $\pm <1e^{-5}$, pMCMC < 0.001; *L. guichenoti*: Δ [28°C – 23°C] = $2.2e^{-4} \pm <1e^{-5}$ days⁻¹, pMCMC < 0.001). Similarly, growth models predicted that lizards treated with CORT would reach larger asymptotic body masses (L_{inf} , grams) than Control lizards (*L. delicata*: Δ [CORT – Control] = 0.008 g $\pm <0.001$; pMCMC < 0.001; *L. guichenoti*: Δ [CORT – Control] = 0.012 g $\pm <0.001$; pMCMC < 0.001), and the same was true of lizards incubated at 28°C compared to those incubated at 23°C (*L. delicata*: Δ [28°C – 23°C] = 0.016 g $\pm <0.001$, pMCMC < 0.001; *L. guichenoti*: Δ [28°C – 23°C] = 0.039 g $\pm <0.001$, pMCMC < 0.001). Taken together, growth models predicted that CORT-treated and warm-incubated lizards grow more rapidly when approaching their adult body size, and that these lizards are expected to ultimately reach larger adult body sizes given that they continue to grow at accelerated rates.

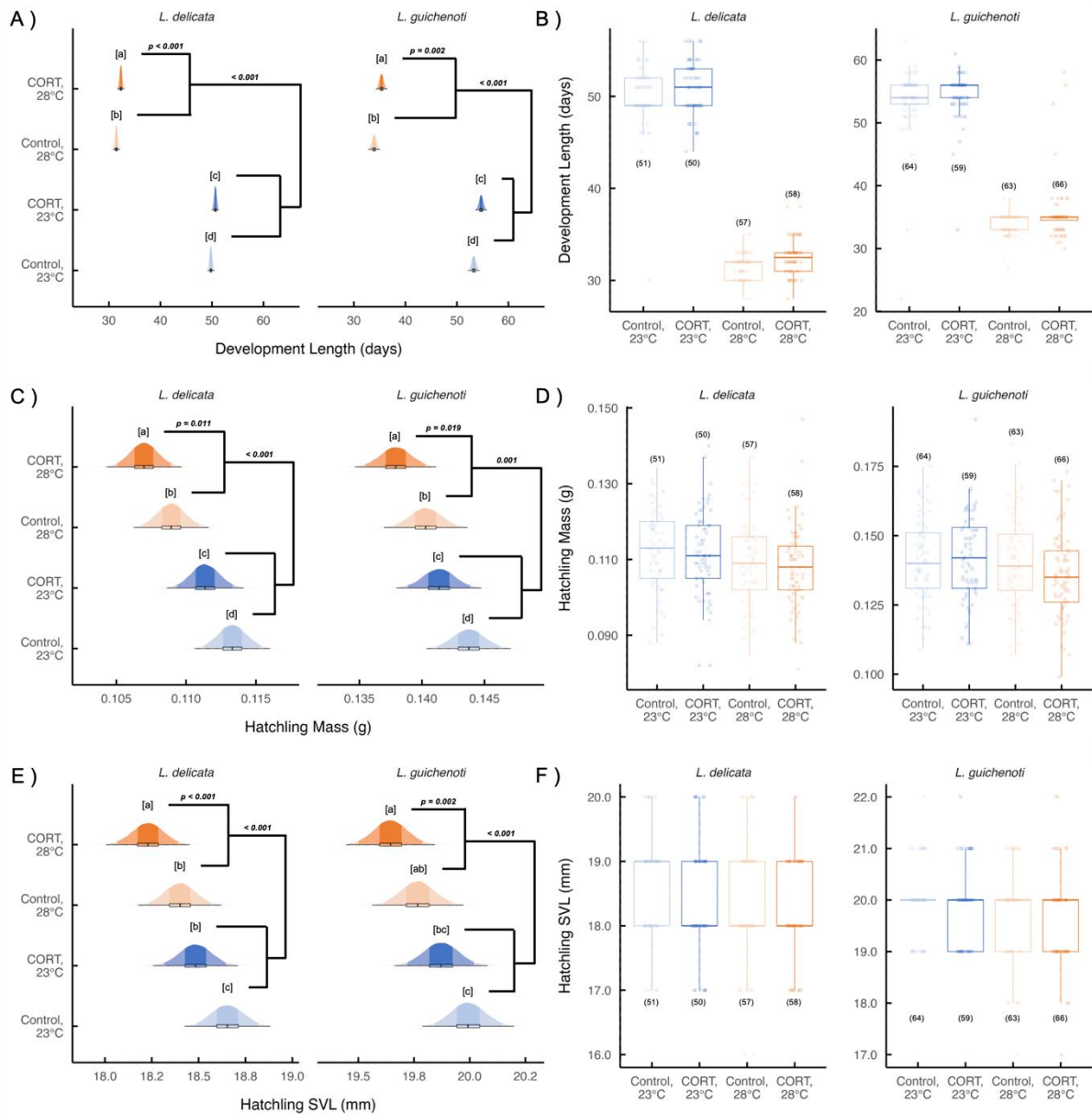


Figure 2. Effects of CORT treatment and incubation temperature on *L. delicata* and *L. guichenoti* developmental length and hatchling size. Developmental length is the number of days between finding an egg (potentially up to 3 days after oviposition) and the egg hatching. Hatchling size is the mass and snout-vent-length (SVL) of lizards at hatching. Panels A), C), and E) are density plots of the predicted posterior distributions estimated for each developmental stress (CORT or Control) by incubation temperature (23°C or 28°C) treatment, generated by Bayesian regression models. Shared letters in squared brackets indicate similar distributions among treatment groups. Whiskers connecting distributions denote from left to right, respectively, the main effects of developmental stress treatment and incubation temperature. The p values above each whisker are the pMCMC value testing whether the distribution of each main effect differs from zero. Significant pMCMC values ($p < 0.05$) are highlighted through solid whiskers and bolded and italicised p -values. Panels B), D), and F) are boxplots summarising the distributions of raw data overlaid with individual data points (empty circles). Numbers in rounded brackets are the sample size of each treatment group.

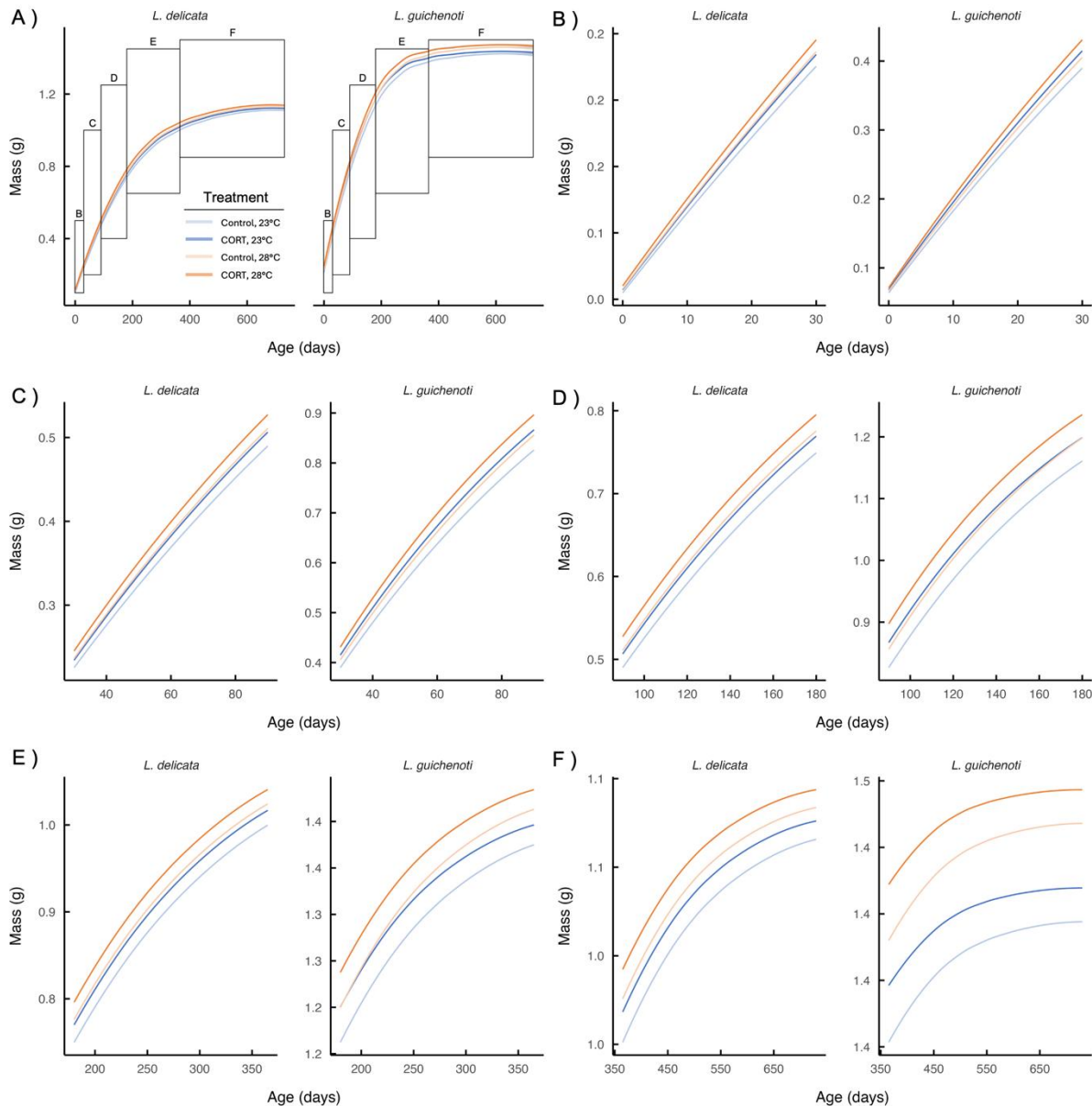


Figure 3. Effects of CORT treatment and incubation temperature on growth curves of *L. delicata* and *L. guichenoti* mass. Growth curves are the mass of *L. delicata* and *L. guichenoti* throughout 730 days post-hatching (DPH) as predicted by Von Bertalanffy growth models (VBGM). VBGM used as input repeated measures of lizard mass at 0 DPH, 30 DPH, 90 DPH, 270 DPH, 365 DPH, and 730 DPH. VBGM predicted the growth rate coefficient K (days^{-1}) and asymptotic body size L_{inf} (g) for each developmental stress by incubation temperature treatment, which were then imputed into the Von Bertalanffy growth equation to predict mass at any given age. A) is the full predicted growth curve from 0 DPH to 730 DPH. Rectangles represent periods of time between each measure, with the letters above each rectangle denoting the corresponding panel in the figure. B) through F) are, respectively, the growth curves from 0-30 DPH, 30-90 DPH, 90-180 DPH, 180-365 DPH, and 365-730 DPH.

Accelerated growth does not fully compensate for small hatch sizes

Consistent with the differences in mass observed at hatching, and despite the faster growth rates and larger asymptotic body sizes predicted by growth models, CORT-treatment and incubation temperature had persistent negative effects on mass into late adulthood ($\sim 760 \pm 21$ DPH, both *L. delicata* and *L. guichenoti*) (Figure 5A, Supplemental Table 22). Lizards treated with CORT during embryonic development weighed less both at hatching and at adulthood than those that received an ethanol control treatment (*L. delicata*: Δ [CORT – Control] = $-0.084 \text{ g} \pm <0.001$, pMCMC = 0.001; *L. guichenoti*: Δ [CORT – Control] = $-0.074 \text{ g} \pm <0.001$, pMCMC = 0.014). Likewise, lizards incubated at 28°C hatched at reduced mass compared to lizards incubated at 23°C and remained smaller in adulthood (*L. delicata*: Δ [28°C – 23°C] = $-0.128 \text{ g} \pm <0.001$, pMCMC < 0.001; *L. guichenoti*: Δ [28°C – 23°C] = $-0.092 \text{ g} \pm <0.001$, pMCMC = 0.006).

Like their effects on hatchling size, the effects of CORT-treatment and incubation temperature on the mass of adult lizards were additive (Figure 5B, Supplemental Table 23). When accounting for sex differences and the independent influence of population density on adult mass (see *Supplemental Results*), lizards both treated with developmental CORT and incubated at 28°C weighed the least in adulthood (*L. delicata*: $N_{\text{CORT},28^\circ\text{C}} = 40$, Estimate $\pm$ SE = $1.148 \text{ g} \pm <0.001$; *L. guichenoti*: $N_{\text{CORT},28^\circ\text{C}} = 55$, Estimate $\pm$ SE = $1.459 \text{ g} \pm <0.001$), while Control lizards incubated at 23°C weighed the most as adults (*L. delicata*: $N_{\text{Control},23^\circ\text{C}} = 41$, Estimate $\pm$ SE = $1.361 \text{ g} \pm <0.001$; *L. guichenoti*: $N_{\text{Control},23^\circ\text{C}} = 51$, Estimate $\pm$ SE = $1.625 \text{ g} \pm <0.001$). Lizards either treated with CORT and incubated at 23°C (*L. delicata*: $N_{\text{CORT},23^\circ\text{C}} = 37$, Estimate $\pm$ SE = $1.276 \text{ g} \pm <0.001$; *L. guichenoti*: $N_{\text{CORT},23^\circ\text{C}} = 48$, Estimate $\pm$ SE = $1.552 \text{ g} \pm <0.001$) or those receiving the ethanol control and incubated at 28°C (*L. delicata*: $N_{\text{Control},28^\circ\text{C}} = 40$, Estimate $\pm$ SE = $1.233 \text{ g} \pm <0.001$; *L. guichenoti*: $N_{\text{Control},28^\circ\text{C}} = 53$, Estimate $\pm$ SE = $1.533 \text{ g} \pm <0.001$) were of an intermediate mass.

The effects of CORT-treatment and incubation temperature on mass were notably more pronounced in adulthood than they were at hatching. CORT-treatment and incubation at 28°C accounted for an $\sim 0.213 \text{ g}$ ($\sim 16\%$ of total body mass) lower mass of *L. delicata* and $\sim 0.166 \text{ g}$ ($\sim 10\%$ of total body mass) lower mass in *L. guichenoti*, up from the $\sim 6.2\%$ reduction in mass at hatching for *L. delicata* and $\sim 3.2\%$ reduction for *L. guichenoti*.

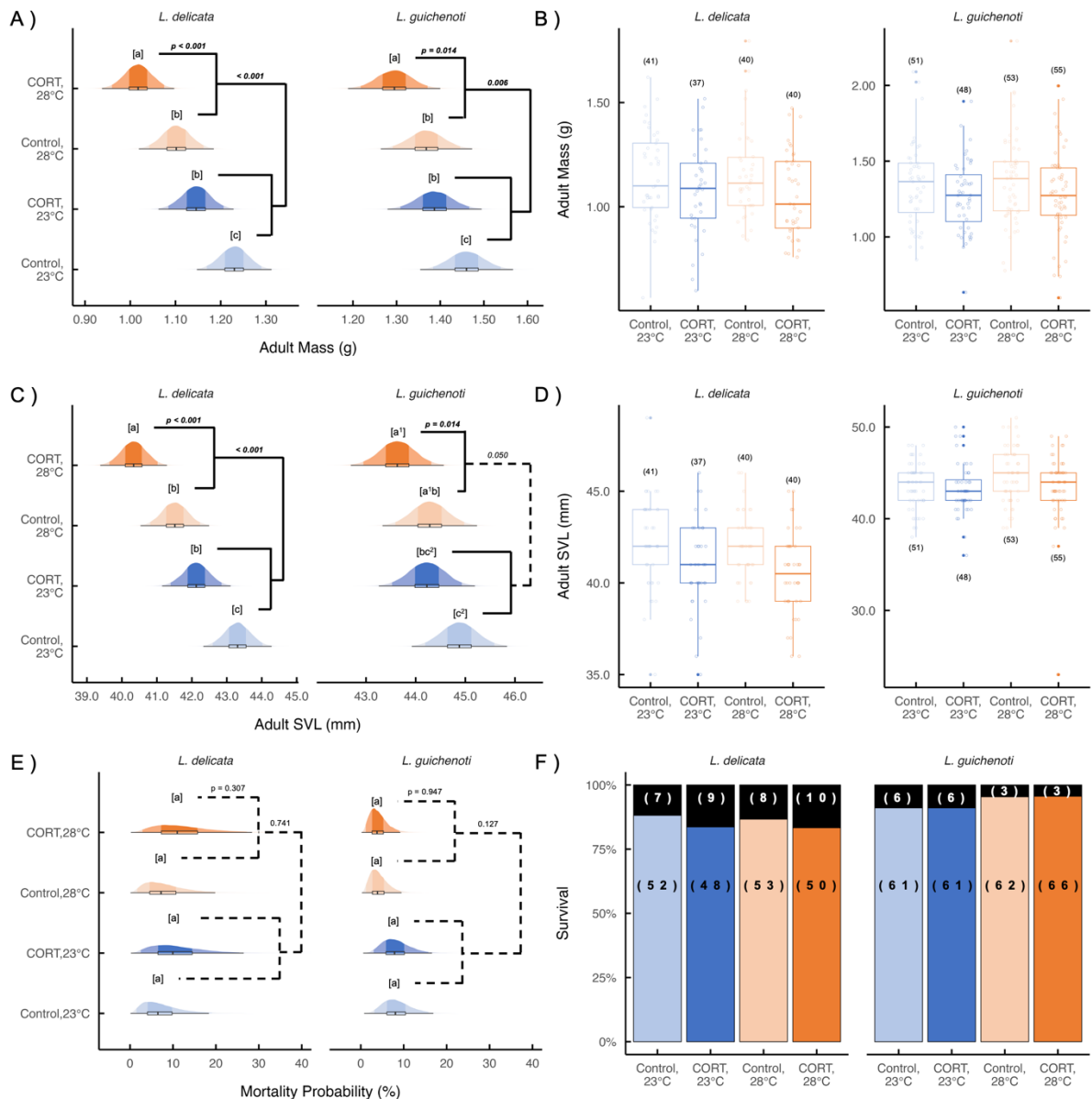


Figure 5. Effects of CORT treatment and incubation temperature on adult body size and survival to late adulthood. Adult sizes are measures of mass and SVL from *L. delicata* and *L. guichenoti* at ~730 days post-hatching (DPH). Mortality was quantified as whether an animal was found during either of the final two remeasurements (365 or 730 DPH). A), C), and E) are density plots of the predicted posterior distributions estimated for each developmental stress (CORT or Control) by incubation temperature (23°C or 28°C) treatment, generated by Bayesian regression models. Shared letters in squared brackets indicate similar distributions among treatment groups. Whiskers connecting distributions denote from left to right, respectively, the main effects of developmental stress treatment and incubation temperature. The *p* values above each whisker are the pMCMC value testing whether the distribution of each main effect differs from zero. Significant pMCMC values ($p < 0.05$) are highlighted through solid whiskers and bolded and italicised *p*-values. Non-significant pMCMC values are highlighted through dashed whiskers and non-bolded or italicised *p*-values. B) and D) are boxplots summarising the distributions of raw data overlaid with individual data points (empty circles). F) is a box plot depicting the proportion of each treatment group that survived (coloured portion) or died (black portion) to late adulthood (~760 DPH). Numbers in rounded brackets are the sample size of each treatment group.

Accelerated growth does not trade off against longevity

Although developmental stress hormones and variation in incubation temperature induced persistent changes in patterns of growth and body size in *L. delicata* and *L. guichenoti*, the same could not be said for long-term patterns of survival (Figure 5E, Supplemental Table 30). Neither CORT exposure (*L. delicata*: Δ [CORT – Control] = 0.442 ± 0.002 , pMCMC = 0.307; *L. guichenoti*: Δ [CORT – Control] = -0.034 ± 0.002 , pMCMC = 0.947) nor incubation temperature (*L. delicata*: Δ [28°C – 23°C] = 0.142 ± 0.002 , pMCMC = 0.741; *L. guichenoti*: Δ [28°C – 23°C] = -0.778 ± 0.002 , pMCMC = 0.127) affected lizard survival to two years post-hatching (~75% of total expected lifespan).

When back-transformed from the logit scale to relative probabilities, CORT-treatment could induce anywhere from a 33% lower probability of mortality to 266% greater probability in *L. delicata*, and from a 64% lower to 173% higher probability in *L. guichenoti*. Incubation temperature was similarly variable, with incubation at 28°C expected to induce from 51% lower to 173% higher probability of mortality in *L. delicata*, and from 85% lower to 23% greater probability in *L. guichenoti*. This high degree of uncertainty in mortality probability is likely driven by the low number of mortalities in both species.

Mortality was low in general for both *L. delicata* and *L. guichenoti* and regardless of CORT-exposure or incubation temperature. In total, we failed to find only 14.3% of *L. delicata* (34 of 237 total animals) and 6.7% of *L. guichenoti* (18 of 268 total animals) in late adulthood. The greatest rate of mortality, 16.7% (10 of 60 total animals), occurred in the *L. delicata* that were treated with CORT and incubated at 28°C. However, only 7-10 animals died in any treatment group for *L. delicata*, and 3-6 animals for any treatment group in *L. guichenoti* (Figure 5F, Supplemental Table 29).

Developmental effects are not mediated by elevated metabolism

Although elevated developmental CORT and incubation at 28°C induced *L. delicata* and *L. guichenoti* to hatch at smaller sizes and grow at faster rates as juveniles, this pattern of growth was likely not facilitated by elevated resting metabolic rates (Figure 6, Supplemental Table 34). Neither CORT-treatment (*L. delicata*: Δ [CORT – Control] = $-0.0005 \text{ mL O}_2 \cdot \text{min}^{-1} \pm <0.0001$, pMCMC = 0.302; *L. guichenoti*: Δ [CORT – Control] = $0.0003 \text{ mL O}_2 \cdot \text{min}^{-1} \pm <0.0001$, pMCMC = 0.470) nor incubation temperature (*L. delicata*: Δ [28°C – 23°C] = $0.0001 \text{ mL O}_2 \cdot \text{min}^{-1} \pm <0.0001$, pMCMC = 0.852; *L. guichenoti*: Δ [28°C – 23°C] = $-0.0008 \text{ mL O}_2 \cdot \text{min}^{-1} \pm <0.0001$, pMCMC = 0.050) influenced resting metabolic rates of oxygen consumption (RMR $\dot{V}\text{O}_2$) in juvenile *L. delicata* or *L. guichenoti*. Likewise, resting metabolic rates of carbon dioxide production (RMR $\dot{V}\text{CO}_2$) were unaffected by developmental CORT exposure (*L. delicata*: Δ [CORT – Control] = $-0.0004 \text{ mL CO}_2 \cdot \text{min}^{-1} \pm <0.0001$, pMCMC = 0.371; *L. guichenoti*: Δ [CORT – Control] = $0.0005 \text{ mL CO}_2 \cdot \text{min}^{-1} \pm <0.0001$, pMCMC = 0.186) or incubation temperature (*L. delicata*: Δ [28°C – 23°C] = $0.0001 \text{ mL CO}_2 \cdot \text{min}^{-1} \pm <0.0001$, pMCMC = 0.724; *L. guichenoti*: Δ [28°C – 23°C] = $-0.0006 \text{ mL CO}_2 \cdot \text{min}^{-1} \pm <0.0001$, pMCMC = 0.113).

There was some evidence that *L. guichenoti* incubated at 28°C had depressed metabolic rates compared to those incubated at 23°C (~11.7% lower $\dot{V}\text{O}_2$; ~8.2% lower $\dot{V}\text{CO}_2$), in contrast to our prediction that warm incubation temperatures would elevate metabolic rates (Supplemental Table 35). However, the effect of incubation temperature on juvenile *L. guichenoti* oxygen consumption was only marginally non-significant (pMCMC = 0.05), and the effect of temperature on carbon-dioxide production was non-significant (pMCMC = 0.113). Similarly, *L. delicata* exposed to developmental CORT had ~7.8% lower rates of $\dot{V}\text{O}_2$ and ~4.9% lower rates of $\dot{V}\text{CO}_2$ (Supplemental Table 33), but the effects of CORT on RMR $\dot{V}\text{O}_2$ and RMR $\dot{V}\text{CO}_2$ did not approach significance (pMCMC's ≥ 0.302). Thus, CORT exposure may depress the metabolism of juvenile *L. delicata*, and incubation at 28°C may depress the metabolism of juvenile *L. guichenoti*, but our data is unable to support these effects with certainty.

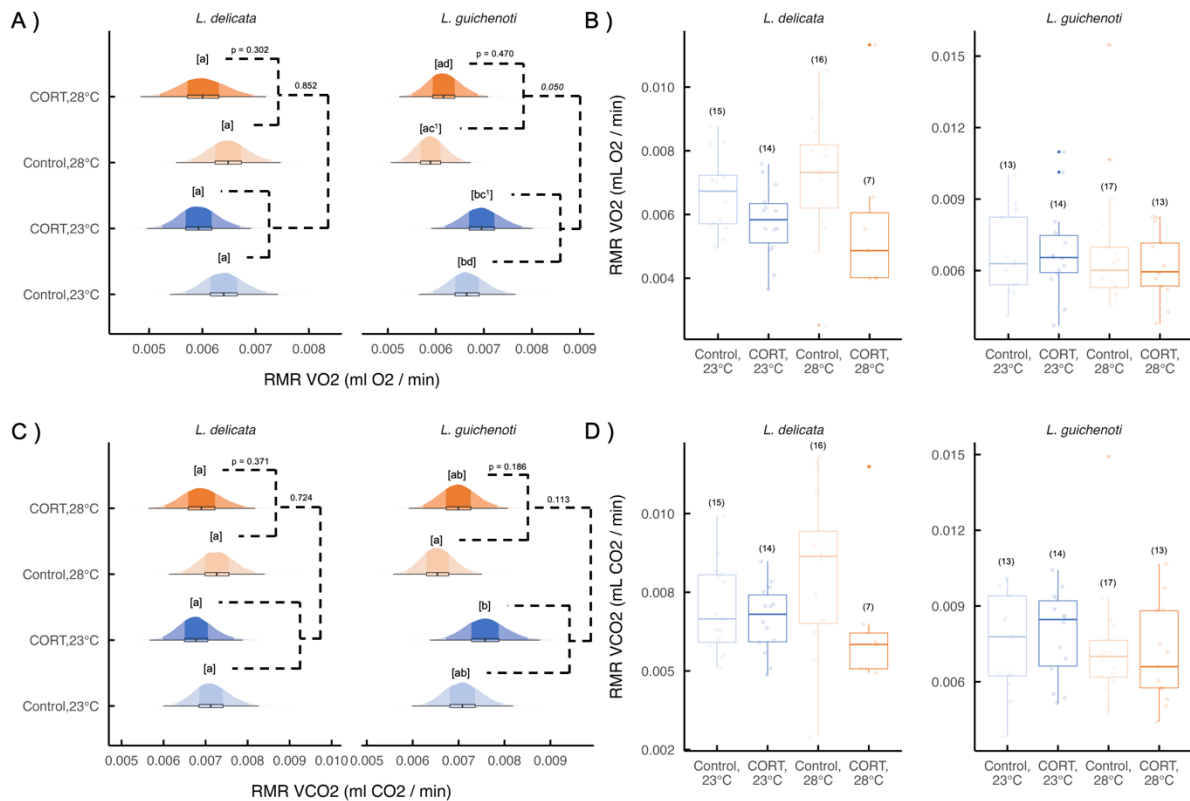


Figure 6. Effects of CORT treatment and incubation temperature on whole-organismal metabolism in juvenile *L. delicata* and *L. guichenoti*. Whole-organismal metabolism was measured as units of gas exchange (oxygen – $\dot{V}O_2$; carbon dioxide – $\dot{V}CO_2$) per minute. Metabolism was measured as resting metabolic rate (RMR) overnight and at a standardised preferred body temperature of 32°C. Panels A) and C) are density plots of the predicted posterior distributions estimated for each developmental stress (CORT or Control) by incubation temperature (23°C or 28°C) treatment, generated by Bayesian regression models. Shared letters in squared brackets indicate similar distributions among treatment groups. Shared numbers above each letter denote distributions that differ from each other non-significantly ($p_{MCMC} \approx 0.1$). Whiskers connecting distributions denote from left to right, respectively, the main effects of developmental stress treatment and incubation temperature. The p values above each whisker are the p_{MCMC} value testing whether the distribution of each main effect differs from zero. Significant p_{MCMC} values ($p < 0.05$) are highlighted through solid whiskers and bolded and italicised p -values. Panels B) and D) are boxplots summarising the distributions of raw data overlaid with individual data points (empty circles). Numbers in rounded brackets are the sample size of each treatment group.

Developmental effects have limited influence on cellular physiology

In addition to whole-organismal metabolism, we predicted that the effects of developmental stress and temperature on patterns of body size and growth, as well as longevity, would be mediated by systemic changes in cellular physiology. We assessed the effects of developmental CORT exposure and incubation temperature on six parameters of cellular morphology (cell size, mitochondrial mass), mitochondrial function (mitochondrial membrane potential, mitochondrial superoxide), and oxidative damage (lipid peroxidation, 8-OHdG oxidative DNA damage).

Generally, there was limited support that developmental CORT exposure and incubation temperature affected the parameters of cellular morphology (liver – Figure 7, Supplemental Table 38; heart – Figure 8, Supplemental Table 43), mitochondrial function (liver – Figure 9, Supplemental Table 49; heart – Figure 10, Supplemental Table 54), and oxidative damage (liver – Figure 11, Supplemental Table 60; heart – Figure 12, Supplemental Table 65) that we measured. This was broadly true of both livers and hearts from both *L. delicata* and *L. guichenoti*, and both as juveniles and adults. However, we found some mixed support for CORT and incubation temperature to affect mitochondrial mass in both hearts and livers, but only in adult *L. delicata*. There was also a tendency for CORT to induce oxidative DNA damage in the livers of both *L. delicata* and *L. guichenoti* as juveniles, but not as adults. CORT also tended to reduce, rather than elevate, oxidative damage in the hearts of *L. delicata* as juveniles, indicating tissue-specific effects of developmental CORT exposure. Finally, warm incubation temperatures may induce greater oxidative damage in the hearts of adult *L. delicata*. We describe these selected effects and tendencies below.

Developmental effects on mitochondrial mass are tissue- and age- specific

Our results suggested that exposure to elevated CORT during embryonic development upregulated mitochondrial mass, after accounting for the differences in mitochondrial content due to cell size variation. However, this upregulation of mitochondrial mass in response to CORT was only found in the heart cells of adult *L. delicata* ($\Delta [\text{CORT} - \text{Control}] = 0.165 \pm <0.001$, pMCMC = 0.010) (Figure 8G). Likewise, incubation temperature influenced mitochondrial mass in the heart cells of adult *L. delicata*, with lizards incubated at 23°C having a greater density of mitochondria than lizards incubated at 28°C, but this effect was non-significant ($\Delta [28^\circ\text{C} - 23^\circ\text{C}] = -0.088 \pm <0.001$, pMCMC = 0.095) (Figure 8G). However, due to technical issues during physiological assays, our sample sizes for adult hearts of *L. delicata* were relatively small compared to adult livers, or juvenile livers and hearts, for which we did not detect significant effects of CORT or incubation temperature (sample sizes – Figure 8H). Thus, we urge caution in drawing strong conclusions from the results of physiological assays on hearts of adult *L. delicata*.

Like in hearts, incubation temperature had a non-significant effect on mitochondrial mass in the livers of only adult *L. delicata* ($\Delta [28^\circ\text{C} - 23^\circ\text{C}] = 0.083 \pm <0.001$, pMCMC = 0.098) (Figure 7G). Unlike in hearts, however, *L. delicata* livers showed the opposite response to incubation temperature: lizards incubated at 28°C had greater mitochondrial mass as adults than lizards incubated at 23°C. While the effects of incubation temperature on both the livers and hearts of adult *L. delicata* are non-significant, their opposite directionalities may indicate tissue-specific responses to developmental thermal environments. Indeed, comparisons of effect sizes supported that there were tissue-specific effects of both incubation temperature (Temperature: $\Delta [\text{Liver} - \text{Heart}] = 0.171 \pm <0.001$, pMCMC = 0.02) and CORT-treatment (CORT: $\Delta [\text{Liver} - \text{Heart}] = -0.204 \pm <0.001$, pMCMC = 0.02) on mitochondrial mass in adult *L. delicata*.

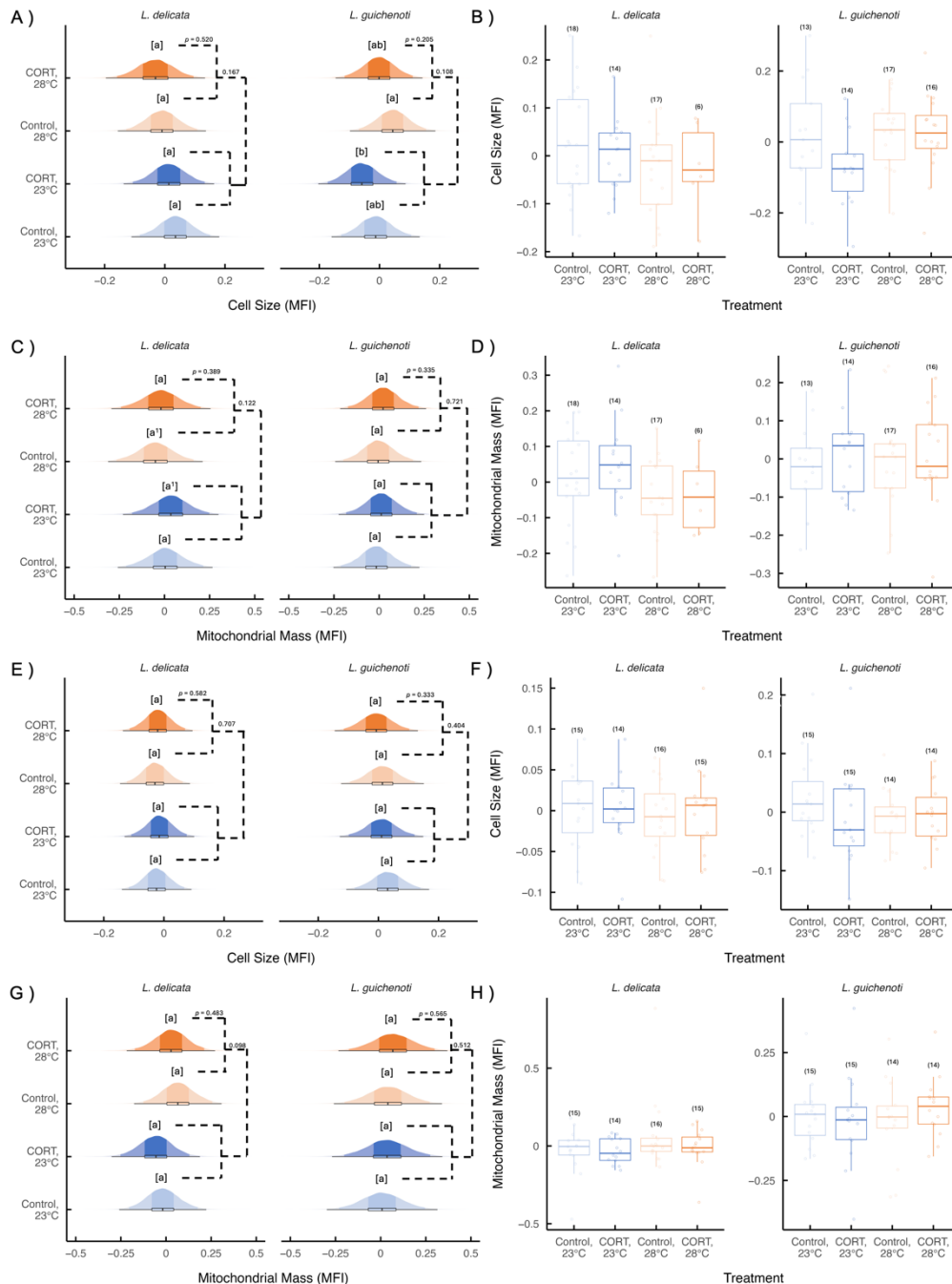


Figure 7. Effects of CORT treatment and incubation temperature on cellular morphology in juvenile and adult *L. delicata* and *L. guichenoti* livers. Data are derived from fluorescent signals emitted by probes that represent cell size and mitochondrial mass and measured via flow cytometric analysis. Individual data points are the mean fluorescent intensity (MFI) of signals from a population of intact, viable liver cells and averaged across three replicates per lizard. Panels A-D) are data from a subset of juvenile lizards, while panels E-H) are data from a separate subset of adult lizards. Panels A), C), E), and G) are density plots of the predicted posterior distributions estimated for each developmental stress (CORT or Control) by incubation temperature (23°C or 28°C) treatment, generated by Bayesian regression models. Shared letters in squared brackets indicate similar distributions among treatment groups. Letters marked with * or + differ non-significantly ($p < 0.1$). Whiskers connecting distributions denote from left to right, respectively, the main effects of developmental stress treatment and incubation temperature. The p values above each whisker are the pMCMC value testing whether the distribution of each main effect differs from zero. Significant pMCMC values ($p < 0.05$) are highlighted through solid whiskers and bolded and italicised p -values. Panels B), D), F), and H) are boxplots summarising the distributions of raw data overlaid with individual data points (empty circles). Numbers in rounded brackets are the sample size of each treatment group.

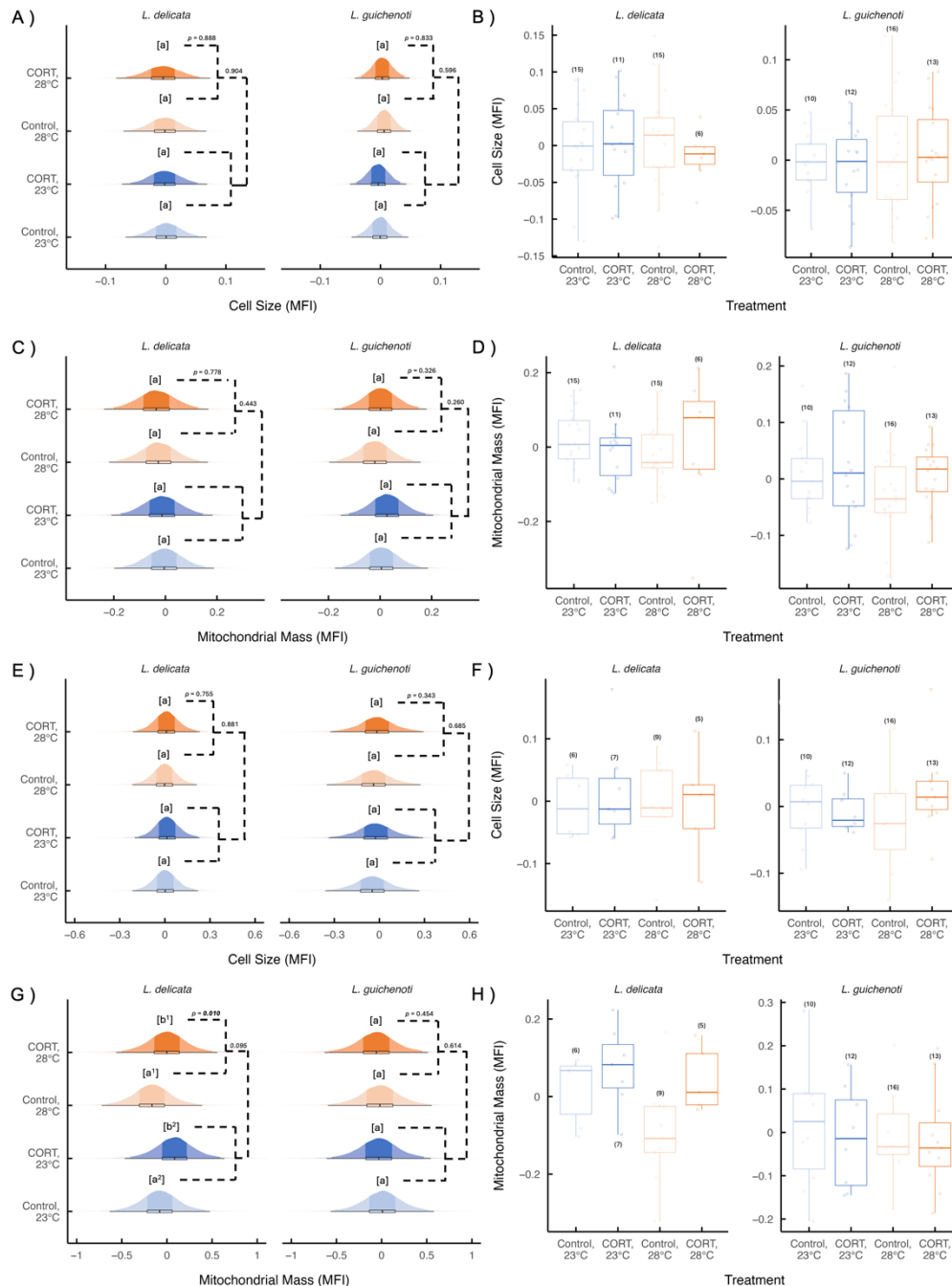


Figure 8. Effects of CORT treatment and incubation temperature on cellular morphology in juvenile and adult *L. delicata* and *L. guichenoti* hearts. Data are derived from fluorescent signals emitted by probes that represent cell size and mitochondrial mass and measured via flow cytometric analysis. Individual data points are the mean fluorescent intensity (MFI) of signals from a population of intact, viable liver cells and averaged across three replicates per lizard. Panels A-D) are data from a subset of juvenile lizards, while panels E-H) are data from a separate subset of adult lizards. Panels A), C), E), and G) are density plots of the predicted posterior distributions estimated for each developmental stress (CORT or Control) by incubation temperature (23°C or 28°C) treatment, generated by Bayesian regression models. Shared letters in squared brackets indicate similar distributions among treatment groups. Letters marked with * or + differ non-significantly ($p < 0.1$). Whiskers connecting distributions denote from left to right, respectively, the main effects of developmental stress treatment and incubation temperature. The p values above each whisker are the pMCMC value testing whether the distribution of each main effect differs from zero. Significant pMCMC values ($p < 0.05$) are highlighted through solid whiskers and bolded and italicised p -values. Panels B), D), F), and H) are boxplots summarising the distributions of raw data overlaid with individual data points (empty circles). Numbers in rounded brackets are the sample size of each treatment group.

Mitochondrial physiology is robust to developmental CORT and temperatures

Neither developmental CORT nor incubation temperature affected mitochondrial membrane potential or mitochondrial superoxide in *L. delicata* or *L. guichenoti*, and no effects approached significance (all pMCMC > 0.1). This was true for both livers (Figure 9, Supplemental Table 49) and hearts (Figure 10, Supplemental Table 54), and developmental effects on mitochondrial function were neither present in juveniles, nor did they manifest by adulthood. There was perhaps some evidence that developmental CORT affected mitochondrial membrane potential in heart cells, but only in *L. delicata*, and the effect varied between juveniles and adults. As juveniles, *L. delicata* exposed to prenatal CORT had elevated mitochondrial membrane potential in heart cells ($\Delta[\text{CORT} - \text{Control}] = 0.045 \pm \leq 0.001$, pMCMC = 0.155), but as adults, *L. delicata* treated with CORT had depressed mitochondrial membrane potential ($\Delta[\text{CORT} - \text{Control}] = -0.151 \pm \geq 0.001$, pMCMC = 0.113). Comparisons of effect sizes between juvenile and adult *L. delicata* supported that CORT had age-specific effects on mitochondrial membrane potential in heart cells ($\Delta[\text{Adult} - \text{Juvenile}] = -0.151 \pm \leq 0.001$, pMCMC = 0.045), suggesting that developmental effects on mitochondrial respiration shifted throughout life (Supplemental Table 58). However, we note small sample sizes for hearts in *L. delicata* due to technical issues that rendered some samples unusable.

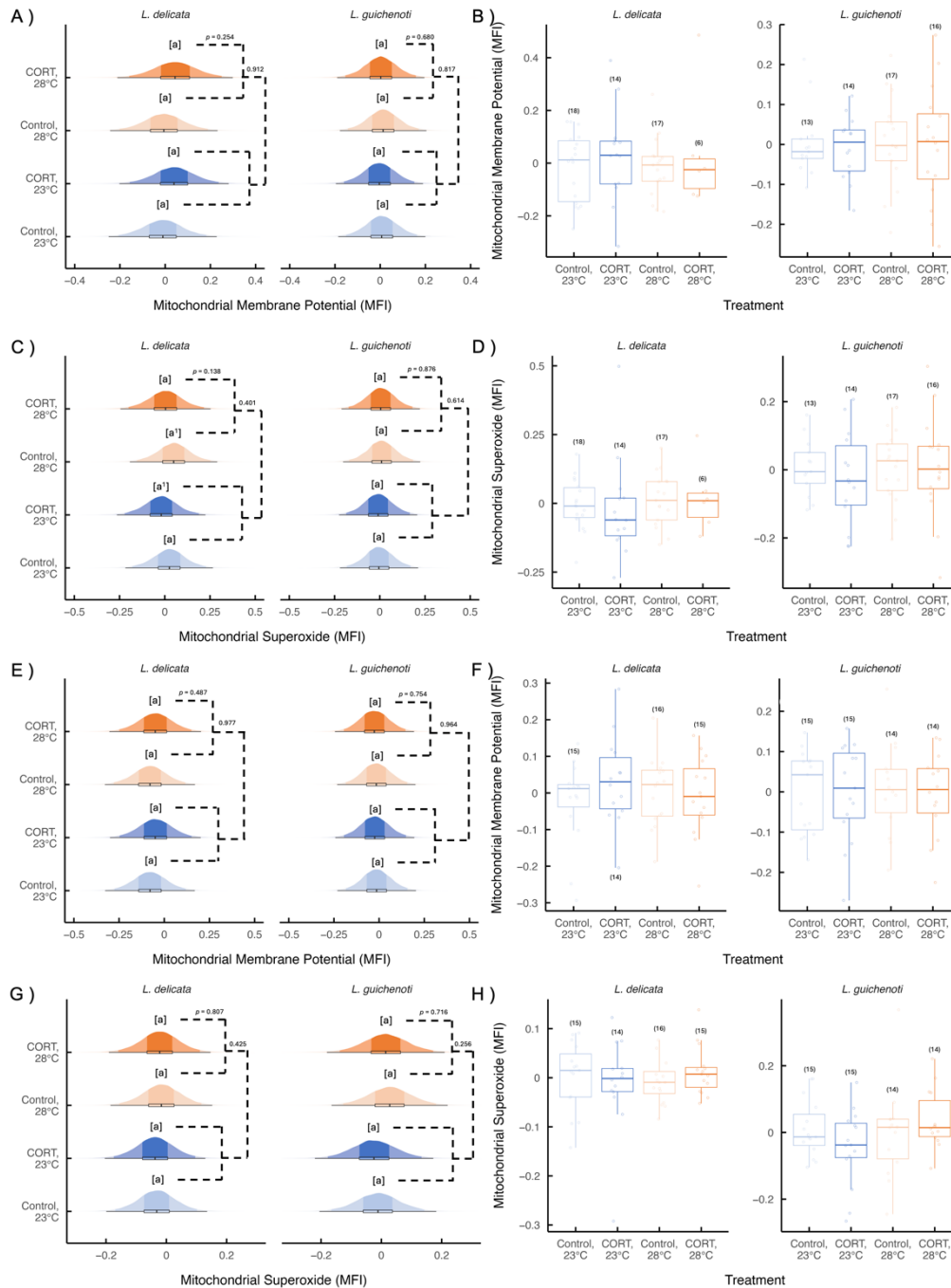


Figure 9. Effects of CORT treatment and incubation temperature on mitochondrial function in juvenile and adult *L. delicata* and *L. guichenoti* livers. Data are derived from fluorescent signals emitted by probes that represent mitochondrial membrane potential and mitochondrial superoxide concentrations and measured via flow cytometric analysis. Individual data points are the mean fluorescent intensity (MFI) of signals from a population of intact, viable liver cells and averaged across three replicates per lizard. Panels A-D) are data from a subset of juvenile lizards, while panels E-H) are data from a separate subset of adult lizards. Panels A), C), E), and G) are density plots of the predicted posterior distributions estimated for each developmental stress (CORT or Control) by incubation temperature (23°C or 28°C) treatment, generated by Bayesian regression models. Shared letters in squared brackets indicate similar distributions among treatment groups. Letters marked with * or + differ non-significantly ($p \approx 0.1$). Whiskers connecting distributions denote from left to right, respectively, the main effects of developmental stress treatment and incubation temperature. The p values above each whisker are the pMCMC value testing whether the distribution of each main effect differs from zero. Significant pMCMC values ($p < 0.05$) are highlighted through solid whiskers and bolded and italicised p -values. Panels B), D), F), and H) are boxplots summarising the distributions of raw data overlaid with individual data points (empty circles). Numbers in rounded brackets are the sample size of each treatment group.

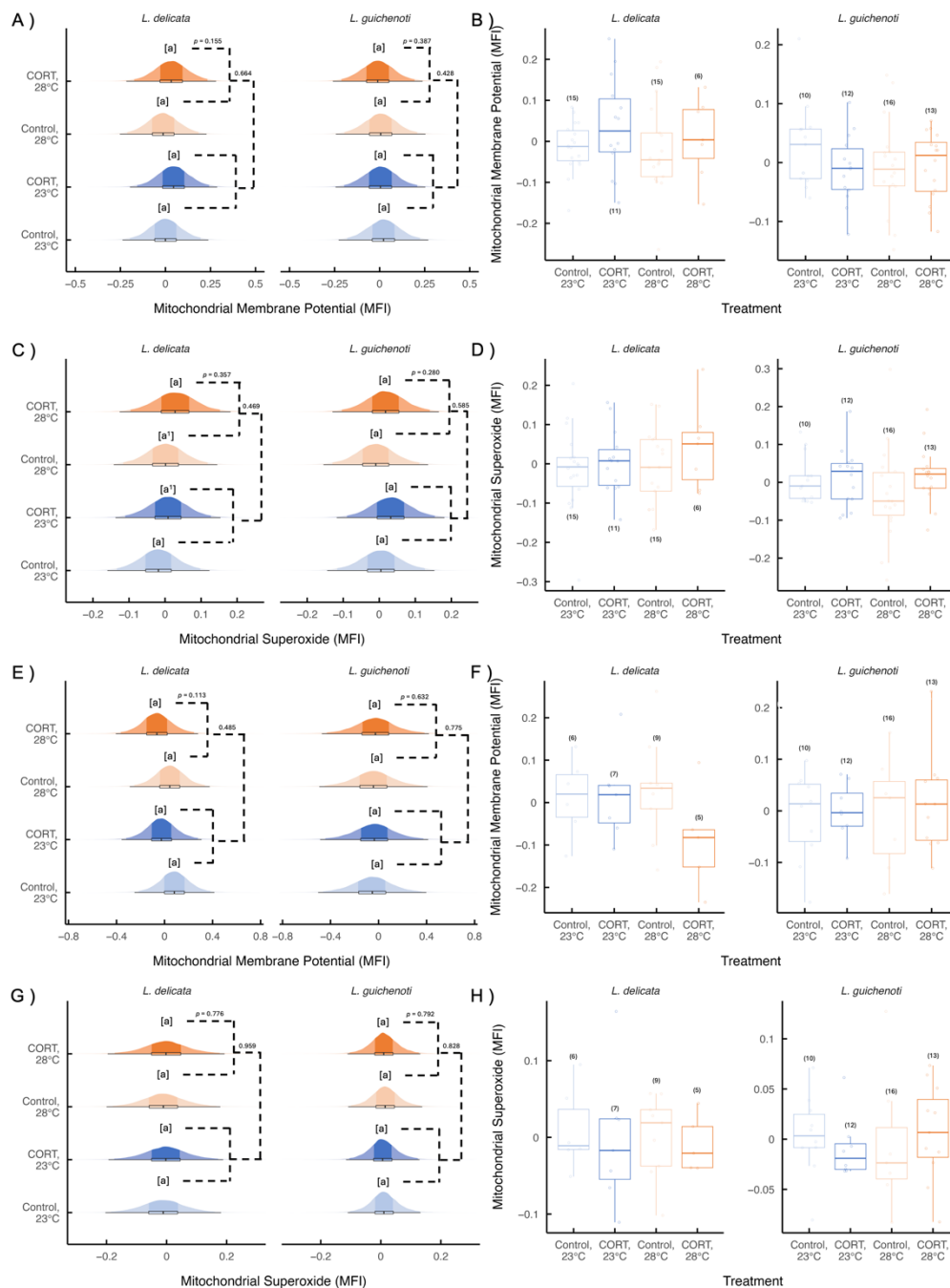


Figure 10. Effects of CORT treatment and incubation temperature on mitochondrial function in juvenile and adult *L. delicata* and *L. guichenoti* hearts. Data are derived from fluorescent signals emitted by probes that represent mitochondrial membrane potential and mitochondrial superoxide concentrations and measured via flow cytometric analysis. Individual data points are the mean fluorescent intensity (MFI) of signals from a population of intact, viable liver cells and averaged across three replicates per lizard. Panels A-D) are data from a subset of juvenile lizards, while panels E-H) are data from a separate subset of adult lizards. Panels A), C), E), and G) are density plots of the predicted posterior distributions estimated for each developmental stress (CORT or Control) by incubation temperature (23°C or 28°C) treatment, generated by Bayesian regression models. Shared letters in squared brackets indicate similar distributions among treatment groups. Letters marked with * or + differ non-significantly ($p < 0.1$). Whiskers connecting distributions denote from left to right, respectively, the main effects of developmental stress treatment and incubation temperature. The p values above each whisker are the pMCMC value testing whether the distribution of each main effect differs from zero. Significant pMCMC values ($p < 0.05$) are highlighted through solid whiskers and bolded and italicised p -values. Panels B), D), F), and H) are boxplots summarising the distributions of raw data overlaid with individual data points (empty circles). Numbers in rounded brackets are the sample size of each treatment group.

Oxidative Damage

As juveniles, there was a non-significant tendency for developmental CORT exposure to induce greater oxidative DNA damage (8-OHdG) in the livers of both *L. delicata* and *L. guichenoti* (*L. delicata*: Δ [CORT – Control] = $0.086 \pm <0.001$, pMCMC = 0.074; *L. guichenoti*: Δ [CORT – Control] = $0.140 \pm <0.001$, pMCMC = 0.073) (Figure 11, Supplemental Table 59). However, any effects of CORT on oxidative DNA damage in livers were not present in adult lizards. There was also a tendency for CORT-treated *L. delicata* to have lower levels of both lipid peroxidation (Δ [CORT – Control] = $-0.125 \pm <0.001$, pMCMC = 0.105) and 8-OHdG (Δ [CORT – Control] = $-0.212 \pm <0.001$, pMCMC = 0.082) in heart cells as juveniles, but such effects of CORT were not present in adult *L. delicata*, and in neither juvenile nor adult *L. guichenoti* (Figure 12, Supplemental Table 64). Effect size comparisons supported there being tissue-specific effects of CORT on levels of oxidative DNA damage in juvenile *L. delicata* (Δ [Liver – Heart] = $0.297 \pm <0.001$, pMCMC = 0.027). Likewise, there was some indication of tissue-specific effects of CORT on lipid peroxidation in juvenile *L. delicata*, but the difference in effects was weak (Δ [Liver – Heart] = $0.147 \pm <0.001$, pMCMC = 0.114) (Supplemental Table 69).

We found support that incubation temperature affected oxidative damage in adult *L. delicata*, but only in hearts (Figure 12, Supplemental Table 64). Adult *L. delicata* incubated at 28°C had greater levels of lipid peroxidation in their heart cells than *L. delicata* incubated at 23°C, but this was a non-significant tendency (Δ [28°C – 23°C] = $0.085 \pm <0.001$, pMCMC = 0.089). Incubation at 28°C also induced greater levels of 8-OHdG DNA damage in adult *L. delicata* hearts compared to incubation at 23°C (Δ [28°C – 23°C] = $0.153 \pm <0.001$, pMCMC = 0.043). These findings lend support to developmental temperatures having delayed effects on oxidative damage that only manifest in adulthood, but like with cellular morphology, we urge some caution in the interpretation of effects in adult hearts due to reduced sample sizes for *L. delicata*.

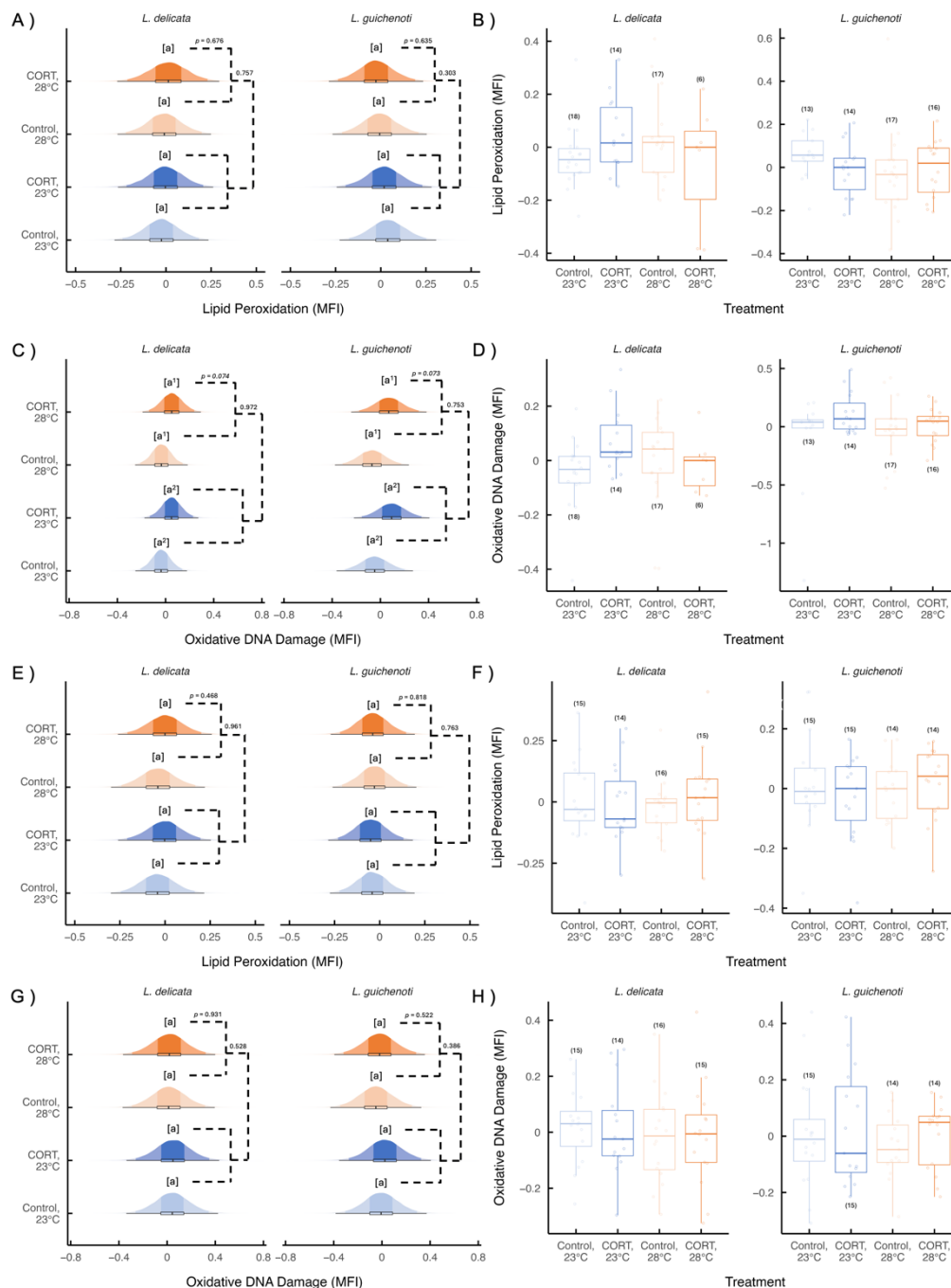


Figure 11. Effects of CORT treatment and incubation temperature on oxidative damage in juvenile and adult *L. delicata* and *L. guichenoti* livers. Data are derived from fluorescent signals emitted by probes that represent lipid peroxidation and oxidative DNA damage (8-OHdG) and measured via flow cytometric analysis. Individual data points are the mean fluorescent intensity (MFI) of signals from a population of intact, viable liver cells and averaged across three replicates per lizard. Panels A-D) are data from a subset of juvenile lizards, while panels E-H) are data from a separate subset of adult lizards. Panels A), C), E), and G) are density plots of the predicted posterior distributions estimated for each developmental stress (CORT or Control) by incubation temperature (23°C or 28°C) treatment, generated by Bayesian regression models. Shared letters in squared brackets indicate similar distributions among treatment groups. Letters marked with * or + differ non-significantly ($p < 0.1$). Whiskers connecting distributions denote from left to right, respectively, the main effects of developmental stress treatment and incubation temperature. The p values above each whisker are the pMCMC value testing whether the distribution of each main effect differs from zero. Significant pMCMC values ($p < 0.05$) are highlighted through solid whiskers and bolded and italicised p -values. Panels B), D), F), and H) are boxplots summarising the distributions of raw data overlaid with individual data points (empty circles). Numbers in rounded brackets are the sample size of each treatment group.

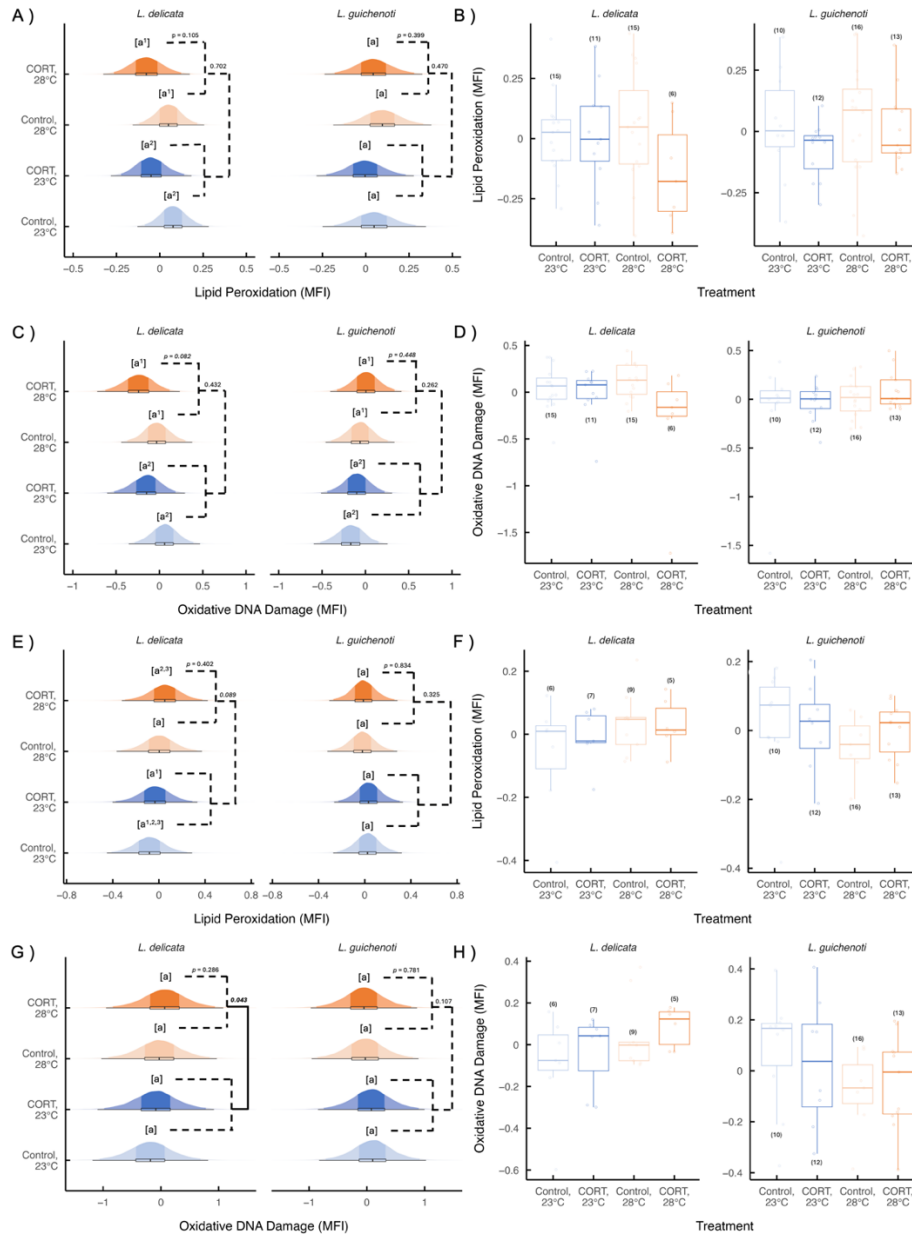


Figure 12. Effects of CORT treatment and incubation temperature on oxidative damage in juvenile and adult *L. delicata* and *L. guichenoti* hearts. Data are derived from fluorescent signals emitted by probes that represent lipid peroxidation and oxidative DNA damage (8-OHdG) and measured via flow cytometric analysis. Individual data points are the mean fluorescent intensity (MFI) of signals from a population of intact, viable liver cells and averaged across three replicates per lizard. Panels A-D) are data from a subset of juvenile lizards, while panels E-H) are data from a separate subset of adult lizards. Panels A), C), E), and G) are density plots of the predicted posterior distributions estimated for each developmental stress (CORT or Control) by incubation temperature (23°C or 28°C) treatment, generated by Bayesian regression models. Shared letters in squared brackets indicate similar distributions among treatment groups. Letters marked with * or + differ non-significantly ($p < 0.1$). Whiskers connecting distributions denote from left to right, respectively, the main effects of developmental stress treatment and incubation temperature. The p values above each whisker are the pMCMC value testing whether the distribution of each main effect differs from zero. Significant pMCMC values ($p < 0.05$) are highlighted through solid whiskers and bolded and italicised p -values. Panels B), D), F), and H) are boxplots summarising the distributions of raw data overlaid with individual data points (empty circles). Numbers in rounded brackets are the sample size of each treatment group.

Discussion

The conditions an individual experiences during development are expected to shape that individual's phenotype, potentially with consequences for their fitness (West-Eberhard, 2003; Monaghan, 2007). However, few studies have tracked how developmental conditions affect key phenotypes across the lifespan or measured direct components of fitness (i.e., survival or reproductive success), especially in naturalistic environments. Of those studies which do, most only consider one developmental condition, despite the likelihood that animals are simultaneously subjected to multiple, potentially interacting, developmental effects. Additionally, there is a paucity of studies testing the persistent effects of developmental conditions on physiological systems which could modulate lifelong phenotypic traits like growth and longevity (Monaghan, 2008; Flatt and Heyland, 2010). Here, we assessed how prenatal CORT and incubation temperature jointly influenced the growth and survival of two closely related lizards in naturalistic environments and tested whether these developmental effects were mediated by changes in metabolism, mitochondrial function, and oxidative stress. The comprehensive scope of this design answers fundamental questions linking multiple developmental conditions to life-history traits and their underlying mechanisms. This design also addresses long-standing research needs such as the call for longitudinal studies under naturalistic conditions and the need to account for interspecific variation in developmental plasticity (Speakman et al., 2018; Uller et al., 2024; Muell et al., 2025).

Our longitudinal, naturalistic study found that early developmental environments additively, rather than interactively, affected key life-history traits like body size and growth throughout the lifespan of *Lampropholis delicata* and *Lampropholis guichenoti*. Yet the effects of prenatal CORT and incubation temperature on body size varied throughout life, resulting in a more complex growth pattern than we had anticipated given the results of previous work on these species (de Jong et al., 2023; Crino et al., 2024). Specifically, we observed that lizards exposed to developmental CORT and warm temperatures hatched smaller, grew faster to adult sizes, but ultimately remained smaller at late adulthood. Despite the expectation that accelerated growth typically trades off against long-term survival, CORT treatment and incubation temperature did not affect survival in our study system. Rather, survival of *L. delicata* and *L. guichenoti* was high overall in naturalistic enclosures, regardless of developmental conditions. Mechanistically, we predicted that the effects of developmental CORT and incubation temperature on growth and survival would be mediated through inducing persistent changes in metabolism, mitochondrial function, or oxidative damage. While previous studies found persistent effects of CORT and incubation temperature on the mass-scaling of metabolism and mitochondrial efficiency, we did not find strong evidence in support of similar mechanisms, thereby suggesting alternative or more nuanced drivers of developmental effects (de Jong et al., 2023; Crino et al., 2024, but see Kar et al., 2022). Furthermore, despite some evidence that CORT induced oxidative damage markers associated with ageing, these effects did not accumulate or persist over time, refuting predictions of the “oxidative stress theory of life-history” (Costantini, 2019). Finally, the effects of CORT and incubation on growth, survival, and physiology did not differ between *L. delicata* and *L. guichenoti*. Thus, phenotypic responses to developmental conditions were relatively conserved among these closely related species despite their ecological differences.

Why did CORT and incubation temperature induce accelerated growth?

We predicted that simultaneous exposure to developmental CORT and variation in incubation temperature would have persistent influence on the body size and growth of *L. delicata* and *L. guichenoti*. Consistent with our predictions and with recent work on *Lampropholis* skinks, hatchlings from eggs treated with CORT and incubated at warm temperatures hatched at reduced body sizes than hatchlings receiving an ethanol control or incubated at cool temperatures (Crino et al., 2024; Leibold et al., 2026). Also consistent with previous work on *L. delicata* (de Jong et al., 2023; Crino et al., 2024), CORT treatment and warm incubation temperatures also induced both *L. delicata* and *L. guichenoti* to grow more rapidly as juveniles. Despite elevated growth rates, however, CORT-treated and warm-incubated

lizards in our study remained smaller at 2-years post-hatching than control-treated and cool-incubated lizards. In fact, the disparity in body sizes was even greater at adulthood than at hatching. A growth pattern wherein animals hatch at smaller body sizes, grow rapidly as juveniles, but ultimately remain smaller as adults is consistent with “incomplete” compensatory growth (Metcalf and Monaghan, 2001; Jobling, 2010). Our results contrast with previous work testing the effects of incubation temperature (de Jong et al., 2023) and developmental CORT exposure (Crino et al., 2024) on growth in *L. delicata*, which find that size differences caused by developmental conditions shrunk or disappeared by adulthood. Developmental CORT exposure has been consistently shown to induce reduced body sizes at hatching or birth in reptiles, but its effects on postnatal growth are equivocal. For example, developmental CORT induced faster growth in *Bassiana duperreyi* but slower growth in *Amphibolurus muricatus* (Warner et al., 2009), no change in growth in *Niveoscincus ocellatus* (Cadby et al., 2010), and an initial increase in the growth of *Thamnophis elegans*, but long-term growth patterns depended on postnatal environments (Palacios et al., 2023). Likewise, the effects of incubation temperature on reptile body size and growth vary by ecological context (Noble et al., 2018; While et al., 2018). In some species, cooler incubation temperatures induce faster growth, in other species warmer temperatures do, and in some cases the effects of temperature within a species vary among populations (Goodman, 2008; Esquerré et al., 2014; Dayananda et al., 2017). This raises the question: Why do the effects of developmental environments on growth patterns vary so much across species or studies?

Variability in growth responses across studies may be the result of differences in postnatal environments, such as resource abundance and quality, that are known to influence whether compensatory growth is observed or not (Stoks et al., 2006; Criscuolo et al., 2008; Xie et al., 2015; Livingston et al., 2014). One likely possibility, then, is that the “incomplete” compensatory growth we observed, compared to other studies finding full compensation, was driven by differences in relative resource availability. In our longitudinal study, *L. delicata* and *L. guichenoti* were almost entirely reared in communal naturalistic mesocosms, while previous work on *L. delicata* reared lizards individually in laboratory conditions for their full lifespan (de Jong et al., 2023; Crino et al., 2024). Therefore, lizards in our study had to compete with both conspecific and interspecific competitors for access to food or suitable thermal habitats. Furthermore, the size and environmental heterogeneity of mesocosms likely increased the energetic costs of foraging and effective thermoregulation relative to laboratory conditions with functionally *ad libitum* food access and controlled thermal conditions. In support of our hypothesis that naturalistic conditions imposed an energetic constraint on growth and body size, *L. delicata* in our study weighed ~1.15 g on average at 2 years post-hatching, after accounting for sex differences and population density. By comparison, laboratory reared *L. delicata* in previous studies weighed ~1.5 g on average at 1 year post-hatching, approximately 30% heavier than in our study (de Jong et al., 2023; Crino et al., 2024). In further support of this hypothesis, population density affected the growth and body size of lizards in our study. Both *L. delicata* and *L. guichenoti* reared in high density enclosures grew slower and were smaller as adults than lizards reared in low density enclosures, suggesting that resources were more limited when spread amongst more numerous individuals. Consequently, lizards in naturalistic enclosures may have been able to grow rapidly early on in life but were unable to sustain this growth into adulthood due to limited resources.

Resource limitation would especially constrain postnatal growth if CORT and incubation temperature induced concomitant changes in thermal behaviour or foraging that regulate energy use, acquisition, and allocation (Refsnider et al., 2019; Buchholz et al., 2024). Likewise, rapid growth rates may become unsustainable under resource limitation if faster growth is associated with greater energetic maintenance costs or reduced energetic efficiency (e.g., reduced mitochondrial efficiency – Crino et al., 2024). It follows then, that changes in behaviour or physiology that facilitate compensatory growth in juveniles later constrain growth in adulthood as animals must allocate increasingly more energy to competing demands (i.e., reproduction, maintenance). The difference in growth trajectories of lizards in our study compared to previous work on *L. delicata* highlights the importance of testing how developmental

environments affect life-history traits in naturalistic conditions. These disparities also indicate that both behavioural and physiological mechanisms regulating energy use and efficiency should be considered in studies of compensatory growth, including how these mechanisms may change throughout life-history stages.

What mechanisms underlie the developmental effects of CORT and incubation temperature on growth?

The ‘oxidative stress theory of life-history’ (*sensu* Costantini, 2019) proposes that key life-history traits and trade-offs are driven by underlying variation in metabolism, mitochondrial function, and oxidative stress dynamics (Koch et al., 2021; Speakman et al., 2015; Monaghan et al., 2009; Costantini, 2024). In the context of compensatory growth and its trade-offs against survival, this theory proposes that faster growth rates are facilitated by more rapid metabolism and mitochondrial respiration. In turn, elevated metabolic activity produces reactive oxygen species (ROS) that damage cellular components associated with faster ageing, earlier senescence, and reduced lifespan. The predictions of the oxidative stress theory of life-history are well-supported in mammals and birds (Naguib and Gil, 2005; Spencer and Verhulst, 2007; Dupont et al., 2019). However, evidence in support of the theory is less clear in reptiles. For example, long-lived snakes produced less ROS than short-lived snakes, but mitochondrial respiration and whole-organismal metabolism were unrelated to lifespan (Robert et al., 2007). In lizards, ROS were not associated with mortality, but high antioxidant activity correlated with reduced survival in only females (Isaksson, 2011). And in another study on lizards, Zhang et al. (2023) found that warming induced oxidative damage early in life that did not persist into adulthood yet was still associated with higher mortality. We found that developmental CORT and warm incubation temperatures induced rapid juvenile growth in *L. delicata* and *L. guichenoti*, but this accelerated growth was not accompanied by an expected increase in metabolic rates or changes in mitochondrial function. Developmental conditions also did not affect long-term patterns of survival, nor the accumulation of oxidative damage markers associated with longevity. Our findings challenge the predictions of the oxidative stress theory of life-history that metabolic, mitochondrial, and oxidative physiology underlie trade-offs between growth and survival in ectotherms. If the physiological mechanisms we quantified in our study do not explain the growth patterns we observed, then what alternative mechanisms are responsible?

Animals have limited energy to allocate between somatic maintenance and energetically costly activities like growth (Kooijman, 1993; Nisbet et al., 2000; Sousa et al., 2008; van Leeuwen et al., 2010). Accelerated growth, therefore, demands that animals must either (a) acquire more energy through increased food intake or (b) reduce the amount of energy spent on somatic maintenance or other activities. Compensatory growth is frequently associated with increased food intake when food becomes abundant following some early-life nutritional deficit that reduces juvenile body sizes (Jobling and Koskela, 1996; Nicieza and Metcalfe, 1997; Radder et al., 2007; Holden et al., 2019; Ali et al., 2003; Hector and Nakagawa, 2012). One possibility, then, is that the compensatory growth we observed in CORT-treated and warm-incubated lizards was driven by developmental effects on feeding rate. Glucocorticoids like CORT stimulate increased begging in bird chicks and overeating in humans, suggesting a role in stimulating food seeking behaviour (Bowers et al., 2016; Possenti et al., 2018; Kitaysky et al., 2001; Kitaysky et al., 2003; Smiseth et al., 2011; Dallman et al., 2004; Kuckuck et al., 2022). Incubation temperature has been linked to increased feeding in birds, fish, and reptiles, though studies testing this relationship are rare (Hope et al., 2020; Albokhadaim et al., 2007; Rueda-Zozaya et al., 2021; Cheng et al., 2020). Increased food intake, and its associated costs could explain rapid growth in juveniles followed by impaired growth in adulthood, as we found in CORT-treated and warm-incubated *L. delicata* and *L. guichenoti*. As juveniles, lizards must expend a relatively lower amount of energy towards metabolic maintenance due to their smaller body sizes (White et al., 2019) and they do not have to directly allocate energy towards reproduction (Angilletta & Sears, 2000; Olsson, Shine, & Wapstra, 2001; but see Shine, 1980). Therefore, increased food intake facilitates rapid growth in juveniles, when the greatest portion of

net energy intake can be allocated towards growth. However, as lizards grow increasingly larger, they must expend more energy on competing energetic demands. Without a concomitant increase in food abundance, the same greater foraging activity that facilitates rapid growth early in life could instead constrain continued growth, especially if increased feeding rates are accompanied by physiological changes in energy use and efficiency.

In addition to greater energy intake, variation in body size and growth may also be facilitated by changes in energy use and efficiency. We had expected that if developmental conditions induced increased growth rates in lizards, that this would be facilitated through an increase in metabolism or mitochondrial respiration. However, CORT and warm temperatures potentially depressed, rather than elevated, resting metabolic rates in juvenile *L. delicata* and *L. guichenoti*. Though we did not predict depressed metabolic rates, this is perhaps not entirely unexpected. Depressed metabolic rates may reflect lower baseline metabolic maintenance costs, thereby allowing lizards to spend a greater portion of energy intake on growth (Sears, 2004). However, metabolism is a multivariate trait and plasticity in resting metabolic rate is likely to be accompanied by changes in other components of metabolism (Pettersen, Marshall, & White, 2018). Resting metabolic rates are generally positively associated with maximum metabolic capacity in vertebrates, but the relationships between resting and maximum metabolism may be decoupled by environmental conditions like temperature (Auer, Killen, & Rezende, 2017). The allometric scaling or thermal dependence of metabolic rates may also vary across ecological contexts or life stages (White et al., 2008). Developmental CORT and incubation temperature may, therefore, influence growth through their effects on metabolic capacity, the mass-dependence of metabolic rates, or metabolic thermal reaction norms, rather than resting metabolic rates at a singular temperature (Pettersen, Marshall, & White, 2018; White et al., 2022; White & Marshall, 2023). Similarly, we quantified mitochondrial membrane potential as an indicator of routine mitochondrial respiration, but mitochondrial respiration is a complex physiological system comprised of interrelated measures (Koch et al., 2021). Developmental conditions may, then, affect mitochondrial respiratory capacity, efficiency, or proton leak which cannot be captured in a broad measure of respiration.

Previous work on *L. delicata* suggests that the effects of developmental environments (particularly incubation temperature) are more nuanced than direct changes in resting metabolic rate or overall mitochondrial respiration. A study by de Jong et al. (2023) found that lizards incubated at 22°C exhibited a shift in metabolic mass-scaling compared to lizards incubated at 26°C or 30°C, but only as subadults (200 days post-hatching). Another study (Kar et al., 2022) found that incubation temperature (23°C or 29°C) had no influence on the thermal dependency of metabolic rates in juvenile (90 days post-hatching) *L. delicata*. Crino et al. (2024) found that lizards incubated at 28°C had impaired mitochondrial efficiency in adulthood (1-year post-hatching) compared to lizards incubated at 23°C. While the effects were not significant, we found that *L. delicata* treated with CORT had elevated mitochondrial membrane potential as juveniles, but depressed membrane potential in adulthood. These findings from *L. delicata* support the hypothesis that the physiological mechanisms underlying variation in growth are likely more nuanced than direct changes in metabolism or mitochondrial respiration, and that such effects may only manifest during narrow windows or at specific points during the lifespan. Recent work on fishes (*Dicentrarchus labrax*) further supports this notion, as Quéméneur et al. (2022) found that not only were growth rates related to mitochondrial physiology, but the specific mechanism that governed growth changed depending on life-history stage. Other studies on fish (*Salmo trutta*) find that flexibility in metabolic capacity, rather than resting metabolic rate alone, facilitates variation in feeding capacity and growth (Auer et al., 2015a; 2015b; 2015c). Thus, alternative physiological pathways like metabolic capacity, metabolic scaling, or mitochondrial efficiency and their relationships to behavioural traits like thermoregulation and feeding behaviour are likely to underlie variation in growth responses.

What costs (if any) are there to developmental effects?

The concept of trade-offs is central to life-history theory (Stearns, 1989). Compensatory growth – as we found in CORT-treated and warm-incubated lizards — is expected to confer fitness benefits through either maximizing the survival of juveniles to adulthood or *via* expediting development to sexual maturity (Metcalf & Monaghan, 2001). However, accelerated growth is also frequently associated with reduced longevity, impaired reproduction, or physiological costs that manifest later in life (Monaghan and Metcalfe, 2001; Hector and Nakagawa, 2012; Smith et al. 2016). Under the life-history trade-off framework, compensatory growth therefore represents a trade-off between short-term survival and long-term reproductive opportunities (Metcalf & Monaghan, 2003; Monaghan, 2008; Mangel & Munch, 2005). If there were no costs associated with growing at rates closer to maximal, then we should expect more rapid growth to be the norm, especially in taxa like lizards for whom body size often correlates with fitness (Blanckenhorn, 2005; Kingsolver and Huey, 2008). Indeed, longitudinal studies on free-living lizard populations find that compensatory growth is associated with reduced long-term survival (Olsson and Shine, 2007; Bestion et al., 2015). However, despite *L. delicata* and *L. guichenoti* exhibiting faster growth in response to prenatal CORT and warm incubation temperatures, these same treatments did not decrease survival across the lifespan. Rather, mortality was low overall in outdoor enclosures for both *L. delicata* and *L. guichenoti*, regardless of developmental treatments. Likewise, we found little evidence that developmental CORT or warm incubation increased oxidative stress and damage markers associated with longevity, thereby suggesting limited oxidative costs associated with accelerated growth. Our finding that developmental conditions did not affect survival or induce persistent oxidative damage raises unresolved questions: what costs, if any, are there to the rapid growth we observed? And if there are such costs, why did we not detect them?

One possibility is that the effects of developmental conditions on survival only manifested extremely early in development. Previous work on *L. delicata* and *L. guichenoti* found that prenatal CORT and warm incubation induced greater embryonic mortality (Leibold et al., 2026), which might suggest the individuals most sensitive to developmental effects had been removed from the population before hatching (“viability selection”; Hadfield, 2008; Mittell & Morrissey, 2024). Viability selection may function to buffer the population from the deleterious effects of poor environmental conditions on offspring phenotypes, at the cost of lower recruitment, therefore has implications for constraining trait evolution and in population persistence (e.g., Sinervo & McAdam, 2008; Mojica & Kelly, 2010; Jarayseh, Masoero, & Bize, 2026). It is also possible that the effects of prenatal CORT and incubation temperature on survival were most pronounced immediately after hatching, and we would have missed this window by virtue of using only lizards that survived to at least 30 days post-hatching. However, studies testing developmental effects on early hatchling survival in lizards generally do not support this hypothesis (Cui et al., 2022; Wang et al., 2021; Hao et al., 2021). Even so, identifying selection acting on this “invisible fraction” (*sensu* Hadfield, 2008) could reveal critical information on developmental effects and their fitness consequences in shifting environments.

Beyond survival, CORT and incubation temperature had no clear effects on mitochondrial superoxide, oxidative DNA damage, or lipid peroxidation markers typically associated with longevity. However, broad metrics of oxidative stress may not capture complexity in oxidative damage dynamics such as the quenching of ROS or damage repair mechanisms. Another possibility, then, is that other biomarkers of oxidative stress and damage, like telomere dynamics or antioxidant activity, are more relevant indicators of developmental effects and life-history trade-offs (Olsson et al., 2018). Telomere dynamics especially are of relevance in life-history studies, as telomere length and attrition are strongly associated with long-term patterns of survival and alternative life-history strategies (Zhang et al., 2023; Rollings et al., 2017; Fitzpatrick et al., 2021). Telomere dynamics are also sensitive to incubation temperature and glucocorticoids (Hansson et al., 2024; Zhang et al., 2023; Friesen et al., 2022; Angelier et al., 2018). However, if telomere dynamics were influenced by developmental CORT or incubation temperature in our study, we should still expect differences in mortality among treatment groups, owing to

variation in rates of ageing and senescence. Indeed, we have observed lizards in our laboratory breeding colony surviving 5 or more years, suggesting the expected 2-3 year lifespan in natural *L. delicata* and *L. guichenoti* populations is not driven primarily by ageing (Joss & Minard, 1985). That we did not detect such differences in survival between treatment groups suggests that postnatal environmental pressures like predation or food scarcity are more important determinants of survival than oxidative damage induced by prenatal conditions.

The costs associated with compensatory growth may be dependent on postnatal environmental conditions, rather than driven by direct physiological effects that reduce longevity or impair reproduction. The absence of predators and the relative abundance of food resources in the outdoor enclosures in our study may, then, explain the lack of survival costs to compensatory growth we observed. If, as we might suspect, prenatal CORT and warm incubation temperatures facilitate rapid growth through elevated food intake, then under natural conditions or under poorer resource availability, additional time spent foraging would expose lizards to greater predation risk (Verdolin, 2006; Stamps, 2007). Foraging also requires substantial energetic investment, and there are no guarantees that sufficient resources are acquired to cover these energetic costs (Pyke et al., 1977). Although resource limitation seemingly constrained growth to some degree in our study, food was relatively abundant and easily accessed by lizards, likely minimising any lethal effects of food scarcity. Under more natural conditions where animals are subject to predation pressure or poor foraging success, these same CORT-treated and warm-incubated animals may not only be unable to sustain the rapid growth that they exhibited as juveniles but may suffer increased mortality in the process. Variation in postnatal conditions like predation pressure may, therefore, explain the discrepancies among studies relating compensatory growth to fitness costs.

Alternatively, developmental effects may carry reproductive costs that do not become apparent until the following generation. In birds and fish, compensatory growth in response to developmental conditions has been associated with reduced reproductive performance (Auer et al., 2010; Hsu et al., 2017). Incubation temperature has been associated with variation in reproductive success among adult lizards, but evidence directly linking accelerated growth to reproduction is rare in reptiles (Warner & Shine, 2009; Warner et al., 2010). Even so, incubation temperature influenced sperm morphology but not count in *L. delicata*, yet whether this directly influences reproductive success is unknown (de Jong et al., 2023). More likely, reproductive costs are indirectly mediated through developmental effects on body size. We found that *L. delicata* and *L. guichenoti* treated with CORT or incubated at 28°C were smaller at adulthood than their control and cool incubated counterparts, despite accelerated growth rates early in life. In at least *L. delicata*, and presumably also in *L. guichenoti*, female body size is positively correlated with the size of individual eggs in each clutch (Forsman & Shine, 1995). Previous work on *L. delicata* and *L. guichenoti* found that egg size was the strongest predictor of both embryonic mortality and hatchling size: smaller eggs result in reduced hatching success and smaller hatchlings, regardless of CORT exposure or incubation temperature (Leibold et al., 2026). Therefore, if developmental effects cause individuals to be smaller as adults, those individuals produce smaller eggs, which in turn become smaller adults that lay smaller eggs themselves, resulting in developmental effects that carry across multiple generations ('transgenerational effects'). Transgenerational effects are currently understudied in non-mammalian vertebrates (but see e.g., Shama & Wegner, 2014; Khan et al., 2016; Hellmann, Carlson, & Bell, 2020; Lee et al., 2020) but have implications for population-level responses to developmental stressors which persist or grow more pronounced across generations, as would be expected under climate change. Continued work on this lizard system, quantifying reproductive traits and phenotypes in the resulting offspring, as well as population dynamics, would inform how developmental conditions experienced by one generation cascade throughout generations.

Conclusions and Future Directions

In summary, we found that developmental conditions (prenatal CORT exposure and incubation temperatures) influenced growth patterns throughout the lifespan of *L. delicata* and *L. guichenoti* in

naturalistic conditions. However, developmental effects on growth were not accompanied by long-term costs to survival or concomitant changes in organismal and cellular physiology. Our findings generally support the prediction that developmental environment carry lifelong consequences for important phenotypes like growth but suggest that the associated fitness costs are more complex than direct trade-offs between growth and longevity. Additionally, our findings challenge the hypothesis that mitochondrial respiration and oxidative stress underlie developmental plasticity, and alternative behavioural mechanisms or physiological pathways may drive variation in life-history traits (Koch et al., 2021; Gyllenhammer et al., 2020; Monaghan et al., 2009; Constantini, 2019). Support for these hypotheses is equivocal, but studies tracking key fitness traits like growth and survival throughout life alongside associated physiological biomarkers are exceedingly rare, especially outside of laboratory conditions (Speakman et al., 2015). Integrative, longitudinal studies on populations in naturalistic conditions, such as ours, are therefore critical for elucidating the long-term effects of developmental conditions on life-history traits and the mechanisms that drive them. However, questions remain: (1) Why do developmental effects cause such varied growth responses among species or studies?; (2) Was compensatory growth in response to developmental conditions facilitated by changes in feeding behaviour or some alternative physiological pathway not measured here?; (3) If rapid growth did not trade off against survival or induce oxidative damage, then what costs (if any) does it carry? Future work tackling these questions will be needed to fully appreciate the consequences of interacting developmental stressors on animals in a rapidly changing environment.

Author Contributions

D. C. L. – Conceptualisation, Methodology, Data Collection, Data Curation, Formal Analysis, Writing – All Drafts

P. R. – Methodology, Data Collection, Writing – Review & Editing;

O. L. C. – Conceptualisation, Methodology, Writing – Review & Editing

C. R. Friesen – Conceptualisation, Writing – Review & Editing, Funding Acquisition

D. W. A. N. – Conceptualisation, Methodology, Writing – Review & Editing, Funding Acquisition

Ethics Statement

All experimental procedures were approved by the Australian National University Animal Experimentation Ethics Committee (Protocol Number A2021/56).

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Chapter 4: Prenatal conditions do not affect brain physiology and learning in a lizard

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Abstract

Early environmental factors like heat or stress hormones can impair learning through brain metabolic function, crucial for neural development and synaptic plasticity. However, whether early environments always result in cognitive impairment through changes in neural physiology is not well-established outside of a few model systems. Here, we investigated the effects of prenatal temperature and corticosterone (CORT) on brain mitochondrial activity and spatial learning in the delicate skink (*Lampropholis delicata*). We treated eggs with either CORT or a control vehicle and incubated at cold ($23 \pm 3^\circ\text{C}$) or hot ($28 \pm 3^\circ\text{C}$) temperatures. Juveniles were tested in a spatial learning task over 40 days after which mitochondrial function in the medial cortex was assessed. Despite among-individual variation in learning ability, mitochondrial physiology and spatial learning in *L. delicata* remained robust to prenatal temperature and CORT exposure. No significant relationship was found between mitochondrial function and cognitive performance, contrary to predictions. Increased metabolic capacity correlated with higher ROS production but did not affect oxidative damage, possibly due to protective mechanisms. These findings highlight the physiological and cognitive resilience of *L. delicata* to early-life challenges. Future research should explore whether this robustness extends to other brain regions, cognitive domains, and life stages.

Introduction

Learning - the acquisition and consolidation of new information - enables animals to create new associations between events, which can be essential for coping with environmental change (Buchanan et al., 2013; Dukas, 2004; Leal and Powell, 2012). However, the capacity to form new associations varies among individuals, potentially affecting their responses to environmental challenges (Ward-Fear et al., 2016; Welklin et al., 2024). Learning faster may imply better exploitation of resources or responding more efficiently to novel threats (Ward-Fear et al., 2016). Consequently, individual differences in learning abilities can profoundly affect fitness, ultimately influencing population growth rates and stability (Ward-Fear et al., 2016; Welklin et al., 2024). Therefore, understanding the mechanisms that drive these differences is crucial for predicting how populations will respond to novel conditions.

Factors like age, sex, or early-life conditions can have important effects on learning abilities (Amiel and Shine, 2012; Amiel et al., 2014; Carazo et al., 2014; Lemaire et al., 2000; Noble et al., 2014; Szuran et al., 1994; Zhu et al., 2004). Adverse developmental environments are particularly influential, as the brain is highly sensitive to environmental inputs during early life (Zhu et al., 2004). For example, early experiences can alter neurotransmitter production (Amani et al., 2021), gene expression (Zhou et al., 2020), or brain structure (Amiel et al., 2017), with lasting effects on cognition. Among the physiological mechanisms underlying learning, mitochondrial activity is considered a key factor (Du et al., 2009; Picard and McEwen, 2014; Picard et al., 2018; Siegel and Albers, 1994). The neural processes involved in learning impose substantial energetic demands (Alexandrov and Pletnikov, 2022; Mann et al., 2021; McNay et al., 2000), making efficient mitochondrial respiration essential (Du et al., 2009; Picard and McEwen, 2014; Picard et al., 2018). Additionally, learning depends on a dense network of functional neurons (Amiel et al., 2017; Lefebvre, 2011), which can be compromised by excessive oxidative stress - a byproduct of mitochondrial metabolism (Du et al., 2009; Finkel and

Holbrook, 2000; Gong et al., 2011; Hoffmann and Spengler, 2018; Zhu et al., 2004). Studies on mammals have shown the pervasive effects of mitochondrial physiology on cognitive abilities (Cao et al., 2014; Hara et al., 2014; Zhu et al., 2004). However, the extent to which these effects are generalisable to other taxa remains largely unexplored.

Since mitochondria are maternally inherited, maternal condition plays a fundamental role in shaping offspring mitochondrial activity (Picard and McEwen, 2014). Maternal stress can also influence how mitochondria operate in offspring (Zhu et al., 2004). Stressful situations experienced by mothers can elevate glucocorticoids (GCs) (Sapolsky et al., 2000), which can affect developing embryos (Uller et al., 2009) altering mitochondrial physiology through transgenerational effects (Picard and McEwen, 2014). For instance, maternal stress has been shown to contribute significantly to oxidative stress in the brain of rats (*Rattus norvegicus*) with impacts on spatial learning abilities (Cao et al., 2014; Haussmann et al., 2012; Zhu et al., 2004).

Temperature is also a significant source of maternal stress in ectotherms. Thermal environments outside the optimal range can elevate GCs in mothers, which can be passed to the offspring (see Crino et al., 2023). Temperature can also directly influence offspring development, particularly during early life (Crino et al., 2024; Noble et al., 2018). The prenatal thermal environment is crucial in shaping mitochondrial function, affecting energy metabolism and oxidative stress (Crino et al., 2024; Stier et al., 2022). Thus, the combined effects of prenatal GCs and temperature may profoundly influence mitochondrial function, with important consequences for brain development and cognition. However, the extent to which prenatal GCs and temperature interact to shape cognitive abilities via mitochondrial physiology remains largely unknown outside of a few model species (Cao et al., 2014; Haussmann et al., 2012; Zhu et al., 2004).

Here, we examined how prenatal temperature and corticosterone (CORT) — the primary GC in reptiles — affect brain mitochondrial physiology and spatial learning in the delicate skink (*Lampropholis delicata*). We hypothesized that prenatal CORT and temperature would influence learning abilities by impacting brain mitochondrial activity. Specifically, we predicted that prenatal CORT would decrease energy production while increasing oxidative damage (Costantini et al., 2011; Gong et al., 2011; but see Crino et al., 2024), especially if CORT can alter cellular components increasing ROS production without enhancing ATP synthesis or making them more vulnerable to oxidative damage. Similarly, we predicted that high temperatures would decrease mitochondrial efficiency (Crino et al., 2024; Závorka et al., 2021), but would reduce oxidative stress (Treidel et al., 2016). We further predicted that the combined effects of CORT and temperature would lead to complex interactions, with both factors negatively impacting mitochondrial efficiency but having opposite effects on oxidative stress. These effects would lead to differences in learning abilities, which could be affected by the balance between energy production and oxidative stress (Alexandrov and Pletnikov, 2022; Du et al., 2009; Picard et al., 2018). By examining these interactions, we aim to clarify how prenatal environmental factors shape learning abilities through mitochondrial function, clarifying the mechanisms that mediate the role of early-life conditions on cognitive development.

Methods

Experimental animals

Lizards came from a breeding colony established in the laboratory in 2019 from wild populations in Sydney, Australia. The colony consisted of 270 adults in groups of two males and four females. Eggs were collected from these groups between November 2023 to January 2024. After collecting the eggs, we treated them with CORT or vehicle control and incubated them under two different temperature regimes (see below). Clutch and egg identity were assigned immediately after egg collection, and the eggs were incubated in individual cups until hatching. Hatchlings were also kept in individual enclosures until the end of the experiment. For details on husbandry and breeding conditions see *Methods 1: Animal husbandry in Supplementary Material*.

Corticosterone and temperature manipulations

We tested the combined effects of prenatal CORT and temperature by elevating CORT concentrations in eggs and then incubating them under one of two temperature regimes (Cold - 23 ± 3 °C or Hot - 28 ± 3 °C). We used a partial split clutch design where eggs from a given clutch were distributed equally across the four treatments when clutch sizes were larger than four and randomly across treatments when less than four. CORT-treated eggs were topically supplied with 5 µL of crystalline corticosterone (Sigma, Cat. No. C2505) dissolved in 100% ethanol at a final concentration of 10 pg CORT/mL (CORT treatment), while Control eggs received an equal volume of 100% Ethanol. CORT dose increased mean yolk CORT levels ~3.7x higher than control eggs in previous studies (Crino et al., 2024). Eggs were then incubated in one of the two previously mentioned temperature regimes that are within the natural limits of nest temperatures in *L. delicata* (Cheetham et al., 2011).

Spatial learning

The spatial learning task involved training lizards to navigate a 6-arm maze to reach an exit connected to a transport box that allowed us to return the lizards to their enclosure without further contact. In each trial, lizards were placed by hand in the center of the maze and left to acclimatise for two minutes. During acclimatisation, the central area was surrounded by a yellow device mounted on a pulley system. At the start of each trial, this device was lifted to startle the lizard. If the lizard did not immediately choose an arm, it was gently prodded with a brush at the end of the tail. Once the lizard made a choice, the brush encouraged movement but without guiding it towards any specific arm. If the lizard did not choose correctly after 20 errors — at which point it typically ceased responding — the lizard was gently guided to the correct arm.

We assessed lizards' spatial learning using external cues for navigation while avoiding intra-maze cues. Subtle intra-maze cues were avoided by replacing the maze every three trials with one of four identical mazes. Each replacement preserved the correct arm's orientation and the maze's position within the room, ensuring consistency for each individual. Additionally, the maze was cleaned with 70% ethanol between trials to avoid the influence of chemical cues. The correct arm was randomly assigned to one of the six arms for each trial to control for potential

side biases. We employed four maze orientations to counterbalance the number of lizards assigned to each orientation across treatments.

The task was repeated once daily for 40 consecutive days, and the number of errors made by each lizard was recorded. An error was defined as the lizard inserting its head into one of the incorrect arms with each incorrect choice, including consecutive choices of the same arm, counted as independent errors.

Brain mitochondrial function

Immediately after completing the tests, we quantified mitochondrial physiology in the brains of lizards. We euthanized lizards via intraperitoneal injection of 10 mg/kg of a 10 mg/mL alfaxan solution (a potent anesthetic), followed by decapitation. Before decapitation, we evaluated lizard righting responses and the pinching reflexes in one of the front limbs.

We extracted the medial cortex in the telencephalon as this brain region is considered homologous to the mammalian hippocampus, where spatial cognition is encoded (Naumann et al., 2015; Rodríguez et al., 2002). The tissue was transferred to 1X PBS solution and then homogenised mechanically using a 100 µm mesh filter (pluriStrainer). The resultant homogenate was divided into two aliquots: one was used fresh for measuring mitochondrial density, membrane potential - a metric of mitochondrial metabolic capacity (Martínez-Reyes et al., 2016) - and superoxide (ROS) production; and the other was cryopreserved for later measurements of DNA damage and lipid peroxidation.

Fresh homogenate suspensions were stained with 5 µM MitoTracker Deep Red FM, 2.5 µM MitoTracker Orange CMTMRos, and 50 µM MitoSOX Red. We used these fluorescent probes to assess mitochondrial density, membrane potential, and ROS, respectively. We also added 5 µL of 10 µg/mL Hoechst 33342 Nuclear Viability Dye to each sample to differentiate viable cells from debris. These samples were analyzed in a flow cytometer within 2 hours of brain extraction.

The aliquots reserved for oxidative damage assays were stained with 10 µg/mL Hoechst 33342 Nuclear Viability Dye and 100 µM BODIPY 665/676 Lipid Peroxidation Sensor before cryopreservation. These dyes were used to measure cell viability and lipid peroxidation, respectively. The samples were then fixed in 1% Neutral-Buffered Formalin, washed, and preserved in a 1X Tris-EDTA solution with 10% DMSO at -20 °C. On the day of the oxidative damage assays, the samples were thawed, and DMSO was removed. Then, cell membranes were permeabilised in 200 µL of 1X PBS containing 20µM digitonin. Following permeabilisation we stained the samples with 20µL of 70µM 8-OHdG Polyclonal Antibody - a marker of DNA damage - and allowed them to incubate overnight at 4 °C. The following day we counterstained the cells with 20µL of 100 µg/mL H+G Goat Anti-Rabbit Conjugate Antibody with Alexa-Fluor 488 and analyzed the samples using the flow cytometer. Oxidative damage assays were performed within 6 months of the initial preparation of fresh samples.

Flow cytometry was performed on a 5-lasers flow cytometer (Becton Dickson LSRFortessa X-20) using the default wavelength filters and a high-throughput plate reader. Detectors and voltage settings for each assay were determined during pilot trials and remained consistent throughout the experiment. Data was processed using FlowJo (v. 10.1) software. We obtained the mean fluorescent intensity for mitochondrial density, metabolic capacity, ROS,

DNA damage, and lipid peroxidation. For further details on the homogenisation, staining, or flow cytometry assays, see *Methods 2: flow cytometry in Supplementary Material*. Sample sizes for DNA damage and lipid peroxidation were smaller than for the other variables because of a plater reader malfunction; however, data was missing randomly and we dealt with missing data in our analyses (see below). We validated that our homogenates contained neurons in a pilot study that used dyes specifically targeting neuronal nuclei (See *Brain validation in Supplementary Material*). This pilot study also ensured that our gating strategy identified these neurons using Flow cytometry.

Statistical analyses

We performed all analyses using the *brm* package (Bürkner, 2017), which fits Bayesian multilevel models with *Stan* (Stan Development Team, 2024) with R version 4.4.0 (R Core Team, 2021). We ran a series of univariate and multivariate models to test the effects of early environment on each variable separately plus quantify the relationships between physiology and learning. All models consisted of four MCMC chains of 8000 iterations, with a warmup interval of 2000 iterations.

Univariate models were used to test the effects of early environment on each variable recorded: mitochondrial density, metabolic capacity, ROS, DNA damage, lipid peroxidation, and the number of errors as a measure of learning. In all the models, we included hormone treatment (CORT vs Control), incubation temperature (Cold vs Hot), and their interaction. Sex and age were included in preliminary models and excluded from the final models when they were not significant. For all univariate models, clutch identity was included as a random factor. Mitochondrial-related variables were log-transformed when necessary, and all were mean-centered and standardized by dividing them by two times the standard deviation (Gelman, 2008). These variables were considered to follow a normal distribution.

Learning was modeled as a function of trial, CORT, temperature, and their three-way interaction. In this model, we included the trial within each level of lizard identity as a random slope. The error structure was modelled using a negative binomial distribution with a logit link function [negbinomial(link = "log")]. Otherwise, the procedure was as in the other models.

We used the posterior distributions of parameters from these models to test for differences between treatments. Learning slopes were obtained using the 'trial' estimates and its interaction with hormone and temperature treatments. Slope estimates less than 0 provide evidence of learning. *pMCMC* was used to test the hypothesis that posterior distributions of slopes and slope contrasts differed from zero. We considered an effect statistically significant when *pMCMC* < 0.05.

We fitted a multivariate structural equation model (SEM) using *brms* to explore direct and indirect links between mitochondrial function and learning. The model was structured based on theoretical expectations shown in Fig. 3. Any missing data was imputed during model fitting using data augmentation (Noble and Nakagawa, 2021), but this was largely restricted to DNA damage and lipid peroxidation. To obtain a measure of learning performance we extracted the posterior distribution of individual learning slopes (i.e., changes in error over time) by including a random trial slope for each lizard. Learning slopes and the rest of variables, were standardized as before (see above). We assumed a Gaussian error distribution for all the variables in this

model. Factors found to be non-significant in the univariate models were excluded. Clutch identity remained a random factor in our SEM model. Direct, indirect, and total effects were derived from posterior estimates (Kline, 2005) (see Table S4).

Results

We started with 80 lizards - 20 per treatment - from 33 clutches. However, due to natural mortality, only 79 lizards were included in the learning analyses, and 78 in the mitochondrial analyses. We provide final sample sizes in all our figures.

We found that the lizards improved their choices over time, making less mistakes as the trials progressed (see Fig. 1 and Table S1). However, the learning rate was not influenced by CORT, temperature, or their interaction (see contrasts in Table S2).

Mitochondrial density, metabolic capacity, ROS, DNA damage, and lipid peroxidation were also not affected by prenatal conditions (see Fig. 2 and Table S2). We did not find effects of sex and age on mitochondrial physiology (see Table S3 and Tables S5-S9).

Our SEM showed that mitochondrial physiology was unrelated to learning abilities (see Fig. 3 and Table S4). While we found that ROS production increased with metabolic capacity ($\beta = 1.090$, 95% CI = [0.316, 1.862], $pMCMC < 0.05$), ROS was not related to oxidative damage (see Fig. 3 and Table S4).

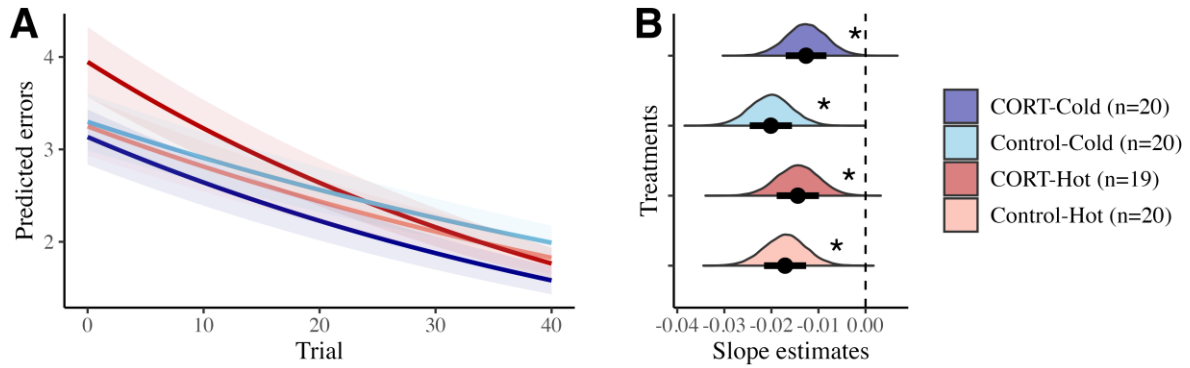


Fig. 1. Model estimates for learning analyses. (a) the predicted number of errors over trials. The lines represent the mean predicted number of errors for each trial, and the shaded areas indicate the standard error of the mean; both were obtained using the slope and intercept estimates from the posterior distributions. (b) shows the distribution of the estimates of slopes per each treatment. The x-axis represents the slope estimate, and in the y-axis are the density of the estimates. Points and bars represent the mean and standard error of the estimated slopes, respectively. Dashed lines indicate value 0. The different colours in both panels indicate the different treatments. Asterisks in (b) indicate significant differences from 0.

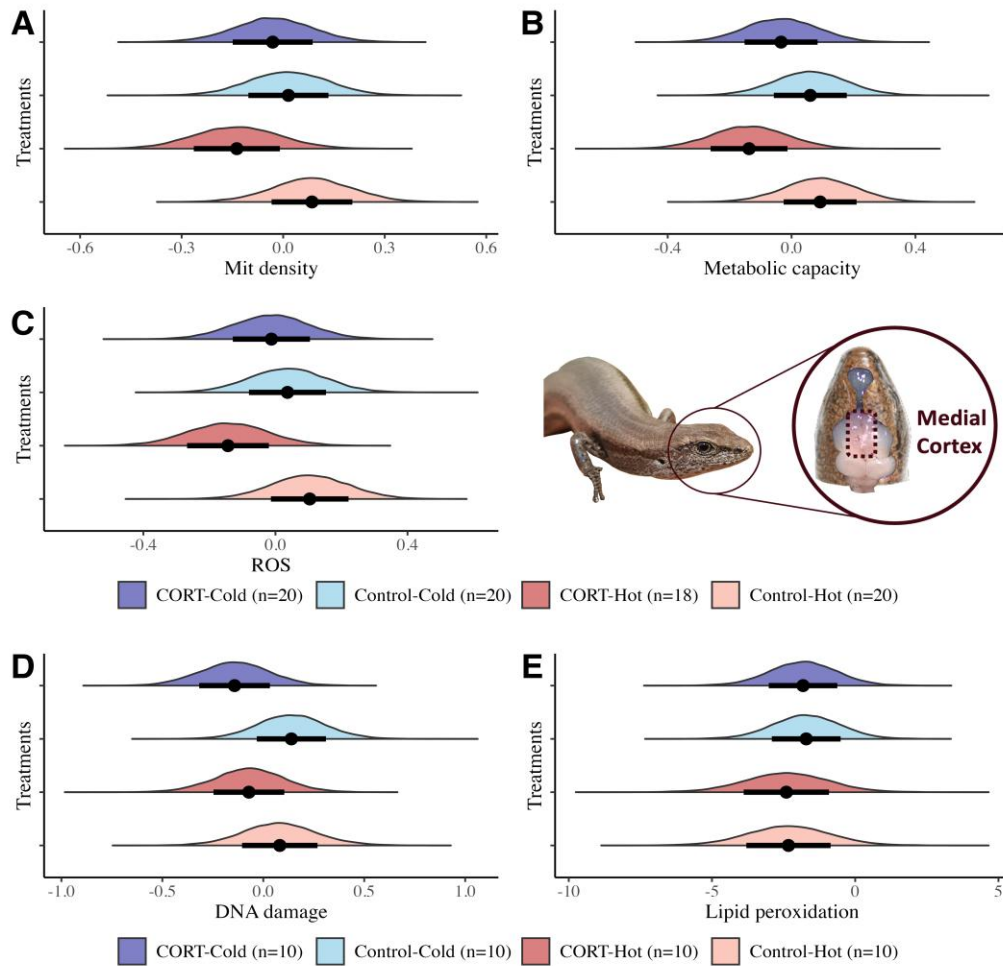


Fig. 2. Model estimates of mitochondrial density (a), metabolic capacity (b), ROS (c), DNA damage (d), and lipid peroxidation € in the medial cortex as a function of the different prenatal conditions. Note that for (d) and €, these analyses do not account for missing data resulting from a flow cytometer malfunction that impacted one plate, so sample sizes are lower for univariate analyses. The x-axis represents the estimated values while the y-axis shows the density of the estimates. Points and bars represent the mean and standard error of the estimated values, respectively. The different colours in both panels indicate the different treatments.

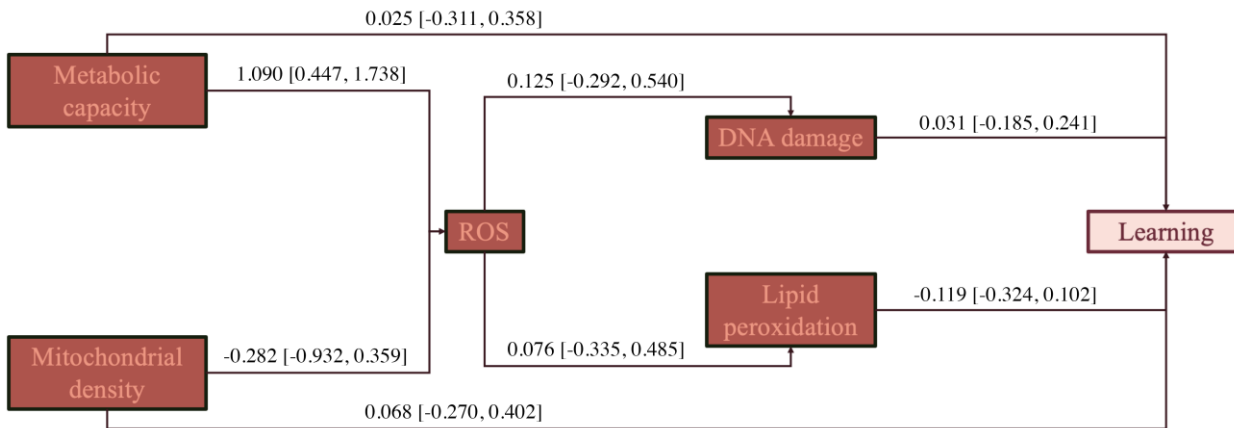


Fig. 3. Structural equation model testing hypothesized direct and indirect effects of physiology on learning. Arrows indicate the directionality of the estimate, and the values show the mean predicted coefficient and its 95% confidence interval.

Discussion

Lizards were able to learn a complex spatial task with marked improvements over time in their ability to choose the correct location. However, learning was neither influenced by prenatal conditions nor linked to mitochondrial function in the medial cortex, counter to what we predicted. Despite ROS increasing with greater metabolic capacity as expected, this did not result in a corresponding increase in oxidative damage in the medial cortex in *L. delicata*. We discuss our results in relation to existing work exploring the underlying mechanisms of learning and how early environments impact it below.

Spatial learning is robust to prenatal conditions

Lizards exhibited clear evidence for learning, but learning rates were not influenced by prenatal CORT or temperature as we predicted, contrasting with findings in other taxa where incubation temperature and prenatal GCs impacted learning and brain development (Amiel and Shine, 2012; Amiel et al., 2017; Dayananda and Webb, 2017; Lemaire et al., 2000; Zhu et al., 2004). Our results suggest that spatial learning abilities in *L. delicata* are buffered from adverse early environmental conditions, consistent with our previous findings showing that behavioural flexibility in this species is also unaffected by prenatal CORT or temperature (Recio et al., 2025). *L. delicata*'s resilience may stem from the relevance of spatial cognition in lizards. For example, spatial learning in velvet geckos (*Amalosia lesueurii*) enhances survival in the wild (Dayananda and Webb, 2017). Future studies should investigate the mechanisms underlying this robustness and the role of cognition in *L. delicata*'s fitness.

Brain metabolic physiology is not affected by prenatal conditions

Contrary to our predictions, prenatal temperature and CORT did not significantly influence mitochondrial physiology in the medial cortex of *L. delicata*. Studies in other taxa have shown that incubation at high temperatures decreases energy production and oxidative stress (Treidel et al., 2016; Závorka et al., 2021), while elevated GCs or maternal stress is related to lower mitochondrial efficiency and higher oxidative damage (Costantini et al., 2011; Gong et al., 2011; but see Crino et al., 2024). In *L. delicata*, high incubation temperatures are known to decrease mitochondrial efficiency in the liver (Crino et al., 2024). However, our study shows that mitochondrial physiology in *L. delicata*'s brain is robust to both incubation temperature and prenatal CORT. Differences between liver and brain could reflect tissue-dependent responses to early-life conditions, highlighting the importance of studying these effects across multiple tissues (Casagrande et al., 2023).

Variation in brain metabolic physiology is not related to learning abilities

Mitochondria play a critical role in cognitive function by synthesizing energy (Du et al., 2009; Picard and McEwen, 2014; Picard et al., 2018; Siegel and Albers, 1994), and by affecting the rate of cell senescence and death caused by oxidative damage (Alexandrov and Pletnikov, 2022; Mann et al., 2021; McNay et al., 2000). Our findings contrast with past studies and suggest that energy limitations do not impact spatial learning in *L. delicata*. The lack of association between mitochondrial density, mitochondrial capacity, and learning abilities may result from

lizards being able to maintain energy production above the threshold required to prevent cognitive dysfunction. The generally low energetic demands of ectothermic organisms compared to endotherms may make this possible and explain why we found no effect when they seem common in mammals (Cao et al., 2014; Hara et al., 2014). Alternatively, individuals with higher respiration and ROS may be upregulating antioxidant production (Rice et al., 2002).

Interestingly, despite finding a significant positive relationship between metabolic capacity and ROS in the medial cortex, the heightened ROS production did not lead to greater oxidative damage. The strong relationship between ROS and mitochondrial capacity aligns with the role of mitochondrial membrane potential in driving ATP synthesis and electron transport, processes that inherently generate ROS as byproducts (Rice et al., 2002). Normally, moderate ROS levels serve essential signaling functions and are controlled by antioxidants (Rice et al., 2002; Terman and Brunk, 2006). However, excessive ROS production can exceed antioxidant action, leading to oxidative stress and cellular dysfunction (Rice et al., 2002; Terman and Brunk, 2006). The lack of oxidative damage from increased ROS strongly suggests that antioxidants could deal with these levels of ROS. As such, DNA damage and lipid peroxidation in the medial cortex were not linked to spatial learning in *L. delicata* possibly because neurons were effectively buffered from damaging free radicals. Nevertheless, since oxidative damage can accumulate over time (Terman and Brunk, 2006), the relationships between oxidative damage and cognitive dysfunction could become more pronounced in older individuals (Hara et al., 2014). Future research should investigate the long-term consequences of mitochondrial activity on brain health and the potential mechanisms that sustain cognitive resilience in *L. delicata*.

Conclusions

We found that spatial learning in *L. delicata* was not influenced by prenatal CORT or temperature, nor was mitochondrial physiology in the medial cortex. Additionally, there was no relationship between mitochondrial function and learning abilities in a spatial task. However, we found that ROS production increased with metabolic capacity, suggesting that links between mitochondrial respiration and oxidative stress may become more pronounced under higher energetic demands or with aging, potentially influencing cognitive function over time.

Author Contributions

Conceptualization: P.R., D.C.L., O.L.C., C.R.F., D.W.A.N.; Data curation: P.R., D.C.L.; Formal analysis: P.R., D.C.L.; Funding acquisition: C.R.F., D.W.A.N.; Investigation: P.R., D.C.L.; Methodology: P.R., D.C.L., O.L.C., C.R.F., D.W.A.N.; Project administration: P.R., D.C.L.; Resources: D.W.A.N.; Supervision: D.W.A.N., C.R.F.; Validation: P.R., D.C.L., D.W.A.N.; Visualization: P.R., D.C.L.; Writing – original draft: P.R., D.C.L.; Writing – review & editing: P.R., D.C.L., O.L.C., C.R.F., D.W.A.N.

Ethical Statement

All the protocols complied with Australian law and were approved by the Australian National University Animal Experimentation Ethics Committee (A2022_33).

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General Discussion

Overview

My thesis has addressed fundamental questions about how developmental plasticity shapes fitness and fitness-related phenotypes across life. Specifically, I tested how exposure to multiple, potentially interacting developmental conditions – elevated corticosterone (CORT) and altered incubation temperature – affected growth and survival in two lizard species (*Lampropholis delicata* and *L. guichenoti*) during both early (Chapter 1) and later life (Chapter 3). I also examined whether these developmental conditions influenced adult cognitive performance (*L. delicata*, Chapter 4) and evaluated the physiological mechanisms underlying developmental plasticity in growth, survival, (Chapter 3) and cognition (Chapter 4). To achieve this, I developed novel flow cytometry methods that enabled simultaneous quantification of mitochondrial function and multiple indices of oxidative damage (Chapter 2).

My findings indicate that developmental CORT exposure and incubation temperature influence early survival and hatchling size (Chapter 1), consistent with general expectations of immediate effects of incubation temperature or glucocorticoids on offspring phenotypes (Noble, Stenhouse, and Schwanz, 2018; Crino et al., 2024). Maternal investment, however, was a powerful mediator of developmental effects on hatchling survival and body size. Regardless of developmental treatment, larger eggs were more likely to survive to hatching and resulted in larger hatchlings than smaller eggs within both *L. delicata* and *L. guichenoti* (Chapter 1). Yet, the “minimum” size for eggs to hatch increased as eggs were subjected to prenatal CORT, warm incubation temperatures, or both (Chapter 1). The selective mortality of smaller eggs suggests that energetic mismatches due to CORT or temperature drove their effects on survival and body size, which can be overcome with greater maternal investment. Furthermore, the effects of CORT and incubation temperature on hatching success and hatchling body size were more pronounced in *L. delicata* (smaller eggs) than in *L. guichenoti* (larger eggs) (Chapter 1). Therefore, Chapter 1 supports that variation in maternal investment both within- and between- species buffers offspring from the most immediate detrimental effects of poor environmental conditions. Yet, offspring also exhibit plasticity in growth during early life, which may further protect them from lethal developmental effects (Chapter 3). While prenatal CORT and warm incubation temperatures resulted in smaller hatchling body sizes, these individuals also grew more rapidly to adult body sizes, potentially improving survival to early adulthood (Chapter 3).

Despite prenatal CORT and incubation temperatures inducing lizards to grow at accelerated rates early in life, compensation for initial disparities in body size was incomplete (Chapter 3). Instead, body size disparities grew even more pronounced in adulthood, demonstrating that developmental effects on body size persisted throughout the lifespan of *L. delicata* and *L. guichenoti* (Chapter 3). Even so, developmental treatments did not impose any detectable long-term costs on survival, contradicting the prediction that accelerated growth trades off against longevity (Chapter 3). Moreover, CORT and incubation temperature did not influence the metabolic rates, mitochondrial function, or oxidative damage markers expected to underlie changes in growth and survival trade-offs (Chapter 3). In contrast to the prediction that elevated metabolic rates facilitate the rapid growth observed, CORT exposure may have depressed metabolism in juvenile *L. delicata* and *L. guichenoti* (Chapter 3). I also expected rapid growth to be driven by an upregulation of mitochondrial content or mitochondrial respiration. However, prenatal CORT and developmental temperatures had no effects on mitochondrial dynamics in the livers or hearts of juvenile *L. delicata* or *L. guichenoti*, and any tendencies for an effect vanished by adulthood (Chapter 3). Similarly, I had anticipated greater oxidative damage in CORT-treated and warm-incubated lizards, which would contribute to a trade-off between growth and longevity. Yet, while oxidative damage may

have been elevated by developmental treatments in juveniles, this effect was not present in adulthood, consistent with the lack of differences in survival between treatment groups (Chapter 3). Following from the effects on hatchling size observed in Chapter 1, Chapter 3 supports that developmental conditions have sustained effects on growth and body size but do not necessarily impose a trade-off against long-term survival. Mechanistically, these effects are not driven by the expected changes in organismal or cellular physiology, but potentially by alternative physiological or behavioural pathways.

When extended beyond the scope of basic life-history traits, developmental CORT and incubation temperature did not exert any influence on cognition. Regardless of developmental treatment, *L. delicata* performed spatial learning tasks at the same levels of success, suggesting that cognitive ability is robust to developmental conditions (Chapter 4). Mitochondrial function and oxidative damage in the medial cortex region of the brain was also unaffected by CORT or incubation temperatures, further supporting that physiological mechanisms associated with cognitive processes are similarly resilient to developmental stress (Chapter 4). Even so, mitochondrial membrane potential in the brain was generally correlated with superoxide production, but not oxidative DNA damage or lipid peroxidation (Chapter 4). This association suggests that neurons are potentially buffered from oxidative damage through upregulation of antioxidants, and that cognitive ability could be hindered under conditions of elevated metabolic activity that overwhelms antioxidant capacity.

Collectively, the results of Chapters 1, 3, and 4 support that developmental CORT and incubation temperatures program fundamental life-history traits like growth and body size, and that this pattern is conserved among closely related species, despite their ecological differences. However, developmental effects do not affect cognitive ability, nor do these effects induce direct trade-offs between growth and survival. From a mechanistic perspective, my findings do not support that mitochondrial or oxidative physiology underlie lifelong phenotypic variation, but do not necessarily rule out more nuanced physiological pathways or behavioural mechanisms. Nevertheless, these findings provide valuable insight into the mechanisms underpinning developmental plasticity and life-history variation across multiple levels of biological organization. In service of this work, I developed novel techniques which simultaneously quantified multiple interrelated markers of mitochondrial respiration and oxidative damage (Chapter 2). These flow cytometric assays were highly repeatable and applicable across diverse tissue types, thereby expanding the toolkit available for integrating physiological biomarkers with organismal traits — an essential step toward understanding how physiology shapes developmental and life-history outcomes.

Multi-factored studies lay a foundation for targeted experiments

This thesis provides a comprehensive dataset linking developmental environments, life-history outcomes, and underlying physiological mechanisms. To my knowledge, it represents one of the most extensive studies of its kind, incorporating multiple simultaneous developmental stressors, two species with differing reproductive strategies, and long-term monitoring from the egg stage through adulthood under naturalistic conditions. It also integrates diverse biomarkers of cellular physiology across multiple tissues (liver, heart, and brain) alongside key life-history metrics such as growth, survival, and cognition. The scope of this work directly addresses long-standing research needs, such as the call for longitudinal studies under naturalistic conditions and the need to account for interspecific variation in developmental plasticity (Speakman et al., 2018; Uller et al., 2024; Muell et al., 2025). The novel flow cytometry methods developed here further address a common limitation in mechanistic physiology studies, which often focus on single biomarkers (e.g., telomere length, 8-OHdG, or total antioxidant capacity) or pathways (e.g., mitochondrial respiration, telomere dynamics) [Beaulieu and Costantini, 2014; Speakman et al., 2015; Isaksson, 2020]. My thesis therefore provides both a broad empirical foundation and a

methodological advance, which enhances our capacity to study developmental plasticity across organizational levels and lay the groundwork for future investigation into the mechanisms of life-history variation.

The multi-factored, comprehensive design of these experiments provides an important foundation for future studies addressing alternative mechanisms of life-history variation. The strength of this approach lies in revealing both where more detailed investigation is warranted and where experimental complexity can be streamlined. My thesis demonstrates that such designs are feasible when suitable model systems are chosen, when the phenotypic scope is well defined (e.g., growth, survival, cognition), and when emerging methods from other fields are adapted effectively (e.g., flow cytometric functional assays). However, this level of complexity remains logistically challenging for most research programs. Including multiple factors (e.g., multiple species, multi-factored experimental treatments, longitudinal design, multiple tissues) in follow-up studies should, therefore, be weighed for the additional information these designs provide against the constraints they impose. Thus, while comprehensive experimental designs such as this are necessary for elucidating broad scale developmental effects on key life-history traits, and for testing the generality of these effects across ecological contexts, they also serve as a framework for addressing targeted questions in follow-up studies.

One direction for streamlining additional work in this space, with the context of the current longitudinal study, would be to focus on discrete life-history stages. For example, Chapter 1 suggests that developmental effects have notable physiological effects during embryonic development that result in increased mortality or reduced body size at hatching. The longitudinal study of Chapter 3 supports that developmental effects on growth can change between the juvenile and subadult stages. There is a possibility that developmental effects on physiology and mortality are most pronounced in the earliest juvenile stage, between hatching and ~30 days post-hatching, which was not captured in this design. And developmental effects may influence reproductive traits that do not become apparent until the next generation. Another way to streamline further studies is in the selection of tissues used for physiological measures. Developmental CORT and incubation temperature had consistent effects across tissues (liver, heart, brain; Chapters 3 and 4), and methodological validation showed that liver and red blood cells exhibited comparable physiological profiles (Chapter 2). This suggests that future work could employ more accessible tissues, such as blood, as proxies for cellular physiology in other organs. Alternatively, male gonadal tissue showed a distinct physiological profile from somatic tissues (Chapter 2), indicating that gametes may be differently affected by developmental conditions. Therefore, additional work targeting these discrete life-history stages, or utilising only one tissue, would provide valuable context to the results I observed in my thesis work while reducing experimental complexity.

A lack of physiological programming suggests alternative mechanisms driving phenotypic variation

This thesis reveals key insights into how early-life conditions shape fitness and fitness-related phenotypes, and the potential (and limitations) of mitochondrial and oxidative physiology as mediators of developmental plasticity. In Chapter 1, I found that simultaneous exposure to developmental CORT and elevated incubation temperatures reduced both hatching success and hatchling body size. However, variation in maternal investment — both within and among species — was the primary determinant of survival and size. Larger eggs were more likely to hatch and produced larger hatchlings within each species, and *L. guichenoti*, which lays larger eggs, exhibited higher hatching success and larger hatchlings than *L. delicata*. Chapter 3 revealed that CORT-treated and warm-incubated hatchlings of both species grew rapidly early in life, ostensibly to compensate for their smaller sizes at hatching, but that they ultimately remained smaller at adulthood than their control or cool-incubated counterparts. Despite these early-life effects on hatching success and persistent effects on growth and body size, developmental

treatments did not influence long-term survival (Chapter 3). Likewise, early exposure to CORT or warm incubation had no effect on adult cognitive performance in *L. delicata* (Chapter 4). Furthermore, I found no evidence that developmental treatments induced lasting changes in mitochondrial or oxidative physiology across tissues (liver, heart, or brain; Chapters 3 and 4). The lack of physiological programming by developmental CORT or incubation temperature, despite their observed phenotypic effects, points to alternative mechanisms besides mitochondrial function or oxidative damage *per se* as drivers of developmental plasticity and a potential role of the postnatal environment in facilitating this plasticity.

I quantified multiple broad parameters of mitochondrial function and oxidative damage: mitochondrial mass, membrane potential, superoxide production, 8-OHdG, and lipid peroxidation. Evidence for CORT and incubation temperature affecting these parameters was scant (Chapters 3 & 4). Yet this does not rule out that the physiological pathways affected by developmental CORT and incubation temperature are more nuanced than could be captured by these metrics alone. There remain several potential mechanisms that underlie life-history variation and plastic responses to developmental conditions. Most notably among these include the discrete phases of the mitochondrial electron transport chain, the regulation of antioxidant activity or damage repair mechanisms, or telomere dynamics implicated in ageing and senescence. Likewise, Chapter 4 assessed developmental effects on a cognitive trait (associative learning) expected to regulate foraging success, which could in turn facilitate life-history variation. However, alternative behavioural or personality traits, like boldness or motivation, that translate into foraging activity, spatial use, or predator avoidance may be more relevant to traits like growth and survival. Additional work targeting these candidate physiological and behavioural mechanisms as mediators of developmental plasticity would more completely elucidate why CORT and incubation temperature induced changes in growth and body size, but not survival, in *L. delicata* and *L. guichenoti*.

Novel flow cytometry methods offer a powerful and accessible tool for mechanistic physiology studies

I developed a novel application of flow cytometry for the simultaneous measurement of multiple physiological biomarkers within a single tissue sample. These methods are low-cost, high-throughput, and require minimal sample volumes while producing highly repeatable and biologically meaningful quantitative data (Chapter 2). This approach therefore removes a major logistical barrier to studies linking physiology with organismal performance. Many mechanistic physiology studies are constrained by the high cost, labour, and sample requirements of existing methods (e.g., enzyme assay kits, high-resolution respirometry), which often force trade-offs between sample size and experimental complexity (e.g., number of species or treatment levels). The limited tissue available from small organisms, or ethical restrictions on sampling volume, further restrict the number of biomarkers that can be measured. The flow cytometric assays developed here overcome these limitations by allowing multiple parameters of mitochondrial function and oxidative damage to be quantified simultaneously from a single small sample. This enables incorporation of physiological biomarkers into a wider range of study designs without compromising statistical power, cost efficiency, or animal welfare.

That these methods performed consistently across tissue types — validated in livers, blood, and gonads (Chapter 2) and applied successfully to livers, hearts, and brains in later chapters (Chapters 3 and 4) — demonstrates their broad applicability. For example, brain tissue can be used to examine the physiological basis of cognition or behaviour (Chapter 4); liver and heart tissue can elucidate the mechanisms of energy metabolism and cellular respiration (Chapter 3); and gonads or sperm can be applied to studies of reproductive physiology (Chapter 2). Importantly, non-terminal blood samples offer a minimally invasive means to repeatedly measure physiology within individuals or to broadly characterize physiological profiles across populations. Blood-based sampling also enables experimental

estimation of physiological reaction norms by comparing profiles before and after treatments. Additionally, flow cytometry data is inherently in relative units of fluorescence, and excels in comparative studies or for testing the effects of experimental treatments. Overall, these flow cytometric methods provide a versatile and accessible framework for integrating cellular physiology into ecological and evolutionary studies. By reducing the logistical and ethical constraints that often limit mechanistic research, they open new opportunities to examine physiological processes underlying key life-history traits, behavioural variation, and responses to environmental change.

Future Directions

Fine-scale temporal data would improve our understanding of developmental programming effects

In Chapter 1, I found that simultaneous CORT exposure and warm incubation temperatures reduced both hatching success and hatchling size, indicating that exposure to multiple developmental stressors can impose immediate fitness and phenotypic costs. However, these effects on hatching success were modest (with egg size exerting a stronger influence), and the underlying mechanisms remain unresolved. In Chapter 3, neither CORT nor incubation temperature influenced mitochondrial or oxidative physiology at ~90 days post-hatching or later in adulthood (~2 years post-hatching). Although the longitudinal design captured broad patterns of growth, survival, and physiology across the lifespan of *L. delicata* and *L. guichenoti*, it lacked the fine-scale temporal resolution needed to detect short-lived, stage-specific, or rapidly compensated physiological changes. Without such resolution, the timing and mechanistic basis of developmental effects remain partially obscured, particularly during ontogenetic windows not sampled in this body of work.

I was unable to measure cellular physiological outcomes during embryonic development, which may be a critical time to focus efforts. A targeted follow-up study tracking embryonic mortality across treatment groups, paired with high-resolution physiological sampling at both whole-organismal (e.g., metabolic rate) and cellular levels during development (e.g., 1-, 7-, and 14-days post-treatment), would help identify the immediate physiological consequences of CORT and thermal stress. One possibility is that embryos are constrained by a finite energetic budget determined by yolk energy content, and developmental stressors reduce hatching success or size by increasing metabolic energy demand or decreasing the efficiency of energy use and assimilation (the Energy Efficiency Hypothesis, Tabh & Nord 2023). The efficiency of energy use and assimilation shifts with variation in incubation temperatures, with temperatures outside of the optimum temperature leading to reduced assimilation of energy into body mass or for the completion of development (Tabh and Nord, 2023). CORT is rapidly metabolised early in development (Carter, Bowden, and Paitz, 2018; Harders, Charboneau, and Paitz, 2024), which may elevate energetic expenditure and mitochondrial respiration. Additionally, acute CORT exposure induces oxidative stress (Majer et al. 2019), suggesting that mortality may occur when oxidative damage exceeds embryonic antioxidant or repair capacity. These mechanisms may therefore act as a sieve, removing embryos with lower energetic or oxidative resilience from the population. The same may be true of early postnatal juveniles.

In Chapter 3, growth, survival, and physiology were assessed beginning at ~90 days post-hatching — after the first major post-hatching growth phase — and necessarily only in individuals that survived to this stage. Because, like many lizards, early juvenile mortality is expected to be high in *Lampropholis*, any physiological or performance effects of developmental CORT exposure or warm incubation may have already manifested, and selectively removed individuals, before sampling began (Heidinger et al., 2012). If so, the absence of treatment effects on mitochondrial or oxidative physiology later in life may reflect a survivor-biased subset of physiologically robust individuals rather than a true

absence of developmental programming. Identifying when, and through which pathways, developmental stressors impose these costs would clarify the mechanistic drivers of early-life mortality and improve predictions of population responses to changing developmental environments. This possibility underscores the need for physiological sampling during the embryonic and early juvenile stages (<90 days), when developmental effects might be strongest and when selective mortality could shape the trajectory of the surviving cohort.

Finally, the physiological data collected in adulthood were obtained near the expected natural lifespan of *L. delicata* and *L. guichenoti* (~2–3 years post-hatching), leaving an intermediate period, from ~90 days to adulthood, largely unexamined. Although delayed physiological responses to developmental environments may be uncommon, they are not unprecedented. For example, in *L. delicata*, warm incubation temperatures influence the mass-specificity of metabolic rates in 1-year-old adults but not in juveniles or 2-year-old adults (de Jong et al. 2023). Fine-scale temporal sampling of mitochondrial and oxidative physiology across this intermediate period could reveal transient, stage-specific, or delayed effects of developmental stressors that were not captured here and may also help explain patterns such as incomplete compensatory growth observed in Chapter 3. Identifying these windows of physiological sensitivity is essential for understanding when developmental plasticity is expressed, when it is compensated, and how it shapes life-history trajectories across ontogeny.

Extended monitoring could reveal long-term effects of developmental conditions on senescence and reproduction

In Chapter 3, I tracked growth and survival in *Lampropholis* across what is typically considered their natural lifespan (~2–3 years), alongside biomarkers of mitochondrial function and oxidative damage. Developmental CORT and incubation temperature had no detectable effects on long-term survival, nor did they influence oxidative DNA damage (8-OHdG) or lipid peroxidation. One possibility is that other biomarkers, particularly telomeres, may have been more sensitive to developmental oxidative stress (Metcalf & Olsson 2021). Telomeres are widely used indicators of ageing and senescence (Monaghan 2010; Haussmann & Marchetto 2010) and are established markers of biological ageing in ectotherms (Olsson et al. 2018). However, if developmental stress had produced meaningful telomere shortening or other unmeasured forms of cumulative oxidative damage, then differences in senescence rates should still translate into detectable survival differences among treatment groups. The absence of such differences, combined with consistently high survival in outdoor enclosures and observations of many individuals living 5+ years in captivity, suggests that *Lampropholis* skinks may live substantially longer in more benign captive environments than in the field. Consequently, detecting any long-term survival costs or delayed physiological consequences of developmental stress may require continued monitoring of this cohort for several more years. However, the biological significance of such late-emerging effects may be limited, given that *Lampropholis* in the wild typically live only 2–3 years and are seemingly more strongly shaped by extrinsic sources of mortality than by ageing itself.

The observation that *Lampropholis* skinks potentially live much longer in captivity (including in outdoor enclosures) suggests that their expected 2–3 year lifespan in the field is not driven primarily by ageing or senescence. Rather, lifespan in these species may be largely determined by environmental factors such as predation pressure, resource availability, or competition. While the outdoor enclosures in which lizards were reared were naturalistic, they offered abundant food and water, ample thermal variability, and reduced predation risk. Consequently, animals in these controlled environments may never encounter the postnatal stressors that reveal the functional consequences of developmental conditions (Monaghan and Haussman, 2015; Sheriff et al., 2017). If developmental conditions alter HPA-axis reactivity (e.g., Crino et al., 2024; McCormick et al., 2016), thermally-sensitive metabolism (e.g., de Jong et al., 2023; Owen et al., 2019; but see Kar et al., 2022), or behaviours such as risk-taking or foraging effort (e.g., MacLeod et al., 2021), developmental effects are likely to manifest as costs only under stressful postnatal conditions. In more benign environments, however, individuals may compensate for

developmental effects without incurring the trade-offs that would normally constrain growth or survival in the wild (de Gasperin et al., 2019). Incorporating more ecologically realistic sources of postnatal stress – whether through true field studies or controlled exposure to factors such as predation cues, food restriction, thermal stress, or routine disturbance – may therefore be necessary to uncover how developmental conditions influence survival, growth, or behaviour in natural populations.

A further possibility is that any fitness consequences of developmental environments may emerge primarily through their effects on future generations, rather than the current generation. Developmental stressors could influence reproductive output directly, such as by altering gamete quality or the allocation of resources to reproduction (Walsh et al., 2019). This idea is tentatively supported by the results of Chapter 2. Gonadal tissues showed a markedly different physiological signature from somatic tissues, suggesting that reproductive cells may be affected by developmental stressors in distinct ways from the livers, hearts, and brains measured in Chapters 3 and 4. In other taxa, oxidative stress has been associated with reduced sperm quality and shorter sperm telomeres (lizards: Friesen et al., 2021; birds: Mora et al., 2015; crickets: Noguera, 2022), indicating plausible pathways through which developmental stress might reduce reproductive success or the quality of offspring. Developmental effects may also manifest by accumulating across generations (‘transgenerational effects’ – Sheriff and Love, 2013). For example, offspring from *Sceloporus undulatus* populations exposed to invasive fire ants across multiple generations exhibited more robust stress responses than offspring from uninvaded populations (McCormick et al., 2016). Thus, the long-term costs of developmental conditions may become apparent only when individuals reproduce, or even only in their offspring. Accordingly, detecting these outcomes may require monitoring populations across multiple generation, or implementing multi-generational experimental designs that allow potential transgenerational effects to accumulate and become measurable.

Stronger developmental perturbations or comparative contexts may reveal hidden plasticity

A potential explanation for the absence of long-term developmental effects on survival (Chapter 3) or cognition (Chapter 4) is that the magnitude of developmental stressors used in these studies were relatively mild perturbations. The CORT dose with which eggs were treated is expected to increase circulating CORT in the yolk to two standard deviations above the mean and within the range of natural variation in our laboratory population (Crino et al., 2024). However, it is likely that circulating CORT levels in natural populations of *Lampropholis* are much higher than those in our laboratory population, owing to exposure to environmental stressors like predation pressure or inter- or intraspecific conflict (e.g., Narayan, Cockrem, & Hero, 2013; Jessop et al., 2015; Telemeco et al., 2019). Thus, while the treatments substantially increased circulating yolk CORT relative to laboratory conditions, they may underestimate the magnitude of CORT variation in natural populations. Even if the amount of CORT used in these studies is representative of CORT levels in natural populations, hormones are rapidly metabolized during early embryonic development (Harders et al., 2024; Crews et al., 1991), so pharmacological levels of CORT elevation may be required to induce long-term physiological effects on mitochondrial function and oxidative stress. Alternatively, CORT may exert long-term physiological effects only in even more mild levels *via* hormetic effects. For example, previous work on this system by Crino et al. (2024) demonstrated that *L. delicata* eggs receiving a ‘low’ CORT dose (half of that used in these experiments) had elevated baseline CORT levels later in life compared to control animals or those receiving a ‘high’ CORT dose (the same as used in these experiments). The findings of Crino et al. (2024), indicate that additional work is needed to characterize dose-dependent responses of developmental CORT on long-term phenotypes and physiology.

While incubation temperatures used (23°C and 28°C) were intended to represent shaded or heat-exposed natural nests, this range of temperatures is relatively narrow and included daily thermal fluctuations ($\pm 3^\circ\text{C}$), therefore thermal treatments overlapped at intermediate temperatures. Thus, these

treatments may simply have been too mild to impose the kinds of energetic, oxidative, or developmental disruptions that drive long-term phenotypic change. Alternatively, any physiological damage associated with extremes may have been repaired when temperatures reach more suitable conditions (Arnold et al. 2025; Buckley, et al. 2025). Pharmacological elevations in CORT or more extreme or constant incubation temperatures might exert larger physiological effects that overwhelm compensatory mechanisms available to embryos and juveniles. For example, de Jong et al. (2023) showed that incubation at a constant 32°C, well above the typical range for *Lampropholis* nests (Cheetham et al., 2011), produced long-term metabolic consequences that were absent under milder or fluctuating regimes. This contrast suggests that both the magnitude and fluctuation of thermal conditions matter (While et al., 2018). Specifically, exposure to constant high temperatures may limit opportunities for physiological repair or metabolic downregulation that fluctuating regimes afford. From a mechanistic standpoint, pushing stressors beyond the ecologically typical range may help clarify the thresholds at which CORT exposure or thermal stress produce measurable changes in oxidative physiology, metabolic dynamics, or growth trajectories. Such work would be useful not because these extreme environments are common in the field, but because identifying the limits of developmental buffering can reveal the processes that maintain phenotypic stability under natural conditions.

While *L. delicata* and *L. guichenoti* have different reproductive strategies, they are closely related and occupy heavily overlapping ecological niches. A broader comparative approach may reveal how life history strategy and thermal ecology shape the sensitivity of developmental stages to stressors. For instance, ectotherms operating at relatively low metabolic rates may tolerate physiological disruption more readily than endotherms, whose tight thermal and metabolic constraints leave less capacity for compensatory adjustments (Clarke & Pörtner, 2010; Buckley, Hulbert, & Jetz, 2012). Oviparous and viviparous species also experience developmental stressors on very different schedules. Acute exposure to maternally derived CORT at oviposition (as in *Lampropholis*) may be metabolized rapidly and primarily affect early mortality, whereas chronic maternal stress during gravidity in viviparous taxa can expose embryos to elevated CORT throughout development, thereby amplifying long-term effects on morphology, metabolism, and behaviour (MacLeod, While, & Uller, 2021). Thermal strategy is also of relevance, as species from stable thermal environments (e.g., tropical habitats) may exhibit narrower reaction norms and higher sensitivity to deviation from optimal temperatures, whereas temperate or arid species accustomed to broad and unpredictable thermal variation may be more robust to developmental thermal stress (Huey & Kingsolver, 1989; Kingsolver, 2009). For example, work on *Drosophila subobscura* found that a more southerly, heat-adapted population was more robust to developmental thermal stress than more northerly population (Porcelli et al., 2019). Tadpoles from more thermally variable (more temperate) populations were developmentally robust to daily thermal fluctuations than tadpoles from less thermally variable (more tropical) populations (Kern, Cramp, & Franklin, 2015). However, experiments directly testing developmental responses in tropical versus temperate reptiles is scarce. Integrating these kinds of comparative contrasts across reproductive modes, metabolic strategies, or climate regimes would help determine why developmental programming of physiology is more prevalent in some taxa than others and elucidate general principles governing developmental plasticity across species (Gaston et al., 2009).

Expanding physiological biomarkers would enhance mechanistic insights and validate novel methods

Chapter 2 presented novel functional assays using flow cytometry to cheaply and rapidly measure several broad metrics of mitochondrial and oxidative physiology relevant to life-history ecology. These methods were repeatable, applicable across tissue types, and were successfully employed in experimental tests in Chapters 3 & 4. Flow cytometry techniques are promising in that they are relatively accessible for incorporation into complex experimental designs, excel in comparative studies or testing for treatment effects, and can give broad overviews of key physiological pathways. Additionally, there is space for

expanding these methods beyond what was presented here. As more functional fluorescent probes become available, additional relevant biomarkers of life-history traits may be incorporated into functional assay panels. Emerging spectral cytometers allow for simultaneous quantification of several dozen biomarkers from a single sample. Furthermore, the flexibility of flow cytometry techniques allows for experimental manipulations at the cellular level, such as exposing cells to varying temperatures, ROS, or antioxidants. Therefore, the techniques presented in Chapter 2 and utilised in Chapters 3 & 4 are a promising new method in ecophysiology with a broad scope of applications and which overcome some of the limitations of established methods in the field. However, the physiological pathways mediating life-history traits and trade-offs are highly complex, and flow cytometry still only captures a subset of potential mechanisms.

I quantified broad-scale mitochondrial traits and oxidative damage biomarkers using flow cytometry, but key mechanisms remain unmeasured. Alternative mechanisms not captured in my work include telomere dynamics (length, attrition, telomerase activity) and antioxidant capacity, which are often linked to ageing, senescence, and stress responses (Monaghan & Haussman, 2006; Metcalfe & Alonso-Alvarez, 2010; Haussman & Heidinger, 2015). Although my findings do not support that developmental stressors impact lifespan, this does not necessarily rule out that these animals exhibit a markedly different physiological strategy to maintain telomere integrity. For example, these individuals may exhibit greater telomerase activity, repairing telomere damage and thereby compensating for faster rates of telomere attrition caused by oxidative damage (e.g., Noguera, da Silva, & Velando, 2020). Or, individuals exposed to prenatal stress cues may have elongated telomeres at hatching, which buffers them from greater rates of attrition (e.g., Noguera, da Silva, & Velando, 2020). However, developmental stressors are generally expected to shorten, rather than elongate telomere length in hatchlings (e.g., Haussman et al., 2011; Noguera & Velando, 2019; Casagrande et al., 2020). Similarly, while CORT and incubation temperature did not impact broad metrics of ROS levels and oxidative damage, this does not rule out that these animals produced more ROS and sustained greater levels of oxidative damage, but overcame them through upregulated antioxidant activity or damage repair mechanisms (e.g., superoxide dismutase, glutathione peroxidase, catalase). A shift in oxidant-antioxidant balances has been proposed as a mediator of oxidative stress responses to glucocorticoids (Costantini & Verhulst, 2009; Costantini, Marasco, & Møller, 2011). Yet, support for this hypothesis is highly variable among studies (e.g., Lin, Decuyper, & Buyse, 2004; Costantini et al., 2008; Cote et al., 2010; meta-analysis: Crino et al., 2026).

The upregulation of telomerase, antioxidant capacity, or oxidative damage repair is expected to impose energetic costs or changes in mitochondrial dynamics, and in turn induce life-history trade-offs (Nager, & Costantini, 2016; Metcalfe & Olsson, 2021). Thus, these more nuanced oxidative damage dynamics could provide a mechanistic basis for the patterns of growth and body size, but not survival, observed in *L. delicata* and *L. guichenoti*, which were not explained by increased ROS or oxidative damage *per se* (Chapter 3). Furthermore, given the correlation between mitochondrial respiration and ROS levels in *L. delicata* brains, an oxidant-antioxidant imbalance under conditions where metabolism is elevated could potentially reveal hidden developmental effects on cognition that I did not detect (Chapter 4). Beyond telomeres and oxidant-antioxidant dynamics, there are also alternative ROS and oxidative damage markers to those quantified here. I measured superoxide, because it is the primary ROS produced by mitochondria and a precursor to other ROS, and two metrics of oxidative damage (lipid peroxidation and 8-OHdG). Yet, ROS like hydrogen peroxide or hydroxyl radicals may be more relevant indicators of oxidative stress levels in cells than superoxide. Additional work quantifying telomere dynamics, antioxidant capacity, or the production of other ROS would, therefore, supplement the findings of the present work and provide a more complete understanding of the pathways underlying developmental effects and life-history trade-offs. Some of these physiological biomarkers can potentially be incorporated into the flow cytometry framework. Notably, telomere length and attrition can be quantified *via* flow cytometric analysis, with some previous work demonstrating its applicability in reptiles (Ballen et al., 2012; Olsson et al., 2018; Olsson et al., 2020). There are also both existing and emerging fluorescent probes capable of quantifying a diverse range of ROS or total ROS, which could be used alongside

superoxide probes to more fully describe the oxidative environment of cells (reviewed in Gomes, Fernandes, & Lima, 2005; Chaturvedi et al., 2025). However, quantification of antioxidant and enzyme activity currently require complementary approaches using established techniques like qPCR and enzyme assay kits.

Using flow cytometry, I was able to quantify mitochondrial membrane potential as a proxy for the overall respiratory capacity of liver, heart, blood, gonad, and brain cells. But like oxidative damage dynamics, mitochondrial respiration is a complex physiological pathway comprised of multiple interrelated mitochondrial traits. While overall respiratory capacity is a valuable metric of a cell's physiological condition, it does not capture nuances in mitochondrial function across distinct complexes of the electron transport chain. Mitochondrial efficiency – the proportion of ATP produced to oxygen consumed – is a key indicator of cellular physiological condition which cannot be captured by broad measures of respiratory capacity. Mitochondrial efficiency determines the conversion of substrates to usable energy for aerobic performance, and is mediated by the degree of proton leak across the mitochondrial membrane. Mitochondrial proton leak, in turn, regulates the production of ROS and is hypothesised to be a determinant of survival and longevity (Brand, 2000). There is potential for expanding flow cytometry methods to allow for measurement of mitochondrial membrane potential across complexes, *via* sequential addition of reagents (pyruvate, ADP) and uncoupling agents (oligomycin, antimycin). But, when possible, mitochondrial dynamics may better be quantified using established methods of high-resolution respirometry (Oroboros O2k oxygraphy) or extracellular flux analysis (SeaHorse XF Analyser). Indeed, while Chapters 3 & 4 found no effects of incubation temperature or developmental CORT on mitochondrial membrane potential, prior work on *L. delicata* using identical developmental treatments found that warm incubation temperatures reduced mitochondrial efficiency into adulthood when assessed *via* high-resolution respirometry (Crino et al., 2024b). Such differences highlight both the complexity of physiological pathways and the importance of selecting appropriate methods for their assessment. Future work integrating novel flow cytometry techniques with complementary approaches like enzyme assays and high-resolution respirometry would elucidate the complex mechanisms underlying life-history variation and developmental responses. Furthermore, an integrative approach employing both novel and established techniques would clarify how flow cytometry fits within the broader landscape of mechanistic physiology and validate their metrics against other physiological biomarkers.

Conclusion

Overall, my thesis provides robust empirical data integrating fundamental fitness traits, cognitive processes, and physiological mechanisms to test the role of simultaneous maternal effects and developmental environments on life-history variation. This work spanned two species with differing reproductive investment strategies, and incorporated a longitudinal design, naturalistic environments, and physiological responses across multiple tissue types. Simultaneous exposure to maternal stress hormones and thermal variation had immediate consequences for fitness and phenotypes, impairing hatching success and body size. But embryos were buffered from developmental effects by greater maternal investment both within- and between- species, suggesting potential directional selection if these same developmental conditions persist across generations. Longitudinal studies revealed that developmental conditions have sustained effects on growth and body size throughout life. Individuals exposed to stress hormones and warm temperatures grew rapidly early in life, thereby compensating for their reduced body sizes at hatching, but ultimately remained smaller in adulthood. However, rapid growth was not accompanied by reduced survival or longevity. These findings suggest that stressful developmental environments shape life-history trajectories but do not necessarily impose direct trade-offs between growth and survival, as expected under a “live fast, die young” framework. Physiological assays on juveniles and adults showed that broad-scale mitochondrial function and oxidative damage markers in livers and hearts were minimally affected by developmental insults. Associative learning trials and

physiological assays on brain tissue also revealed no developmental effects on cognitive processes or mitochondrial and oxidative physiology. This thesis demonstrates that developmental conditions affect a select few, but relevant, phenotypic traits in lizards. These results also highlight the potential role of alternative behavioural or physiological pathways, as well as postnatal environmental factors, in shaping life-history variation. Finally, this thesis demonstrates relatively conserved developmental responses among closely-related species with similar ecologies, but notably differ with findings from other, more-divergent taxa – especially viviparous or endothermic species. Beyond these empirical insights, the novel flow cytometry methods developed here provide a versatile, accessible tool for integrating cellular physiology into ecological and evolutionary studies, enabling future work to explore complex mechanistic questions across tissues, developmental stages, and experimental designs. Collectively, these findings advance our understanding of developmental plasticity, life-history variation, and the physiological mechanisms that drive them, while offering practical frameworks and methodological tools to guide targeted future research.

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Appendix I

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Supplemental Material for Chapter 1

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Appendix I.1: Model Comparisons

Introduction

This document describes the model selection process we used to test whether the joint effects of ‘maternal stress’ (CORT or Control), incubation temperature (23°C or 28°C), and maternal investment (initial egg mass) on the embryonic mortality and hatchling size of delicate skinks (*Lampropholis delicata*) and common garden skinks (*L. guichenoti*) were additive or interactive. For each response variable (embryonic mortality, hatchling mass, hatchling SVL), we constructed (*brms::bf()* function) six model structures in which ‘maternal stress’ (CORT), incubation temperature (Temp), maternal investment (Egg Mass), and each permutation of their two- and three-way interactions were included as fixed effects. Clutch identity was included as a random intercept in all models to account for similarities among siblings. We modelled embryonic mortality (a logical of 1 if eggs died, 0 if they hatched) assuming a Bernoulli (link ‘logit’ function) distribution. We modelled hatchling mass assuming a Gaussian distribution. We ran each model structure as a Bayesian generalized mixed-effects model (GLMM) using the *brms* and *rstan* R packages (R v. 4.2.1; *brms::brm()* function). Each model was run on parallel 4 MCMC chains for 2000 iterations with 1000 warmup iterations at an adapt delta value of 0.99. Models were run separately for each species.

For model comparisons we fit LOO criterion (*loo::fit_criterion()* function) to each GLMM using the *loo* and *brms* R packages, then compared the expected log pointwise predictive density (ELPD) and its error among models (*loo::loo_compare()* function). We selected the best model as the model with the lowest ELPD, but also considered any models whose distribution overlapped with zero as statistically indistinguishable from the best model. If multiple models were indistinguishable from each other, we considered the best model as the simplest model with the fewest interaction terms. In all cases, this defaulted to the ‘additive null model’ in which no interaction terms between CORT, Temp, and Egg Mass were included and with Clutch included as a random effect. Below, we show the outputs of each LOO comparison with short descriptions.

Embryonic Mortality

Lampropholis delicata

In initial model comparisons of *L. delicata* embryonic mortality, only the ‘additive null model’ emerged as a suitable model. However, the inclusion of an interaction term between incubation temperature and egg mass almost resulted in an indistinguishable model. Even so, this model was excluded downstream based on the additive null model having fewer interaction terms. Thus we used the additive null model including clutch as the best model of *L. delicata* embryonic mortality.

Species	Model	Δ ELPD $\pm$ S.E.
L. delicata	Egg Mortality ~ CORT + Temp + Egg Mass + (1 Clutch)	0 $\pm$ 0
	Egg Mortality ~ CORT + Temp * Egg Mass + (1 Clutch)	-1.149 $\pm$ 0.974
	Egg Mortality ~ CORT * Temp + Egg Mass + (1 Clutch)	-1.31 $\pm$ 0.85
	Egg Mortality ~ CORT * Egg Mass + Temp + (1 Clutch)	-2.141 $\pm$ 0.623
	Egg Mortality ~ (CORT + Temp + Egg Mass)^2 + (1 Clutch)	-4.21 $\pm$ 1.355
	Egg Mortality ~ CORT * Temp * Egg Mass + (1 Clutch)	-6.53 $\pm$ 1.668

Lampropholis guichenoti

In initial comparisons of models for *L. guichenoti* embryonic mortality, the model including the three-way interaction between CORT, incubation temperature, and egg mass was the best-fitting model. However, with the exception of the model including all two-way interactions, all other model structures were indistinguishable from the best-fitting model, due to high variance. This high variance is likely driven by the overall low mortality in *L. guichenoti*. Because the inclusion of interaction terms did not make the model more informative, we used the additive null model with clutch as a random effect as the model of best fit.

Species	Model	Δ ELPD $\pm$ S.E.
L. guichenoti	Egg Mortality ~ CORT * Temp * Egg Mass + (1 Clutch)	0 $\pm$ 0
	Egg Mortality ~ CORT * Egg Mass + Temp + (1 Clutch)	-2.991 $\pm$ 4.338
	Egg Mortality ~ CORT + Temp + Egg Mass + (1 Clutch)	-3.246 $\pm$ 4.323
	Egg Mortality ~ CORT + Temp * Egg Mass + (1 Clutch)	-3.485 $\pm$ 5.025
	Egg Mortality ~ CORT * Temp + Egg Mass + (1 Clutch)	-4.137 $\pm$ 4.305
	Egg Mortality ~ (CORT + Temp + Egg Mass)^2 + (1 Clutch)	-5.332 $\pm$ 4.625

Hatchling Mass

Lampropholis delicata

In comparisons of *L. delicata* hatchling mass models, all models except the one including the three-way interaction between CORT, temperature, and egg mass, were indistinguishable. Because the inclusion of any two-way interactions did not improve model fit, the additive null model including clutch as a random effect was selected as the best-fitting model of *L. delicata* hatchling mass.

Species	Model	Δ ELPD $\pm$ S.E.
L. delicata	Hatchling Mass ~ CORT + Temp * Egg Mass + (1 Clutch)	0 $\pm$ 0
	Hatchling Mass ~ CORT * Egg Mass + Temp + (1 Clutch)	-0.652 $\pm$ 1.771
	Hatchling Mass ~ CORT * Temp + Egg Mass + (1 Clutch)	-0.8 $\pm$ 1.693
	Hatchling Mass ~ CORT + Temp + Egg Mass + (1 Clutch)	-0.842 $\pm$ 1.333
	Hatchling Mass ~ (CORT + Temp + Egg Mass)^2 + (1 Clutch)	-0.868 $\pm$ 1.627
	Hatchling Mass ~ CORT * Temp * Egg Mass + (1 Clutch)	-3.047 $\pm$ 1.812

Lampropholis guichenoti

In comparisons of *L. guichenoti* hatchling mass models, the additive null model emerged as the only suitable model. Thus, the additive null model including clutch as a random effect was selected as the best-fitting model of *L. guichenoti* hatchling mass.

Species	Model	Δ ELPD $\pm$ S.E.
L. guichenoti	Hatchling Mass ~ CORT + Temp * Egg Mass + (1 Clutch)	0 $\pm$ 0
	Hatchling Mass ~ CORT * Temp * Egg Mass + (1 Clutch)	-2.289 $\pm$ 2.193
	Hatchling Mass ~ CORT * Egg Mass + Temp + (1 Clutch)	-2.41 $\pm$ 3.023
	Hatchling Mass ~ CORT * Temp + Egg Mass + (1 Clutch)	-3.254 $\pm$ 2.183
	Hatchling Mass ~ CORT + Temp + Egg Mass + (1 Clutch)	-3.788 $\pm$ 2.669
	Hatchling Mass ~ (CORT + Temp + Egg Mass)^2 + (1 Clutch)	-5.584 $\pm$ 1.852

Appendix I.2: Supplemental Tables

Supplemental Table 1. Expanded summary statistics on treatment groups of *L. delicata* and *L. guichenoti*. This is an expanded version of Table 1 from the main document. In addition to the values given in Table 1, Supplementary Table 1 includes statistics on initial egg morphology (Mass, Length, and Width) for eggs that died and hatched, as well as measures of hatchling SVL and tail length. Eggs (Died) and Eggs (Hatched) are counts of eggs that died and hatched, respectively. Egg Mortality is the proportion of eggs that died. All other values are means $\pm$ standard deviation.

Species	Trait	Control,23°C	CORT,23°C	Control,28°C	CORT,28°C
<i>L. delicata</i>	Eggs (Died)	16	21	17	32
<i>L. delicata</i>	Eggs (Hatched)	152	145	149	141
<i>L. delicata</i>	Egg Mortality	9.50%	12.70%	10.20%	18.50%
<i>L. delicata</i>	Egg Mass (All)	0.143 $\pm$ 0.028	0.145 $\pm$ 0.026	0.145 $\pm$ 0.026	0.145 $\pm$ 0.024
<i>L. delicata</i>	Egg Length (All)	7.945 $\pm$ 0.731	7.946 $\pm$ 0.59	7.89 $\pm$ 0.567	8.018 $\pm$ 0.618
<i>L. delicata</i>	Egg Width (All)	5.108 $\pm$ 0.544	5.135 $\pm$ 0.522	5.153 $\pm$ 0.558	5.097 $\pm$ 0.499
<i>L. delicata</i>	Egg Mass (Died)	0.126 $\pm$ 0.032	0.138 $\pm$ 0.024	0.142 $\pm$ 0.039	0.137 $\pm$ 0.03
<i>L. delicata</i>	Egg Length (Died)	8.124 $\pm$ 1.656	7.984 $\pm$ 0.646	8.051 $\pm$ 0.489	7.878 $\pm$ 0.592
<i>L. delicata</i>	Egg Width (Died)	4.714 $\pm$ 0.567	5.034 $\pm$ 0.355	4.895 $\pm$ 0.63	4.958 $\pm$ 0.618

<i>L. delicata</i>	Egg Mass (Hatched)	0.145 ± 0.027	0.146 ± 0.026	0.146 ± 0.024	0.147 ± 0.022
<i>L. delicata</i>	Egg Length (Hatched)	7.926 ± 0.56	7.94 ± 0.584	7.871 ± 0.574	8.05 ± 0.621
<i>L. delicata</i>	Egg Width (Hatched)	5.149 ± 0.526	5.15 ± 0.541	5.182 ± 0.544	5.128 ± 0.464
<i>L. delicata</i>	Hatchling Mass (g)	0.112 ± 0.013	0.108 ± 0.013	0.106 ± 0.012	0.105 ± 0.012
<i>L. delicata</i>	Hatchling SVL (mm)	18.549 ± 0.813	18.266 ± 0.881	18.283 ± 0.88	18.14 ± 0.879
<i>L. delicata</i>	Hatchling TL (mm)	25.331 ± 1.662	24.849 ± 1.698	25.538 ± 1.687	24.794 ± 1.993
<i>L. guichenoti</i>	Eggs (Died)	7	9	8	12
<i>L. guichenoti</i>	Eggs (Hatched)	134	127	131	132
<i>L. guichenoti</i>	Egg Mortality	5%	6.60%	5.80%	8.30%
<i>L. guichenoti</i>	Egg Mass (All)	0.182 ± 0.029	0.186 ± 0.032	0.184 ± 0.03	0.186 ± 0.036

<i>L. guichenoti</i>	Egg Length (All)	8.393 ± 0.655	8.494 ± 0.644	8.451 ± 0.683	8.541 ± 0.733
<i>L. guichenoti</i>	Egg Width (All)	5.586 ± 0.49	5.623 ± 0.487	5.596 ± 0.448	5.586 ± 0.529
<i>L. guichenoti</i>	Egg Mass (Died)	0.132 ± 0.026	0.182 ± 0.059	0.176 ± 0.046	0.169 ± 0.043
<i>L. guichenoti</i>	Egg Length (Died)	8.011 ± 0.553	8.677 ± 0.828	8.64 ± 0.58	8.619 ± 0.71
<i>L. guichenoti</i>	Egg Width (Died)	4.881 ± 0.777	5.613 ± 0.862	5.518 ± 0.534	5.388 ± 0.636
<i>L. guichenoti</i>	Egg Mass (Hatched)	0.184 ± 0.027	0.186 ± 0.029	0.185 ± 0.029	0.188 ± 0.035
<i>L. guichenoti</i>	Egg Length (Hatched)	8.413 ± 0.656	8.481 ± 0.631	8.439 ± 0.69	8.534 ± 0.737
<i>L. guichenoti</i>	Egg Width (Hatched)	5.623 ± 0.445	5.624 ± 0.455	5.601 ± 0.444	5.605 ± 0.517
<i>L. guichenoti</i>	Hatchling Mass (g)	0.142 ± 0.016	0.14 ± 0.015	0.14 ± 0.017	0.137 ± 0.017

<i>L. guichenoti</i>	Hatchling SVL (mm)	19.869 ± 0.73	19.842 ± 0.778	19.816 ± 0.865	19.656 ± 0.91
<i>L. guichenoti</i>	Hatchling TL (mm)	26.123 ± 1.941	25.967 ± 1.71	26.384 ± 2.035	26.008 ± 2.241

1 **Supplemental Table 2. Pairwise comparisons of each treatment group within each species.** Values are derived from comparisons of predicted
2 posterior distributions generated by Bayesian GLMMs (see main document and Electronic Supplementary Material – Model Comparisons). The
3 Estimate is the mean of the difference between predicted posterior distributions, Est.Error is the error about the resultant distribution, and l-95% CI
4 and u-95% CI are the lower and upper 95% confidence intervals of the distribution, respectively. The pMCMC value is a test statistic suitable for
5 testing whether two or more predicted posterior distributions overlap, with pMCMC < 0.05 indicating that the difference between distributions is
6 not equal to zero.

Trait	Species	Contrast	Estimate	Est.Error	l-95% CI	u-95% CI	pMCMC
Egg Mortality	<i>L. delicata</i>	[CORT,28°C] - [CORT,23°C]	0.366	0.003	-0.228	0.946	0.22
Egg Mortality	<i>L. delicata</i>	[CORT,28°C] - [Control,28°C]	0.696	0.003	0.109	1.328	0.02
Egg Mortality	<i>L. delicata</i>	[CORT,28°C] - [Control,23°C]	1.062	0.004	0.223	1.913	0.011
Egg Mortality	<i>L. delicata</i>	[CORT,23°C] - [Control,28°C]	0.33	0.004	-0.507	1.196	0.45
Egg Mortality	<i>L. delicata</i>	[CORT,23°C] - [Control,23°C]	0.696	0.003	0.109	1.328	0.02
Egg Mortality	<i>L. delicata</i>	[Control,28°C] - [Control,23°C]	0.366	0.003	-0.228	0.946	0.22
Egg Mortality	<i>L. guichenoti</i>	[CORT,28°C] - [CORT,23°C]	0.22	0.004	-0.561	1.004	0.578
Egg Mortality	<i>L. guichenoti</i>	[CORT,28°C] - [Control,28°C]	0.463	0.004	-0.323	1.273	0.25
Egg Mortality	<i>L. guichenoti</i>	[CORT,28°C] - [Control,23°C]	0.683	0.006	-0.413	1.78	0.215
Egg Mortality	<i>L. guichenoti</i>	[CORT,23°C] - [Control,28°C]	0.243	0.006	-0.909	1.39	0.663
Egg Mortality	<i>L. guichenoti</i>	[CORT,23°C] - [Control,23°C]	0.463	0.004	-0.323	1.273	0.25
Egg Mortality	<i>L. guichenoti</i>	[Control,28°C] - [Control,23°C]	0.22	0.004	-0.561	1.004	0.578
Hatchling Mass	<i>L. delicata</i>	[CORT,28°C] - [CORT,23°C]	-0.004	0	-0.005	-0.003	0
Hatchling Mass	<i>L. delicata</i>	[CORT,28°C] - [Control,28°C]	-0.003	0	-0.004	-0.002	0

Hatchling Mass	<i>L. delicata</i>	[CORT,28°C] - [Control,23°C]	-0.007	0	-0.009	-0.006	0
Hatchling Mass	<i>L. delicata</i>	[CORT,23°C] - [Control,28°C]	0.001	0	0	0.003	0.12
Hatchling Mass	<i>L. delicata</i>	[CORT,23°C] - [Control,23°C]	-0.003	0	-0.004	-0.002	0
Hatchling Mass	<i>L. delicata</i>	[Control,28°C] - [Control,23°C]	-0.004	0	-0.005	-0.003	0
Hatchling Mass	<i>L. guichenoti</i>	[CORT,28°C] - [CORT,23°C]	-0.003	0	-0.005	-0.001	0.001
Hatchling Mass	<i>L. guichenoti</i>	[CORT,28°C] - [Control,28°C]	-0.003	0	-0.005	-0.001	0
Hatchling Mass	<i>L. guichenoti</i>	[CORT,28°C] - [Control,23°C]	-0.006	0	-0.008	-0.004	0
Hatchling Mass	<i>L. guichenoti</i>	[CORT,23°C] - [Control,28°C]	0	0	-0.002	0.002	0.887
Hatchling Mass	<i>L. guichenoti</i>	[CORT,23°C] - [Control,23°C]	-0.003	0	-0.005	-0.001	0
Hatchling Mass	<i>L. guichenoti</i>	[Control,28°C] - [Control,23°C]	-0.003	0	-0.005	-0.001	0.001

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Supplemental Table 3. Pairwise comparisons of each treatment level between species. Values are derived from comparisons of predicted posterior distributions generated by Bayesian GLMMs (see main document and Electronic Supplementary Material – Model Comparisons). The Estimate is the mean of the difference between predicted posterior distributions, Est.Error is the error about the resultant distribution, and l-95% CI and u-95% CI are the lower and upper 95% confidence intervals of the distribution, respectively. The pMCMC value is a test statistic suitable for testing whether two or more predicted posterior distributions overlap, with pMCMC < 0.05 indicating that the difference between distributions is not equal to zero.

Trait	Treatment	Contrast	Estimate	Est.Error	l-95% CI	u-95% CI	pMCMC
Egg Mortality	CORT,23°C	[<i>L. delicata</i>] - [<i>L. guichenoti</i>]	0.586	0.007	-0.688	1.983	0.377
Egg Mortality	CORT,28°C	[<i>L. delicata</i>] - [<i>L. guichenoti</i>]	0.732	0.007	-0.492	2.085	0.257
Egg Mortality	Control,23°C	[<i>L. delicata</i>] - [<i>L. guichenoti</i>]	0.352	0.007	-1.036	1.843	0.621
Egg Mortality	Control,28°C	[<i>L. delicata</i>] - [<i>L. guichenoti</i>]	0.499	0.007	-0.89	1.993	0.5
Hatchling Mass	CORT,23°C	[<i>L. delicata</i>] - [<i>L. guichenoti</i>]	0	0	-0.002	0.003	0.893
Hatchling Mass	CORT,28°C	[<i>L. delicata</i>] - [<i>L. guichenoti</i>]	-0.001	0	-0.004	0.001	0.325
Hatchling Mass	Control,23°C	[<i>L. delicata</i>] - [<i>L. guichenoti</i>]	0	0	-0.002	0.003	0.851
Hatchling Mass	Control,28°C	[<i>L. delicata</i>] - [<i>L. guichenoti</i>]	-0.001	0	-0.004	0.001	0.358

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Appendix II

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Supplemental Material for Chapter 2

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Appendix II.1: Flow Cytometry Full Protocols

Procedures

Tissue Collection

1. Anaesthetise the animal via intraperitoneal injection of 10 μ L 10mg/mL Alfaxan.
2. If not already known, measure the animal's mass, SVL, and TL. If collecting sperm via hemipene eversion, remove the sperm with a pipette tip and add to activation media. Activate following established protocols, then dilute in 1mL cold PBS and store on ice.
3. Clip the animal's tail at ~20mm. Store the tail in cold PBS on ice.
4. Euthanise the animal via decapitation with surgical scissors. If collecting brain, store the head in cold PBS on ice. Remove brain following established protocols and store resultant tissue sample in cold PBS on ice.
5. Collect trunk blood into a microcapillary tube. Immediately blow this blood into an empty 1.5mL centrifuge tube and store on ice.
6. Remove the liver and heart from the body cavity. Rinse each tissue twice in fresh, cold PBS. Store each washed tissue in cold PBS on ice.
 - a. Separate the gallbladder from the liver by suspending the liver in a small amount of cold PBS and punching out the liver section containing the gallbladder using a clipped 1000 μ L pipette tip or a cluster tube.
 - b. If working with a larger animal, use cluster tubes to punch a standardized amount of liver or heart tissue.
7. If collecting sperm via removal of gonads or spermatophores, use a scoop to lift the tissue out of the body cavity. Rinse the tissue once in cold PBS, then store in cold PBS on ice.

Tissue Preparation

1. Tail Tips, Brains, and Hearts:
 - a. Transfer the tissue to a 1.5mL centrifuge tube containing 100 μ L of 125U/mL of pre-warmed collagenase in 1X PBS.
 - b. Using a pair of fine surgical scissors, snip the tissue into small chunks within the solution. Incubate at 32°C for 30min.
 - c. Following incubation, proceed to (2).
2. Liver and digested Tail Tips, Brains, and Hearts:
 - a. Mash the tissue through a 100 μ m mesh filter using the rubber stopper of an insulin syringe.
 - b. Rinse through the mesh filter with 1mL of cold 1X PBS.
3. Whole Blood:
 - a. Centrifuge whole blood at 7000g for 7min to separate plasma from red blood cells (RBC).
 - b. Remove the plasma with a pipette and either store in a fresh tube (if using for enzyme assays) and store at -20°C or dispose.
 - c. Resuspend the RBC in 1mL of cold 1X PBS.
4. Sperm or Gonads:
 - a. Break up sperm samples by vigorous trituration within 1mL of cold 1X PBS.

- b. If working with gonads, homogenise the tissue in the well of 100µm mesh filter using the rubber stopper of an insulin syringe. Rinse through mesh filter with 1mL of cold 1X PBS.
5. Store all tissues on ice until further processing.

Extracellular and Cell Permeant Staining

1. Centrifuge homogenised tissues at 800-1200xg for 10min to separate debris from cells. Remove and dispose of the supernatant. Resuspend the pellet in 1000µL of cold 1X PBS.
2. Centrifuge homogenised tissues at 800-1200xg for 10min to separate debris from cells. Remove and dispose of the supernatant. Resuspend the pellet in 200µL of pre-warmed 1X PBS.
3. Remove 50µL of each sample and add to pooled homogenate for each tissue type. This will be used for making single colour controls. When all homogenates for the day have been pooled, split pooled homogenate into 150µL aliquots in 1.5mL tubes for each of NEG, DR+, MTO+, SOX+, LPX+, H42+, AF488+, OXO+, and POS.
4. Add pre-warmed MitoTracker Deep Red (DR), MitoTracker Orange CMTMRos (MTO), MitoSOX Red (SOX), Hoechst 33342 (H42), and BODIPY 665/676 Lipid Peroxidation Sensor (LPX) to each sample or respective control at 1:10 (dye:sample at pre-established concentrations).
5. Incubate samples at 32°C for 30min to stain.
6. Dilute samples back to 1mL with warm 1X PBS.
7. Centrifuge samples at 800-1200xg for 10min to separate debris from cells. Remove and dispose of the supernatant. Resuspend the pellet in 200µL of warm 1X PBS.
8. Fix the samples with 70% EtOH or 10% Neutral-Buffered Formalin (NBF).
 - a. EtOH: Add a total of 600µL of warm 70% ethanol to each sample. Do this in 3 replicates of 200µL. Mix by gentle inversion between each replicate. Incubate at -20°C for 20min to fix.
 - b. NBF: Add NBF to sample at 1:10 to reach a final concentration of 1% NBF. Incubate at 32°C for 20min to fix.
9. Centrifuge samples at 800-1200xg for 10min to pellet cells. Remove supernatant. Resuspend the cells in 1mL cold 1X Tris-EDTA and 10% DMSO if freezing. Store on ice until transfer to cryoboxes. If using fresh, instead skip to Intracellular and Nuclear Staining step 2.
10. Store the sample at -20°C to freeze, then transfer to -80°C for long-term storage.

Intracellular and Nuclear Staining

1. Quickly thaw samples by briefly (~2min) submerging them in near-boiling water.
2. Centrifuge samples at 800-1200xg for 10min to pellet cells. Remove the supernatant. Resuspend the pellet in 1000µL 1X Tris-EDTA.
3. Centrifuge samples at 800-1200xg for 10min to pellet cells. Remove the supernatant. Resuspend the pellet in 200µL warm 1X PBS.
4. Add pre-warmed digitonin to all samples and controls. Incubate at 32°C for 20min.
5. Centrifuge samples at 800-1200xg for 10min to pellet cells. Remove the supernatant. Resuspend the pellet in 200µL warm 1X PBS.
6. Add 8-OHdG Polyclonal Antibody (OHdG) to all samples and respective controls at 1:10.
7. Incubate samples at 32°C for 30min.
8. Dilute samples up to 1mL in cold 1X PBS to stop permeabilisation and staining.
9. Centrifuge samples at 800-1200xg for 10min to pellet cells. Remove the supernatant. Resuspend the pellet in 200µL of pre-warmed 1X PBS.
10. Add pre-warmed Alexa Fluor 488 Conjugate Antibody (AF488) at 1:10 to each sample and respective control at 1:10.
11. Incubate samples at 32°C for 30min.

- 111 12. Dilute samples up to 1mL in cold 1X PBS to stop staining.
112 13. Centrifuge samples at 800-1200xg for 10min to pellet cells. Remove the supernatant. Resuspend
113 the pellet in 1mL of 1X PBS.
114 14. Plate samples and run within 3 days.

115 Recipes

116 1X PBS and 1X Tris-EDTA

- 117 1. Dilute stock solutions of buffer in ddH₂O to achieve 1X concentration.
118 a. PBS commonly comes in 10X stock, so dilute at 1:10
119 b. Tris-EDTA commonly comes in 100X stock, so dilute at 1:100
120 2. Store indefinitely at room temperature or in a 4°C refrigerator.

121 Cryopreservation Solution (10% DMSO + 1X Tris-EDTA)

- 122 1. Prepare a 1X buffer solution suitable for cryopreserving samples. We are using Tris-EDTA
123 because it chelates Ca⁺ ions that form from the cytotoxic properties of DMSO.
124 2. Add 99.9% DMSO to 1X buffer solution at 1:10.
125 3. Vortex vigorously to mix.
126 4. Store at room temperature until use. Ensure DMSO has been dissolved before adding to samples.

127 Collagenase (Type II)

- 128 1. Dissolve solid collagenase in room temperature or pre-warmed 1X PBS to a concentration of
129 2mg/mL (50 U/μL). Concentrated collagenase may be frozen in small aliquots for later use.
130 2. Add pre-warmed collagenase to samples in 1:10 to reach a final concentration of 5 U/μL to
131 chemically digest.
132 3. Remaining collagenase may be kept for up to one week at 4°C.

133 Digitonin

- 134 1. Dissolve solid digitonin in ddH₂O to a concentration of ~6.14mg/mL (5mM).
135 2. Vortex or triturate the solution until digitonin has completely dissolved. If digitonin still does not
136 dissolve, submerge the tube in near-boiling water for 3 minutes and vortex or triturate again.
137 3. Bring the 5mM stock solution to a 200μM intermediate solution by diluting with room-
138 temperature or pre-warmed 1X PBS in a ratio of 1:25.
139 4. Add pre-warmed digitonin intermediate solution to samples at 1:10 to permeabilise cells.
140 5. Remaining digitonin solution may be stored at 4°C for up to one week. Precipitate is likely to
141 form during storage and must be completely dissolved before use.

142 MitoTracker Deep Red FM

143 Stock Solution (1mM)

- 144 1. Thaw a 50μg vial of MitoTracker Deep Red FM and bring to room temperature.
145 2. Add 92μL of 99.9% DMSO to the 50μg vial and triturate to mix.
146 3. Use immediately to make intermediate solution, Mitochondrial Function Master Mix, or freeze in
147 small aliquots at -20°C. Do not freeze-thaw more than twice.

148 Intermediate Solution (5μM)

- 149 1. Dilute 1mM stock solution in 1X PBS at 1:200 to reach a 5μM intermediate solution.

2. Add pre-warmed intermediate solution to samples in a 1:10 ratio to reach a final concentration of 0.5 μ M.
3. Store remaining intermediate solution at 4°C and use within 1 week.

MitoTracker Orange CMTMRos

Stock Solution (1mM)

1. Thaw a 50 μ g vial of MitoTracker Orange CMTMRos and bring to room temperature.
2. Add 117 μ L of 99.9% DMSO to the 50 μ g vial and triturate to mix.
3. Use immediately to make intermediate solution, Mitochondria Function Master Mix, or freeze in small aliquots at -20°C. Do not freeze-thaw more than twice

Intermediate Solution (2.5 μ M)

1. Dilute 1mM stock solution in 1X PBS at 1:400 to reach a 2.5 μ M intermediate solution.
2. Add pre-warmed intermediate solution to samples in a 1:10 ratio to reach a final concentration of 0.25 μ M.
3. Store remaining intermediate solution at 4°C and use within 1 week.

MitoSOX Red

Stock Solution (5mM)

1. Thaw a 50 μ g vial of MitoSOX Red and bring to room temperature.
2. Add 13 μ L of 99.9% DMSO to the 50 μ g vial and triturate to mix.
3. Use immediately to make intermediate solution, Mitochondria Function Master Mix, or freeze in small aliquots at -20°C. Do not freeze-thaw more than twice

Intermediate Solution (50 μ M)

1. Dilute 5mM stock solution in 1X PBS at 1:100 to reach a 50 μ M intermediate solution.
2. Add pre-warmed intermediate solution to samples in a 1:10 ratio to reach a final concentration of 5 μ M.
3. Store remaining intermediate solution at 4°C and use within 1 week.

BODIPY 665/676 Lipid Peroxidation Sensor

Stock Solution (10mM)

1. Bring a 5mg vial of solid BODIPY 665/676 to room temperature.
2. Dissolve the contents of the 5mg vial in 111.5 μ L of warm 99% DMSO. Triturate or vortex vigorously to mix.
3. Use immediately to make intermediate solution or freeze in small aliquots at -20°C. Do not freeze-thaw more than twice.

Intermediate Solution

1. Dilute 10mM stock solution in 1X PBS at 1:100 to reach a 100 μ M intermediate solution.
2. Add pre-warmed intermediate solution to samples in a 1:10 ratio to reach a final concentration of 5 μ M.
3. Store remaining intermediate solution at 4°C and use within 1 week.

189 Hoechst 33342 Nuclear Viability Dye

- 190 1. Hoechst 33342 comes in 2mg/mL stock solution.
- 191 2. Dilute stock solution in 1X PBS at 1:50 to reach a 100µg/mL intermediate solution. Vortex or
- 192 triturate to mix.
- 193 3. Add pre-warmed intermediate solution to samples in a 1:10 ratio to reach a final concentration of
- 194 10µg/mL.
- 195 4. Remaining intermediate solution may be kept in a 4°C refrigerator for several months.

196 8-OHdG Polyclonal 1° Antibody

- 197 1. 8-OHdG Polyclonal 1° Antibody comes in 1mg/mL (3.5mM) stock solution.
- 198 2. Dilute stock solution in 1X Tris-EDTA at 1:50 to reach a 200µg/mL (70µM) intermediate
- 199 solution.
- 200 3. Add intermediate solution to samples at 1:10 to reach a final concentration of 20µg/mL (7µM).
- 201 4. Store remaining intermediate solution at 4°C and use within 1 week.

202 IgG Goat Anti-Rabbit 2° Antibody + Alexa-Fluor 488

- 203 1. IgG Goat Anti-Rabbit 2° Antibody + Alexa-Fluor 488 comes in 2mg/mL stock solution.
- 204 2. Dilute stock solution in 1X Tris-EDTA at 1:20 to each a 100µg/mL intermediate solution.
- 205 3. Add intermediate solution to samples in a 1:10 ratio to reach a final concentration of 10µg/mL.
- 206 4. Store remaining intermediate solution at 4°C and use within 1 week.

207 Mitochondrial Function Master Mix

- 208 1. Prepare stock solutions of MitoTracker Deep Red FM, MitoTracker Orange CMTMRos,
- 209 MitoSOX Red, and Hoechst 33342. You may also include BODIPY 665/676 and Hoechst 33342
- 210 in this mix if doing the full panel.
- 211 2. Dilute MitoTracker Deep Red FM stock solution with 1X PBS at 1:2.
- 212 3. Dilute MitoTracker Orange CMTMRos stock solution with 1X PBS at 1:4.
- 213 4. Combine equal parts of MitoTracker Deep Red FM, MitoTracker Orange CMTMRos, MitoSOX
- 214 Red, Hoechst 33342, and BODIPY 665/676 in a tube.
- 215 5. Dilute the stain mix with 1X PBS at 1:100.
- 216 6. Add pre-warmed Mitochondrial Function Master Mix to samples at 1:10.

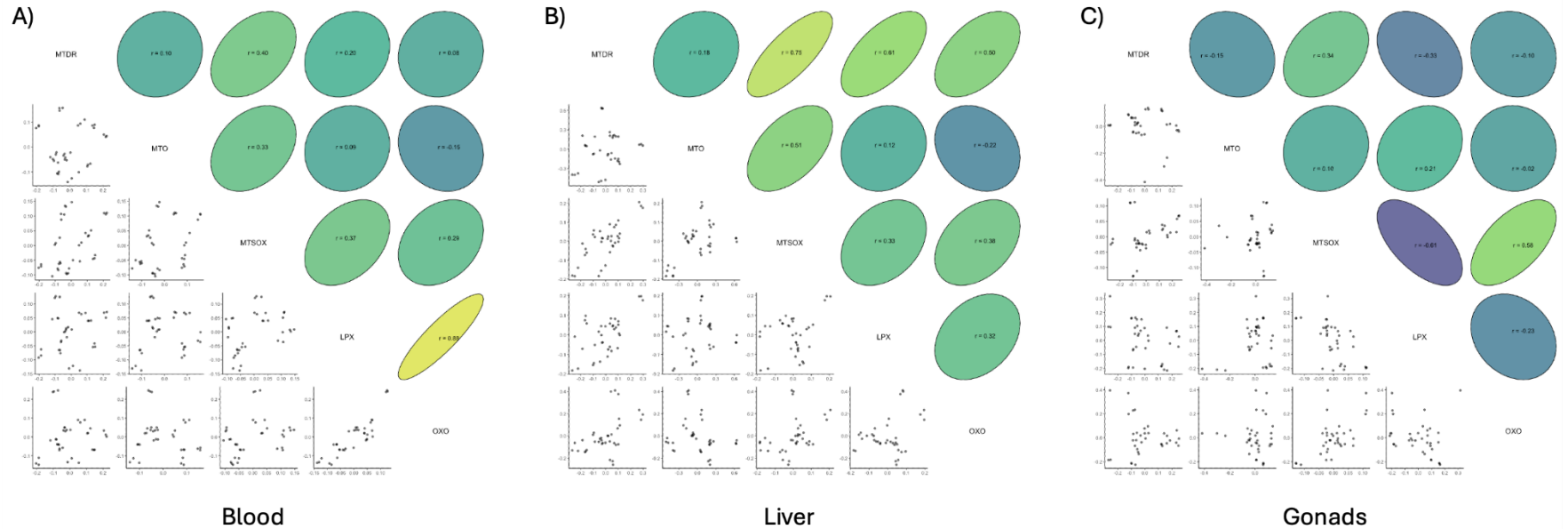
217 Appendix II.2: Supplemental Tables &
218 Figures

219

220 **Supplemental Table 1 – List of supplies used in flow cytometry preparations. For each item, the product name, supplier, and reference number are**
 221 **given, along with a short description of their usage.**

Product	Supplier	Reference	Description
BODIPY 665/676 (Lipid Peroxidation Sensor)	ThermoFisher	B3932	Stain for detecting lipid peroxidation in fixed cells.
8-OHdG/8 Hydroxyguanosine Polyclonal Antibody	ThermoFisher	BS-1278R	Primary antibody for detecting DNA damage in fixed cells. Used in conjunction with Alexa-Fluor 488 Rabbit Anti-Goat as a secondary antibody.
Rabbit anti-Goat IgG (H+L) Superclonal Recombinant Secondary Antibody, Alexa Fluor 488	ThermoFisher	A27012	Secondary antibody. Used in conjunction with the primary antibody 8-OHdG for detecting DNA damage.
MitoTracker Orange CMTMRos	ThermoFisher	M7510	Stain for detecting mitochondrial respiration.
Hoechst 33342 (10 mg/mL in Water)	ThermoFisher	H3570	Nuclear dye used for distinguishing intact cells from cellular debris.
Dimethyl Sulfoxide (DMSO), Anhydrous, >99.9%	Sigma-Aldrich	276855-100ML	Solution used as a cryoprotectant and for preparation of stains.
Tris-EDTA buffer solution (100X Concentrate)	Sigma-Aldrich	T9285-100ML	Buffer used as a cryoprotectant for preserved samples.
Formalin solution, neutral buffered, 10%	Sigma-Aldrich	HT501128	Used to fix and preserve samples.
Phosphate Buffered Saline (PBS), 10X Concentrate	Sigma-Aldrich	P5493-4L	Buffer used for general purposes and for assays on live cells.
MitoSOX Red Superoxide Indicator	ThermoFisher	M36008	Stain for detecting ROS production.
MitoTracker Deep Red FM	ThermoFisher	M22426	Stain for detecting mitochondria in sample - allows for quantifying of mitochondria number, size, and complexity.
Collagenase (Type II)	Sigma-Aldrich	C2-28-100mg	Enzyme used for light digestion of tissue samples. As of now, only used on brain samples. Unnecessary for <i>Lampropholis</i> liver and heart.
100µm Mini Strainer	PluriSelect	43-10100-76	Strainer used for homogenisation of tissues.

0.65 mL Polypropylene Cluster Tubes (Mini Tubes)	Corning Life Sciences	MTS-06-C	Small tubes for flow-cytometry assays.
Digitonin	Sigma-Aldrich	D141-100MG	Non-ionic detergent used for "soft" permeation of outer cell membranes.
96-Well Flat-Bottom Plates	ThermoFisher	167008	Flat-bottom 96-well plate for high throughput flow cytometry experiments.
1.5 mL Polypropylene Microtubes	Corning Life Sciences	MCT-150-C	Centrifuge tubes for sample preparation and storage.
30% Hydrogen Peroxide Solution	Sigma-Aldrich	323381-500ML	Concentrated hydrogen peroxide used for inducing oxidative stress in cells.



Supplemental Figure 1 – Relationships among individual physiological parameters within each of blood (A), liver (B), and gonadal (C) tissues. Each panel shows pairwise relationships among the mean fluorescent intensities of fluorescent probes detecting each of mitochondrial density (MTDR), mitochondrial membrane potential (MTO), mitochondrial superoxide (MTSOX), lipid peroxidation (LPX), and oxidative DNA damage (8-OHdG; OXO). The bottom left half of each panel shows the fluorescent values on each axis of individual replicates of each sample (three replicates per tissue per lizard), while the top right half show the strength of the correlation among these axes.

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Appendix III

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Supplemental Material for Chapter 3

Appendix III.1: Model Comparisons

Overview

This supplemental document describes a model selection process for Chapter 3. This model selection process was conducted to determine model structures for the main analysis of developmental stress exposure (CORT treatment) and incubation temperature on life-history traits. As part of model selection, we considered additional parameters that potentially influenced life-history traits, such as the population density in the naturalistic enclosures in which animals were housed (either low or high density), and the sex of the individual animal.

Introduction

Eggs of *Lampropholis delicata* and *Lampropholis guichenoti* were treated with corticosterone (CORT) or an ethanol control (Control) as eggs, then incubated at either cool (23°C) or warm (28°C) thermal regimes throughout embryonic development. These treatments simulated, respectively, variation in exposure to maternal stress hormones and variation in nest thermal environments. At hatching, we measured the mass and snout-vent-length (SVL) of lizards. Lizards were briefly raised in laboratory conditions before being transferred to naturalistic outdoor enclosures. In outdoor enclosures, lizards were kept in mixed-species populations, which were either low density (N = 16 total lizards; 8 of each species) or high density (N = 32 total lizards; 16 of each species). Throughout the subsequent two years, we performed exhaustive searches of each enclosure for lizards, and lizards were remeasured for their mass and SVL, as well as their sex determined via hemipene eversion. The final remeasurement was conducted at the end of the second full reproductive season, approximately 750 days post-hatching, or about 75% of the expected lifespan of these species in natural settings. Lizards not captured during either of the last two remeasurements were considered as having died, which was used to assess the effects of developmental treatments on long-term survival.

We primarily aimed to test how exposure to multiple stressors during embryonic development impacted key traits throughout the lifespan of *L. delicata* and *L. guichenoti* housed in naturalistic outdoor enclosures. We therefore directly tested the effects of developmental CORT exposure and incubation temperature on hatchling body size, growth, adult body size, and survival. Because CORT exposure and incubation temperature may affect shared physiological pathways, we assessed whether CORT and incubation temperature interacted to affect body size, growth, and survival.

We also considered how the postnatal environment – in this case, variation in population density – affected growth and survival, both independently and through potential interactions with prenatal stressors. Prenatal stress exposure may function to prepare individuals for stressful postnatal environments thereby improving performance in high density enclosures, but may be potentially maladaptive in low density enclosures, or may be a constraint that reduces individual performance regardless of postnatal environment quality. Thus, the context of the postnatal environment may alter the effects of developmental stressors in an independent or interactive manner.

Finally, we considered how patterns of sexual size dimorphism and differences in life-history strategy among sexes may impact growth, body size, and survival in these species, and that the effects of developmental stressors may be sex-specific. Sexual size dimorphism, as is observed in *L. guichenoti*, can cause disparity in body sizes between males and females, with potential differences in growth patterns, regardless of developmental stress exposure. Similarly, sex differences in life-history strategy may lead to alternative patterns of longevity, with one sex being more long-lived than the other. Sexual size

dimorphism or sex differences in life-history strategy may also lead to sex-specific effects of developmental stressors, particularly if the larger body sizes, faster growth rates, or greater longevity of one sex buffers that sex from developmental effects. Thus, we assessed whether sex independently influenced growth, size, and survival, or whether sex interacted with developmental CORT and incubation temperature to drive life-history trajectories.

Model Selection

To assess the contributions of developmental CORT exposure, incubation temperature, population density, and sex, along with any potential interactions, to patterns of body size, growth, and survival, we employed a hypothesis-driven model selection approach. For each of these traits, we compared among models including at least the independent main effects of developmental CORT treatment and incubation temperature, along with models including additional covariates of sex and population density, and any potential 2- or 3-way interactions between developmental treatments and additional covariates. Models for comparison were constructed in a Bayesian regression modelling framework using the *brms* and *rstan* R packages. Each model for comparison was run for 2000 iterations on 4 parallel Markov Chain Monte Carlo chains, with a warmup period of 1000 iterations, at an adapt delta of 0.99 and thinning of 1. We modelled hatchling size, growth, and adult size assuming a Gaussian distribution. Because survival is a logical variable, we instead modelled survival assuming a Bernoulli (link = “logit”) distribution.

Models were compared by the estimated log pointwise predictive density (ELPD) values of each model fit via leave-one-out (LOO) comparisons using the *loo* R package. The ELPD value estimates the accuracy of a model in predicting new data points based on the input dataset. We report Δ ELPD values for each model comparison, which are the difference in ELPD values between each candidate model and the top performing model, and the resulting standard error (SE) about the distribution of each model comparison. We considered multiple models to be indistinguishable in their predictive accuracy if the $|\Delta \text{ELPD}| < 2 \text{ SE}$, as this suggests the distributions of the two models’ predictive accuracy overlap. In cases where multiple models were indistinguishable, we selected the simplest model containing the fewest interaction terms or additional covariates as the best fitting model. Models were run and compared separately for *L. delicata* and *L. guichenoti*, thereby allowing for selected models to most accurately capture the biology of each species. Results of model comparisons are summarised in Supplementary Tables in this document.

Developmental Length

We tested whether developmental stressors influenced the length of development in *L. delicata* and *L. guichenoti*. Incubation temperature is known to exert strong influence over the amount of time to hatch, due to its pervasive influences on biological rates like metabolism, thus was included as a predictor in all models for comparison. Developmental CORT exposure may also affect time to hatching, as it is known to also induce faster developmental rates post-hatching. Alternatively, CORT exposure may prolong developmental length through depressing physiological pathways involved in embryonic development. We also considered that the size of each egg may affect how long that egg takes to hatch, owing to larger eggs having greater yolk resources to metabolise. Finally, because CORT and temperature may both impact the rate of yolk metabolism and subsequently developmental length, we tested for interactions between egg mass and developmental treatments. To account for similarities in developmental length among siblings, we included clutch as a random intercept in all models for comparison.

As a baseline (1), we modelled developmental length as a function of only incubation temperature, excluding CORT treatment and egg mass. We included this model as a baseline under the premise that the effect of incubation temperature is likely to explain most variation in developmental length, regardless of CORT treatment or egg size. We compared against models also including (2) CORT

treatment as an independent covariate and (3) both CORT and initial egg mass as independent covariates. These models tested whether the inclusion of additional predictors improved predictive accuracy over incubation temperature alone. We compared against a model including (4) the interaction between CORT and incubation temperature with egg mass as an independent effect. This model tests whether the effects of CORT exposure depend on incubation temperature, as might be expected if temperature alters the rate of CORT metabolism. We also tested for all (5) two-way interactions and (6) the three-way interaction between CORT, temperature, and egg mass. The inclusion of interactions between treatment and egg mass aimed to test whether egg size ameliorated or exacerbated the effects of developmental treatments on development length.

Supplemental Table 1. Summary of model comparisons for developmental length (hatch time) in *L. delicata*.

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. delicata</i>	Hatch Time $\sim$ CORT + Temp + Egg Mass + (1 Clutch)	0 ± 0
	Hatch Time $\sim$ (CORT + Temp + Egg Mass) ² + (1 Clutch)	-0.922 ± 2.731
	Hatch Time $\sim$ CORT * Temp + Egg Mass + (1 Clutch)	-1.023 ± 0.746
	Hatch Time $\sim$ CORT * Temp * Egg Mass + (1 Clutch)	-1.881 ± 2.877
	Hatch Time $\sim$ CORT + Temp + (1 Clutch)	-4.954 ± 3.33
	Hatch Time $\sim$ Temp + (1 Clutch)	-11.81 ± 4.868

Supplemental Table 2. Summary of model comparisons for developmental length (hatch time) in *L. guichenoti*.

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. guichenoti</i>	Hatch Time $\sim$ (CORT + Temp + Egg Mass) ² + (1 Clutch)	0 ± 0
	Hatch Time $\sim$ CORT + Temp + Egg Mass + (1 Clutch)	-1.119 ± 2.416
	Hatch Time $\sim$ CORT * Temp + Egg Mass + (1 Clutch)	-2.448 ± 2.515
	Hatch Time $\sim$ CORT * Temp * Egg Mass + (1 Clutch)	-3.853 ± 3.464
	Hatch Time $\sim$ CORT + Temp + (1 Clutch)	-3.862 ± 3.872
	Hatch Time $\sim$ Temp + (1 Clutch)	-6.922 ± 4.109

Model comparisons of developmental length found that for *L. delicata*, models including both CORT treatment and initial egg mass improved predictive accuracy over models not including CORT or egg mass. However, model accuracy was not improved by the addition of interaction terms between incubation temperature, CORT treatment, and egg mass. This generally supports that CORT treatment and variation in egg mass influence the length of development. By contrast, models of *L. guichenoti* developmental length were indistinguishable between those including CORT and egg mass and the baseline model including only incubation temperature as a predictor. Thus, for *L. guichenoti*, there is little

support that CORT or initial egg mass, nor their interactions with incubation temperature influenced the length of development. The differences in model performance between *L. delicata* and *L. guichenoti* may be reflective of their differences in reproductive strategy. The relatively larger eggs of *L. guichenoti* are potentially more robust to the effects of CORT treatment or variation in egg mass, due to the dilution or incomplete metabolism of CORT in the yolk, and because embryos do not necessarily utilise the entire yolk mass for development. Thus, for our main analysis of developmental length, we consider the effects of CORT and egg mass for *L. delicata*, but only the effect of incubation temperature for *L. guichenoti*.

Hatchling Size

We tested the immediate effects of developmental stressors on hatchling body size. Hatchling body size was modelled as a multiple-response model including hatchling mass (g) and SVL (mm) as the response variables, and accounting for residual correlations between mass and SVL. We also aimed to address potential sex differences in hatchling body size or developmental stress effects, as well as the influence of maternal investment in egg size on the size of the resultant hatchlings. To account for any similarities among siblings which was not resolved by our split-clutch experimental design, we included the clutch identity as a random effect in all models during model comparisons. We selected the model which best fit hatchling body size using an iterative process wherein we first tested for the inclusion of CORT treatment, incubation temperature, and egg size as main effects, along with any potential interactions between main effects. Then, we used the selected model from the first iteration to compare against models including sex as an independent covariate or interactive effect.

In the first iteration of model selection for hatchling size, the baseline model (1) predicted hatchling size as a function of only initial egg mass, thereby testing whether all variation in hatchling size can be accounted for by variation in maternal investment. Egg mass was included as a covariate in all models for comparison, to account for the strong positive correlation between egg size and hatchling size. We compared the baseline model for hatchling size against candidate models which included (2) CORT treatment and incubation temperature as independent main effects or (3) an interaction between CORT treatment and incubation temperature. These models aimed to test whether CORT and incubation temperature directly and additively affect hatchling size, or whether the effects of incubation temperature and CORT interact, potentially through the effects of temperature on the metabolism of CORT. Similarly, we tested whether the size of eggs influenced the effects of CORT and incubation temperature by comparing models including (4) all two-way interactions and (5) the three-way interaction between CORT, incubation temperature, and egg mass. CORT may be diluted in larger eggs due to the high yolk volume, thereby reducing or slowing CORT exposure, and larger eggs may be buffered from thermal variation, thus egg size may influence the effects of CORT and incubation temperature.

Supplemental Table 3. Summary of first iteration of model comparisons for hatchling size in *L. delicata*.

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. delicata</i>	Hatchling Size $\sim$ CORT + Temp + Egg Mass + (1 Clutch)	0 ± 0
	Hatchling Size $\sim$ CORT * Temp + Egg Mass + (1 Clutch)	-4.747 ± 0.986
	Hatchling Size $\sim$ (CORT + Temp + Egg Mass) ² + (1 Clutch)	-6.111 ± 2.029
	Hatchling Size $\sim$ CORT * Temp * Egg Mass + (1 Clutch)	-9.158 ± 2.69
	Hatchling Size $\sim$ Egg Mass + (1 Clutch)	-23.518 ± 6.052

Supplemental Table 4. Summary of first iteration of model comparisons for hatchling size in *L. guichenoti*.

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. guichenoti</i>	Hatchling Size $\sim$ CORT + Temp + Egg Mass + (1 Clutch)	0 ± 0
	Hatchling Size $\sim$ (CORT + Temp + Egg Mass) ² + (1 Clutch)	-1.812 ± 3.006
	Hatchling Size $\sim$ CORT * Temp + Egg Mass + (1 Clutch)	-3.426 ± 2.001
	Hatchling Size $\sim$ CORT * Temp * Egg Mass + (1 Clutch)	-5.824 ± 3.557
	Hatchling Size $\sim$ Egg Mass + (1 Clutch)	-9.008 ± 4.383

In the second iteration of model selection, we tested for the inclusion of sex as a predictor of hatchling size. The preferred model from the first iteration (1) included CORT treatment, incubation temperature, and egg mass as independent predictor variables with no interactions, thus this was used as our baseline model in the second iteration. We compared the baseline model against a model (2) also including sex as independent covariate, thereby testing for the presence of overall sex differences in body size at hatching. We also tested whether the independent effects of CORT and incubation temperature were sex-specific by comparing against a model (3) including the interactions between CORT treatment and sex, and incubation temperature and sex. Because the previous iteration of model selection did not support the inclusion of an interaction between CORT treatment and incubation temperature, we did not compare against models including this interaction in the second iteration.

Supplemental Table 5. Summary of second iteration of model comparisons for hatchling size in *L. delicata*.

Species	Model	Δ ELPD $\pm$ S.E.
L. delicata	Hatchling Size $\sim$ CORT + Temp + Egg Mass + (1 Clutch)	0 $\pm$ 0
	Hatchling Size $\sim$ CORT + Temp + Sex + Egg Mass + (1 Clutch)	-4.221 $\pm$ 2.365
	Hatchling Size $\sim$ (CORT + Temp) * Sex + Egg Mass + (1 Clutch)	-8.874 $\pm$ 3.059

Supplemental Table 6. Summary of second iteration of model comparisons for hatchling size in *L. guichenoti*.

Species	Model	Δ ELPD $\pm$ S.E.
L. guichenoti	Hatchling Size $\sim$ CORT + Temp + Sex + Egg Mass + (1 Clutch)	0 $\pm$ 0
	Hatchling Size $\sim$ CORT + Temp + Egg Mass + (1 Clutch)	-1.227 $\pm$ 2.502
	Hatchling Size $\sim$ (CORT + Temp) * Sex + Egg Mass + (1 Clutch)	-3.945 $\pm$ 1.562

Model selection revealed that for both *L. delicata* and *L. guichenoti*, the model with the greatest accuracy in predicting hatchling size included CORT treatment, incubation temperature, and initial egg mass as independent main effects. This model structure is identical to the preferred model structure of hatchling size found during model selection in a previous study on the larger colony of *L. delicata* and *L. guichenoti* from which our population is a subset. Models including the two- and three-way interactions between CORT treatment, incubation temperature, and egg mass did not improve model performance over those excluding these interactions. Similarly, models including sex as an independent covariate performed as well as those excluding sex entirely, suggesting that sex offered limited information in predicting hatchling size. Thus, sexual size dimorphism is likely not present at hatching in either *L. delicata* or *L. guichenoti*. Models which included the two-way interactions between sex and CORT treatment, and sex and incubation temperature, did not perform as well as models not including these interactions. This suggests that our data does not support developmental effects on hatchling size being sex-specific.

Growth Curve

Following from hatchling size, we aimed to test how developmental stressors influence growth trajectories over the lifespan of *L. delicata* and *L. guichenoti*. We modelled growth trajectories using the Von Bertalanffy Growth Model (VBGM), which uses the body size of animals at given ages as input to estimate growth parameters t_0 , K , and L_{inf} . Parameter t_0 is a hypothetical age (in days, for this study) at which the animal's size would be equal to zero, and thereby sets the intercept (or starting size) of the growth curve. The parameter K is the growth rate coefficient (in days⁻¹), which represents the rate at which animals grow as they approach the size at which their growth asymptotes (L_{inf}). The parameter L_{inf} , or asymptotic size, is therefore the predicted maximum body size animals are expected to reach given t_0 and K . In a Bayesian framework, a VBGM is modelled as a multiple response model for which predictor variables are defined separately for each of t_0 , K , and L_{inf} , and the size of the animal is modelled following the equation:

$$Size \sim Linf * (1 - e^{-k*(age-t_0)})$$

We aimed to test whether developmental CORT treatment, incubation temperature, population density, and sex modified the growth trajectories of *L. delicata* and *L. guichenoti*. To determine the model structure to be used in the main analysis of growth, we used a two-step model comparison approach. In the first round of model comparisons, we tested whether the predictive accuracy of VBGMs was improved by including interactions between CORT treatment and incubation temperature, the independent effect of population density, and the interactions between population density and developmental treatments. In the second round of model comparisons, we tested for the inclusion of sex as an additional independent or interactive effect. Individual was included as a random intercept in all models to account for repeated measurements of the same animal.

For all candidate growth models, we modelled K and Linf as a function of the specified main effects, but modelled t0 using a simple intercept model. This modelling structure allows for estimating growth trajectories accounting for variation in initial body size. We defined predictive prior distributions for each of t0, K, and Linf to improve VBGM predictive accuracy. Priors for K and Linf were set based on growth patterns observed in the raw data. The prior distribution for K assumed that animal growth asymptotes at 500 days post-hatching, which is roughly equal to the end of the first full reproductive season. The prior distribution for Linf assumed that animals reach a mass of 1-2 g and SVL of 40-50 mm in adulthood. Because t0 is largely a modelling artefact and was modelled following a simple intercept model, prior distributions of t0 were centred about 0 and allowed for variation of ~1 day. We modelled and compared growth in mass and growth in SVL separately, rather than as a multiple response model, to reduce model complexity.

In the first round of model comparisons, we tested whether interactions between CORT treatment and incubation temperature, and the inclusion of population density as an independent or interactive covariate, improved the accuracy of predicting K and Linf. The baseline model (1) estimated K and Linf using only the independent effects of CORT treatment and incubation temperature. We compared the baseline model against a similar model (2) including the interaction between CORT treatment and incubation temperature. We also tested against models including population density as an independent covariate either (3) with no interactions between CORT and incubation temperature or (4) with an interaction between CORT and incubation temperature. Models (3) and (4) therefore tested whether population density independently influence growth regardless of developmental treatments, potentially reflecting overall limited resources for sustaining growth in high density enclosures. Developmental effects may depend on population density. For example, if developmental conditions impact an individual's competitive ability to acquire food resources or suitable thermal habitat, the effects of developmental CORT treatment or incubation temperature may be exacerbated in competitive high-density environments. Therefore, we also compared against a model (5) including all 2- and 3-way interactions between CORT treatment, incubation temperature, and population density.

Supplemental Table 7. Summary of first iteration of model comparisons for growth curves (mass) in *L. delicata*.

Species	Model	Δ ELPD $\pm$ S.E.
L. delicata	Growth (Mass) $\sim$ CORT + Temp + Density + (1 Lizard)	0 ± 0
	Growth (Mass) $\sim$ CORT * Temp + Density + (1 Lizard)	-5.919 ± 2.698
	Growth (Mass) $\sim$ CORT * Temp + (1 Lizard)	-6.123 ± 2.637
	Growth (Mass) $\sim$ CORT + Temp + (1 Lizard)	-8.476 ± 3.571
	Growth (Mass) $\sim$ CORT * Temp * Density + (1 Lizard)	-47.863 ± 22.961

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462 **Supplemental Table 8. Summary of first iteration of model comparisons for growth curves (mass) in**
463 ***L. guichenoti*.**

Species	Model	Δ ELPD $\pm$ S.E.
L. guichenoti	Growth (Mass) $\sim$ CORT + Temp + (1 Lizard)	0 ± 0
	Growth (Mass) $\sim$ CORT * Temp + Density + (1 Lizard)	-1.205 ± 1.885
	Growth (Mass) $\sim$ CORT * Temp + (1 Lizard)	-1.742 ± 2
	Growth (Mass) $\sim$ CORT * Temp * Density + (1 Lizard)	-3.315 ± 2.557
	Growth (Mass) $\sim$ CORT + Temp + Density + (1 Lizard)	-3.87 ± 1.952

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465 **Supplemental Table 9. Summary of first iteration of model comparisons for growth curves (SVL) in**
466 ***L. delicata*.**

Species	Model	Δ ELPD $\pm$ S.E.
L. delicata	Growth (SVL) $\sim$ CORT + Temp + (1 Lizard)	0 ± 0
	Growth (SVL) $\sim$ CORT * Temp + (1 Lizard)	-3.145 ± 0.511
	Growth (SVL) $\sim$ CORT + Temp + Density + (1 Lizard)	-10.265 ± 3.285
	Growth (SVL) $\sim$ CORT * Temp + Density + (1 Lizard)	-13.918 ± 3.407
	Growth (SVL) $\sim$ CORT * Temp * Density + (1 Lizard)	-21.937 ± 3.856

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468 **Supplemental Table 10. Summary of first iteration of model comparisons for growth curves (SVL)**
469 **in *L. guichenoti*.**

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. guichenoti</i>	Growth (SVL) $\sim$ CORT + Temp + (1 Lizard)	0 $\pm$ 0
	Growth (SVL) $\sim$ CORT + Temp + Density + (1 Lizard)	-2.236 $\pm$ 2.093
	Growth (SVL) $\sim$ CORT * Temp + (1 Lizard)	-3.245 $\pm$ 0.527
	Growth (SVL) $\sim$ CORT * Temp + Density + (1 Lizard)	-4.299 $\pm$ 2.2
	Growth (SVL) $\sim$ CORT * Temp * Density + (1 Lizard)	-11.147 $\pm$ 2.683

During the second round of growth model comparisons, we tested for the inclusion of sex differences in growth trajectories. We compared models including sex against the best performing model from the first round of growth model comparisons. The reference model (1) included CORT treatment, incubation temperature, and population density as independent main effects. We compared against a model (2) including sex as an independent covariate and excluding density as a main effect. The purpose of this model was to test whether any effects of population density could also be reasonably explained by imbalances in sex ratios among enclosures. We also compared against a model (3) including both population density and sex as independent covariates alongside CORT treatment and incubation temperature. To test whether the effects of CORT treatment and incubation temperature were sex specific, we also compared against models that included the two-way interactions between CORT and sex, and between incubation temperature and sex. Models including interactions between sex and developmental treatments either (4) included population density as a covariate or (5) excluded population density from the main effects. We did not compare against models including any interactions between CORT treatment and incubation temperature, as this interaction was not supported in the first round of model comparisons. We also did not test for any interactions between sex and population density to limit model complexity.

Supplemental Table 11. Summary of second iteration of model comparisons for growth curves (mass) in *L. delicata*.

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. delicata</i>	Growth (Mass) $\sim$ CORT + Temp + Density + Sex + (1 Lizard)	0 $\pm$ 0
	Growth (Mass) $\sim$ CORT + Temp + Density + (1 Lizard)	-0.2 $\pm$ 3.578
	Growth (Mass) $\sim$ CORT + Temp + Sex + (1 Lizard)	-0.974 $\pm$ 2.975
	Growth (Mass) $\sim$ (CORT + Temp) * Sex + (1 Lizard)	-4.706 $\pm$ 2.921
	Growth (Mass) $\sim$ (CORT + Temp) * Sex + Density + (1 Lizard)	-5.54 $\pm$ 2.502

Supplemental Table 12. Summary of second iteration of model comparisons for growth curves (mass) in *L. guichenoti*.

Species	Model	Δ ELPD $\pm$ S.E.
L. guichenoti	Growth (Mass) $\sim$ CORT + Temp + Density + (1 Lizard)	0 $\pm$ 0
	Growth (Mass) $\sim$ (CORT + Temp) * Sex + Density + (1 Lizard)	-1.728 $\pm$ 3.293
	Growth (Mass) $\sim$ (CORT + Temp) * Sex + (1 Lizard)	-4.415 $\pm$ 3.435
	Growth (Mass) $\sim$ CORT + Temp + Sex + (1 Lizard)	-5.307 $\pm$ 3.599
	Growth (Mass) $\sim$ CORT + Temp + Density + Sex + (1 Lizard)	-5.632 $\pm$ 3.16

Supplemental Table 13. Summary of second iteration of model comparisons for growth curves (SVL) in *L. delicata*.

Species	Model	Δ ELPD $\pm$ S.E.
L. delicata	Growth (SVL) $\sim$ CORT + Temp + (1 Lizard)	0 $\pm$ 0
	Growth (SVL) $\sim$ CORT + Temp + Sex + (1 Lizard)	-7.343 $\pm$ 2.545
	Growth (SVL) $\sim$ CORT + Temp + Density + (1 Lizard)	-9.984 $\pm$ 3.301
	Growth (SVL) $\sim$ (CORT + Temp) * Sex + (1 Lizard)	-12.811 $\pm$ 3.041
	Growth (SVL) $\sim$ CORT + Temp + Sex + Density (1 Lizard)	-16.529 $\pm$ 4.083
	Growth (SVL) $\sim$ (CORT + Temp) * Sex + Density + (1 Lizard)	-22.393 $\pm$ 4.35

Supplemental Table 14. Summary of second iteration of model comparisons for growth curves (SVL) in *L. guichenoti*.

Species	Model	Δ ELPD $\pm$ S.E.
L. guichenoti	Growth (SVL) $\sim$ CORT + Temp + Sex + (1 Lizard)	0 $\pm$ 0
	Growth (SVL) $\sim$ CORT + Temp + Sex + Density (1 Lizard)	-1.061 $\pm$ 1.88
	Growth (SVL) $\sim$ (CORT + Temp) * Sex + (1 Lizard)	-6.039 $\pm$ 0.734
	Growth (SVL) $\sim$ (CORT + Temp) * Sex + Density + (1 Lizard)	-7.939 $\pm$ 2.038
	Growth (SVL) $\sim$ CORT + Temp + Density + (1 Lizard)	-16.732 $\pm$ 6.435
	Growth (SVL) $\sim$ CORT + Temp + (1 Lizard)	-16.973 $\pm$ 6.128

Comparisons of VBGM suggested different preferred model structures for each of *L. delicata* and *L. guichenoti*, and the preferred structure differed by whether we were modelling growth in body mass or growth in SVL. For *L. delicata*, model selection preferred that growth in mass was modelled as a function

of the independent effects of CORT treatment, incubation temperature, and population density, with no interactions and with sex excluded as a covariate. However, for *L. delicata* growth in SVL, neither sex nor density were included as covariates. For *L. guichenoti*, growth in mass was best predicted as a function of only the independent effects of CORT treatment and incubation temperature, without the inclusion of sex or density as predictor variables. However, exploration of the raw growth and body size data suggests that density and sex do influence growth trajectories, thus we performed a secondary analysis of *L. guichenoti* growth including only density and sex as independent main effects. The inclusion of sex improved the predictive accuracy of growth in SVL for *L. guichenoti*. Differences in preferred model structures of growth for *L. delicata* and *L. guichenoti* likely reflect the differences in biology of each species, and differences in model structures between growth in mass and SVL potentially indicate differential effects of pre- and postnatal environments on storage (mass) versus structural growth (SVL).

Sex was not included in preferred models of growth in both mass and SVL for *L. delicata*. The exclusion of sex from models indicates that an individual's sex offers limited information in accurately predicting that individual's growth, which likely reflects a general lack of sexual size dimorphism in *L. delicata*. By contrast, sex was clearly informative in predicting the growth in SVL of *L. guichenoti*, and patterns of sex differences in mass from the raw data were clear enough to justify a secondary analysis directly testing for sex differences. The relevance of sex to growth and body size is consistent with the known biology of *L. guichenoti*, which displays female-biased sexual size dimorphism in adulthood. However, the lack of interactions between sex and developmental treatments suggests that despite overall sex differences in growth, the effects of CORT treatment and incubation temperature on growth are not sex-specific.

Population density was only included as a covariate in models of *L. delicata* growth in mass, but not SVL, and not for *L. guichenoti*. However, like sex, differences in *L. guichenoti* mass between high and low population density were clear enough in the raw data to directly test for density effects on growth in a secondary analysis. That density was informative in VBGM of *L. delicata* growth, but not *L. guichenoti*, suggests that population density exerts more influence on the growth of *L. delicata* than *L. guichenoti*. This difference potentially reflects differences in competitive ability between *L. delicata* and *L. guichenoti*, consistent with the general size disparity between the two species – *L. guichenoti* is larger-bodied than *L. delicata*, which typically corresponds with greater competitive ability. Thus, *L. delicata* growth is likely more impacted by high density environments as they face both high interspecific and intraspecific competition. That population density was relevant to growth in mass, but not SVL, may indicate that structural growth (SVL) is more robust or inflexible in response to postnatal environmental conditions than is the storage of body mass.

Adult Size

In addition to hatchling size and growth trajectories, we wanted to determine whether the effects of developmental CORT exposure and incubation temperature persisted throughout life to affect size at adulthood. We also aimed to test whether CORT and incubation temperature independently or interactively affected adult size. We also tested whether the postnatal environment, in this case population density, directly affected adult size or interacted with developmental treatments. Finally, we tested whether any long-term effects of developmental CORT and incubation temperature were sex-specific, or if there were overall size differences among sexes regardless of developmental conditions. To identify the effects which best explained adult size in *L. delicata* and *L. guichenoti*, we employed an iterative model comparison approach which first tested whether population density informed adult size, and whether there were any interactions between population density, CORT treatment, and incubation temperature. We then compared the best performing models from among these against models including sex as an independent or interactive covariate.

Like hatchling size, adult size was treated as a multiple response variable comprised of both mass and SVL, and accounting for residual correlations between mass and SVL. Unlike hatchling size, however, there was variation in the specific age of animals within the remeasure period, with animals ranging from 730-820 days old. Because growth is indeterminate in *L. delicata* and *L. guichenoti*, thus older animals are generally larger, we accounted for variation in age among adults by including age (in days; centred about the mean age) as an independent covariate in all models of adult size. To account for any persistent similarities in body size among siblings, we also included clutch identity as a random intercept in all models for comparison.

In the first round of model comparisons we tested for the inclusion of an interaction between CORT and incubation temperature, and whether population density directly affected adult size or interacted with CORT and incubation temperature to affect adult size. As a baseline model (1) we included the independent main effects of CORT treatment and incubation temperature. We then compared against a model (2) including an interaction between CORT and incubation temperature. To test for direct effects of population density on adult size, potentially through reducing availability of food resources for sustained growth, we included models including density as an independent covariate, either (3) with CORT and incubation temperature independent or (4) with CORT and incubation temperature interacting. Early developmental effects on adult size may depend on the postnatal environment, owing to preparatory adaptive responses or by reducing competitive performance, thus we also compared against a model (5) including the two-way interactions between CORT treatment or incubation temperature and population density.

Supplemental Table 15. Summary of first iteration of model comparisons for adult size (mass and SVL) in *L. delicata*.

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. delicata</i>	Adult Size ~ CORT + Temp + Density + Age + (1 Clutch)	0 $\pm$ 0
	Adult Size ~ CORT * Temp + Density + Age + (1 Clutch)	-1.765 $\pm$ 0.991
	Adult Size ~ (CORT + Temp) * Density + Age + (1 Clutch)	-4.179 $\pm$ 1.073
	Adult Size ~ CORT + Temp + Age + (1 Clutch)	-16.688 $\pm$ 5.816
	Adult Size ~ CORT * Temp + Age + (1 Clutch)	-17.956 $\pm$ 5.992

Supplemental Table 16. Summary of first iteration of model comparisons for adult size (mass and SVL) in *L. guichenoti*.

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. guichenoti</i>	Adult Size $\sim$ CORT + Temp + Density + Age + (1 Clutch)	0 $\pm$ 0
	Adult Size $\sim$ CORT * Temp + Density + Age + (1 Clutch)	-2.398 $\pm$ 1.092
	Adult Size $\sim$ (CORT + Temp) * Density + Age + (1 Clutch)	-4.241 $\pm$ 1.697
	Adult Size $\sim$ CORT + Temp + Age + (1 Clutch)	-49.976 $\pm$ 7.788
	Adult Size $\sim$ CORT * Temp + Age + (1 Clutch)	-51.1 $\pm$ 7.716

573

574 For the second round of adult size model comparisons, we tested for independent effect of sex on
575 adult body size, or for sex-specific effects of developmental treatments on adult body size. For both *L.*
576 *delicata* and *L. guichenoti*, the preferred model from the first round of model comparisons (1) included
577 the independent main effects of CORT treatment, incubation temperature, and population density, with no
578 interactions. We compared against an otherwise identical model (2) including sex as an additional
579 independent covariate, thereby testing for overall sex differences in adult body size. We also tested
580 whether to account for sex-specific effects of CORT treatment and incubation temperature by comparing
581 against a model (3) which included the two-way interactions between CORT treatment or incubation
582 temperature and sex. Because an interaction between CORT and incubation temperature was not
583 supported in the first round of comparisons, we did not test against models including this interaction in
584 the second round. We also did not test for any interactions between sex and population density, so as not
585 to make models overly complex and risk obscuring the main effects of CORT treatment and incubation
586 temperature. However, we note that it is possible for sex differences in body size to arise under different
587 population densities, owing to either differences in competitive ability among the sexes or alternative life-
588 history strategies between sexes.

589 **Supplemental Table 17. Summary of second iteration of model comparisons for adult size (mass and**
590 **SVL) in *L. delicata*.**

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. delicata</i>	Adult Size $\sim$ CORT + Temp + Sex + Density + Age + (1 Clutch)	0 $\pm$ 0
	Adult Size $\sim$ (CORT + Temp) * Sex + Density + Age + (1 Clutch)	-3.301 $\pm$ 1.587
	Adult Size $\sim$ CORT + Temp + Density + Age + (1 Clutch)	-19.398 $\pm$ 7.208

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592

593 **Supplemental Table 18. Summary of second iteration of model comparisons for adult size (mass and**
594 **SVL) in *L. guichenoti*.**

Species	Model	Δ ELPD $\pm$ S.E.
L. guichenoti	Adult Size $\sim$ CORT + Temp + Sex + Density + Age	0 ± 0
	Adult Size $\sim$ (CORT + Temp) * Sex + Density + Age	-2.919 ± 1.669
	Adult Size $\sim$ CORT + Temp + Density + Age	-37.165 ± 8.123

Survival

Like growth and adult size, we aimed to test the effects of CORT treatment and incubation temperature on long-term patterns of survival and also considered the influence of population density and sex. Developmental CORT and incubation temperature may induce animals to adopt alternative life-history strategies, such as inducing faster growth rates at the expense of shorter lifespans. Alternative life-history strategies are potentially mediated by the effects of CORT and temperature on underlying physiological mechanisms, and CORT and temperature may therefore interact with each other to drive the pace of life. Population density may also affect survival if resources are scarce. The effects of CORT and incubation temperature on survival may also be exacerbated in high density populations if they impact competitive ability (e.g., reduced size). Therefore, we also consider the interactive effects of population density with CORT and temperature treatments. Sex differences in survival are also possible, if males and females maximise reproductive output through alternative life-history strategies. For example, males may maximise reproductive output by reaching maturity younger and increasing their mating opportunities, but potentially die younger. Females may, by contrast, increase reproductive output by surviving through multiple reproductive seasons and producing more egg clutches throughout their lifespan, resulting in greater longevity. Similarly, the effects of CORT and temperature may be sex-specific, if the longer-lived sex is more robust to the effects of developmental stressors on longevity.

To test for the inclusion of interactions between CORT treatment and incubation temperature, the inclusion of density and sex as independent effects, and any interactions between sex or density and developmental treatments, we used an iterative model comparison approach. This approach was similar to the approach described for adult size, except that survival was modelled as a univariate response model and assuming a Bernoulli distribution, because survival is a logical variable (1 if the animal died, 0 if they did not). Briefly, in the first round of model comparisons, we compared amongst models including only CORT and incubation temperature with or without an interaction term, with or without population density as an additional independent covariate, and including the two- and three-way interactions between CORT, temperature, and density. For the second round, we compared a model with only CORT treatment and incubation temperature as independent effects, a similar model including sex as an independent covariate, and a model including the two-way interactions between each of CORT and incubation temperature with sex.

626 **Supplemental Table 19. Summary of first iteration of model comparisons for survival in *L. delicata*.**

Species	Model	Δ ELPD $\pm$ S.E.
L. delicata	Survival $\sim$ CORT + Temp + (1 Mesocosm)	0 $\pm$ 0
	Survival $\sim$ CORT + Temp + Density + (1 Mesocosm)	-0.797 $\pm$ 0.554
	Survival $\sim$ CORT * Temp + (1 Mesocosm)	-1.123 $\pm$ 0.343
	Survival $\sim$ CORT * Temp + Density + (1 Mesocosm)	-1.574 $\pm$ 0.635
	Survival $\sim$ CORT * Temp * Density + (1 Mesocosm)	-5.406 $\pm$ 2.198

627

628 **Supplemental Table 20. Summary of first iteration of model comparisons for survival in *L.***
629 ***guichenoti*.**

Species	Model	Δ ELPD $\pm$ S.E.
L. guichenoti	Survival $\sim$ CORT + Temp + (1 Mesocosm)	0 $\pm$ 0
	Survival $\sim$ CORT + Temp + Density + (1 Mesocosm)	-0.585 $\pm$ 0.99
	Survival $\sim$ CORT * Temp + (1 Mesocosm)	-1.401 $\pm$ 0.373
	Survival $\sim$ CORT * Temp + Density + (1 Mesocosm)	-1.973 $\pm$ 1.165
	Survival $\sim$ CORT * Temp * Density + (1 Mesocosm)	-3.342 $\pm$ 3.143

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631 **Supplemental Table 21. Summary of first iteration of model comparisons for survival in *L. delicata*.**

Species	Model	Δ ELPD $\pm$ S.E.
L. delicata	Survival $\sim$ CORT + Temp + Sex (1 Mesocosm)	0 $\pm$ 0
	Survival $\sim$ (CORT + Temp) * Sex + (1 Mesocosm)	-1.791 $\pm$ 1.298
	Survival $\sim$ CORT + Temp + (1 Mesocosm)	-11.863 $\pm$ 5.339

632

633 **Supplemental Table 22. Summary of first iteration of model comparisons for survival in *L.***
634 ***guichenoti*.**

Species	Model	Δ ELPD $\pm$ S.E.
<i>L. guichenoti</i>	Survival $\sim$ (CORT + Temp) * Sex + (1 Mesocosm)	0 $\pm$ 0
	Survival $\sim$ CORT + Temp + (1 Mesocosm)	-0.483 $\pm$ 1.907
	Survival $\sim$ CORT + Temp + Sex (1 Mesocosm)	-0.534 $\pm$ 1.564

For both *L. delicata* and *L. guichenoti*, model comparisons did not support including interactions between CORT treatment and incubation temperature. Models including sex as an independent effect were preferred in *L. delicata* over models excluding sex, suggesting that one sex was more likely to die than the other. However, models of *L. guichenoti* survival were not improved by the inclusion of sex as a covariate, indicating that sex poorly predicts survival. Population density was not supported as an independent covariate or for interactions with developmental treatments in either *L. delicata* or *L. guichenoti*, suggesting that survival was similar among low and high population enclosures.

Summary

We conducted model comparisons for key life-history traits of developmental time, hatchling size, growth, adult size, and survival in *L. delicata* and *L. guichenoti*. The goal of these comparisons was to identify the most informative model structure for our main analysis of the effects of developmental CORT treatment and incubation temperature. Because developmental stressors can have joint effects on shared mechanistic pathways, we tested whether model predictive accuracy was improved by the addition of interactions between CORT and temperature. We also assessed whether additional covariates (i.e., sex and population density) likely to affect life-history traits were informative enough to include in our analyses. Finally, we tested whether the inclusion of sex-specific or density-dependent developmental effects improved model accuracy.

Across all traits, model comparisons did not support the inclusion of interactions between CORT treatment and incubation temperature, strongly indicating that the effects of each developmental treatment were independent of the other. We also found little support for the effects of CORT and incubation temperature to be sex-specific, as no interactions between CORT and sex or temperature in sex were included in the top performing models. Additionally, developmental effects were not dependent on the postnatal environment, as model comparisons did not support the inclusion of interactions between prenatal CORT or incubation temperature and population density.

The model selection process supported the inclusion of sex as an independent predictor of growth in *L. guichenoti*, survival in *L. delicata*, and adult size in both species. This is potentially indicative of sexual size dimorphism in *L. guichenoti*, consistent with their known ecology, and differences in longevity between male and female *L. delicata*. Population density was similarly supported as an independent predictor of growth and adult size in both species, suggesting that individuals differ in overall growth and size depending on the population density regardless of developmental effects or sex. Most likely, based on exploration of the raw data, individuals in high density enclosures are smaller than those in low density enclosures, potentially due to the higher competition reducing resource availability for sustained growth. Notably, population density was included only in models of growth in mass, but not SVL. This perhaps suggests that structural growth (SVL) is either more robust to resource limitation or has limited plasticity in response to high population density compared to storage (mass).

Appendix III.2: Supplemental Results

Introduction

We tested the long-term effects of developmental corticosterone (CORT) exposure and variation in incubation temperature on long-term patterns of body size, growth, and survival in two lizards (*Lampropholis delicata* and *Lampropholis guichenoti*). During this longitudinal study, lizards were housed in naturalistic outdoor enclosures that were either of low population density ($N_{\text{LOW}} = 16$ total animals; 8 per species) or high population density ($N_{\text{HIGH}} = 32$ total animals; 16 per species). Over ~2 years (~75% of the expected lifespan of these species), we repeatedly measured the mass and snout-vent-length of lizards to estimate growth using von Bertalanffy growth models. We also considered animals as having died if they were not found in either of the last two remeasurement periods, which we used to test for differences in longevity among lizards receiving different developmental treatments.

As a test of the mechanisms facilitating developmental effects on growth and survival, we measured *via* flow-through respirometry the metabolic rates of a subset of juvenile lizards (~90 days post-hatching). Then, we sacrificed juvenile lizards for measurements *via* flow cytometry of six parameters of cellular physiology in liver and heart tissue. On a separate subset of adult lizards (~760 days post-hatching), we again sacrificed lizards for measurements of cellular physiology in liver and heart tissue.

To improve the accessibility of results, the main text includes only reporting of the main effects of CORT treatment and incubation temperature on a subset of the data. The main text reports on hatchling mass, growth curves in mass, adult mass, survival, metabolism, and cellular physiology. This document contains additional reporting on traits that were not of primary interest in our study. This includes development length, hatchling SVL, growth curves in SVL, and adult SVL. Additionally, here we report on independent effects other than the main effects of CORT and incubation temperature, like sex-differences in and the influence of population density on growth and body size.

Results

Embryonic conditions influence the length of development

In total, we used 283 *L. delicata* eggs from 108 unique clutches and 288 *L. guichenoti* from 132 clutches as our study population, which is a subset of a previously described larger population of eggs (Leibold et al., 2026). Consistent with general expectations in oviparous taxa, incubation temperature was a strong predictor of development length, with lizards incubated at 28°C hatching substantially sooner than those incubated at 23°C (*L. delicata*: $\Delta [28^\circ\text{C} - 23^\circ\text{C}] = -18.348 \text{ days} \pm 0.001$, pMCMC < 0.001; *L. guichenoti*: $\Delta [28^\circ\text{C} - 23^\circ\text{C}] = -19.336 \text{ days} \pm 0.002$, pMCMC < 0.001). Developmental CORT exposure also affected the time to hatching, albeit to a lesser degree than incubation temperature, with CORT-treated eggs hatching later than control eggs (*L. delicata*: $\Delta [\text{CORT} - \text{Control}] = 0.878 \text{ days} \pm 0.001$, pMCMC < 0.001; *L. guichenoti*: $\Delta [\text{CORT} - \text{Control}] = 1.434 \text{ days} \pm 0.002$, pMCMC = 0.002) (Figure 2A, Supplemental Table 2).

For both *L. delicata* and *L. guichenoti*, eggs receiving the Control treatment and incubated at 28°C hatched the soonest (*L. delicata*: $N_{\text{Control}, 28^\circ\text{C}} = 71$, Mean $\pm$ SD = 31.479 ± 1.351 days; *L. guichenoti*: $N_{\text{Control}, 28^\circ\text{C}} = 70$, Mean $\pm$ SD = 33.757 ± 1.689 days), with CORT treated eggs incubated at 28°C hatching slightly later (*L. delicata*: $N_{\text{CORT}, 28^\circ\text{C}} = 70$, Mean $\pm$ SD = 32.471 ± 1.878 days; *L. guichenoti*: $N_{\text{CORT}, 28^\circ\text{C}} = 76$, Mean $\pm$ SD = 35.553 ± 4.603 days). Eggs treated with CORT and

incubated at 23°C hatched the latest (*L. delicata*: $N_{\text{CORT},23^{\circ}\text{C}} = 69$, Mean $\pm$ SD = 50.841 ± 2.779 days; *L. guichenoti*: $N_{\text{CORT},23^{\circ}\text{C}} = 72$, Mean $\pm$ SD = 54.458 ± 3.599 days), with Control eggs hatching slightly sooner (*L. delicata*: $N_{\text{Control},23^{\circ}\text{C}} = 73$, Mean $\pm$ SD = 49.918 ± 3.407 days; *L. guichenoti*: $N_{\text{Control},23^{\circ}\text{C}} = 70$, Mean $\pm$ SD = 53.614 ± 5.32 days) (Figure 2B, Supplemental Table 1).

Larger egg mass was also associated with shorter developmental periods across treatment groups in both *L. delicata* and *L. guichenoti* (*L. delicata*: Δ [Egg Mass] = $-23.423 \text{ days} \cdot \Delta \text{ g}^{-1} \pm 0.040$, pMCMC = 0.001; *L. guichenoti*: Δ [Egg Mass] = $-33.930 \text{ days} \cdot \Delta \text{ g}^{-1} \pm 0.049$, pMCMC < 0.001) (Supplemental Table 2). Given that initial egg mass ranged from 0.097–0.253 g (Δ 0.156 g), egg mass variation accounted for up to 3.654 days of variation in development length. For *L. guichenoti*, egg mass ranged from 0.106–0.273 g (Δ 0.167 g), thus accounting for up to 5.666 days of variation in development length. The influence of egg mass on developmental length may indicate that embryos in larger eggs utilise energy more rapidly or more efficiently than embryos in smaller eggs. An alternative explanation, however, is that this effect was caused by larger eggs being slightly older. Lizard eggs absorb moisture after oviposition, so eggs found 2-3 days after oviposition weigh more than eggs found within 1 day of oviposition.

Maternal investment predicts hatchling mass

Consistent with previous work (Leibold et al., 2026) initial egg mass was the strongest predictor of hatchling mass in both *L. delicata* and *L. guichenoti*, with larger eggs generally resulting in heavier hatchlings (*L. delicata*: Δ [Egg Mass] = $0.273 \text{ g} \cdot \Delta \text{ g}^{-1} \pm <0.001$, pMCMC < 0.001; *L. guichenoti*: Δ [Egg Mass] = $0.291 \text{ g} \cdot \Delta \text{ g}^{-1} \pm <0.001$, pMCMC < 0.001) (Supplemental Table 6). The effect of egg mass on hatchling mass was independent of CORT-treatment and incubation temperature, suggesting no interaction between developmental stressors and egg mass to drive hatchling size. Egg mass variation in *L. delicata* (Δ 0.156 g) accounted for up to 0.042 g of hatchling mass, while in *L. guichenoti* egg mass variation (Δ 0.167 g) accounted for up to 0.049 g of hatchling mass. Sex was excluded as a covariate during model selection for hatchling size, suggesting no sex-differences in body mass at hatching for either *L. delicata* or *L. guichenoti* (see *Supplementary Document – Model Comparisons*).

Developmental CORT and temperature induce reduced body length

Developmental treatments affected hatchling SVL in a similar manner to hatchling mass (Figure 2E, Supplemental Table 7). Developmental CORT-treatment induced *L. delicata* and *L. guichenoti* to hatch at reduced SVL compared to Control lizards (*L. delicata*: Δ [CORT – Control] = $-0.171 \text{ mm} \pm <0.001$, pMCMC = 0.044; *L. guichenoti*: Δ [CORT – Control] = $-0.122 \text{ mm} \pm <0.001$, pMCMC = 0.122). Incubation at 28°C also induced lizards to hatch at smaller body lengths than incubation at 23°C (*L. delicata*: Δ [28°C – 23°C] = $-0.254 \text{ mm} \pm <0.001$, pMCMC = 0.003; *L. guichenoti*: Δ [28°C – 23°C] = $-0.227 \text{ mm} \pm <0.001$, pMCMC = 0.006).

Like mass, the effects of CORT exposure and warm incubation temperatures were additive (Figure 2F, Supplemental Table 1). Control lizards incubated at 23°C hatched at the largest SVL (*L. delicata*: $N_{\text{Control},23^{\circ}\text{C}} = 51$, Mean $\pm$ SD = 18.667 ± 0.766 mm; *L. guichenoti*: $N_{\text{Control},23^{\circ}\text{C}} = 64$, Mean $\pm$ SD = 19.953 ± 0.653 mm), and CORT-treated lizards incubated at 28°C hatched at the smallest SVL (*L. delicata*: $N_{\text{CORT},28^{\circ}\text{C}} = 58$, Mean $\pm$ SD = 18.241 ± 0.709 mm; *L. guichenoti*: $N_{\text{CORT},28^{\circ}\text{C}} = 66$, Mean $\pm$ SD = 19.697 ± 0.841 mm). Control lizards incubated at 28°C (*L. delicata*: $N_{\text{Control},28^{\circ}\text{C}} = 57$, Mean $\pm$ SD = 18.439 ± 0.887 mm; *L. guichenoti*: $N_{\text{Control},28^{\circ}\text{C}} = 63$, Mean $\pm$ SD = 19.746 ± 0.879 mm), and CORT-treated lizards incubated at 23°C (*L. delicata*: $N_{\text{CORT},23^{\circ}\text{C}} = 50$, Mean $\pm$ SD = 18.500 ± 0.839 mm; *L. guichenoti*: $N_{\text{CORT},23^{\circ}\text{C}} = 59$, Mean $\pm$ SD = 19.881 ± 0.768 mm) hatched at intermediate body lengths.

CORT effects on hatchling body length differed by species

The effect of CORT on hatchling SVL was non-significant only in *L. guichenoti* (pMCMC = 0.122), but the effects of both developmental stress and incubation temperature were similar between *L. delicata* and *L. guichenoti* (Stress: Δ [*L. delicata* – *L. guichenoti*] = -0.161 ± 0.001 , pMCMC =

0.245; Temperature: $\Delta [L. \text{delicata} - L. \text{guichenoti}] = -0.025 \pm 0.001$, pMCMC = 0.852) (Supplemental Table 10). This suggests a conserved response to developmental stressors in both species, but potentially a difference in the strength of CORT's influence on SVL. Like hatchling mass, the effects of developmental CORT exposure and incubation temperature on the SVL of hatchling *L. delicata* and *L. guichenoti* were significant, but small. Treatment with CORT and incubation at 28°C induced *L. delicata* to hatch at up to ~0.45 mm shorter (~2.4% reduction in total SVL) and *L. guichenoti* only ~0.26 mm shorter (~1.3% reduction in total SVL) (Supplemental Table 8).

Independent of CORT-treatment and incubation temperature, egg mass was the strongest predictor of hatchling SVL in both *L. delicata* and *L. guichenoti* (*L. delicata*: $\Delta [\text{Egg Mass}] = 12.523 \text{ mm} \cdot \Delta \text{g}^{-1} \pm 0.013$, pMCMC < 0.001; *L. guichenoti*: $\Delta [\text{Egg Mass}] = 12.194 \text{ mm} \cdot \Delta \text{g}^{-1} \pm 0.009$, pMCMC < 0.001). Larger eggs generally resulted in longer hatchlings, with egg mass variation ($\Delta 0.156 \text{ g}$) accounting for up to 1.954 mm of SVL in *L. delicata*, and 2.036 mm of SVL in *L. guichenoti* ($\Delta 0.167 \text{ g}$) (Supplemental Table 7). Sex was excluded as a covariate during model selection for hatchling size, suggesting no sex-differences in body length at hatching for either *L. delicata* or *L. guichenoti* (see *Supplementary Document – Model Comparisons*).

High competition promotes rapid mass gain

Beyond the main effects of CORT treatment and incubation temperature on growth, population density also influenced growth trajectories in both *L. delicata* and *L. guichenoti* (Supplemental Table 15). While for *L. delicata*, population density was included as a covariate alongside CORT-treatment and incubation temperature, model comparisons suggested exclusion of population density as a covariate in the main analysis for *L. guichenoti* (see *Supplementary Document – Model Comparisons*). However, exploration of raw growth data and a secondary analysis supported population density as a driver of growth in *L. guichenoti* (Supplemental Table 15). For both *L. delicata* and *L. guichenoti*, lizards held in high density enclosures grew at faster rates (K , days⁻¹) than lizards in low density enclosures (*L. delicata*: $\Delta [\text{High} - \text{Low}] = 4.1\text{e}^{-4} \text{ days}^{-1} \pm <1\text{e}^{-5}$, pMCMC < 0.001; *L. guichenoti*: $\Delta [\text{High} - \text{Low}] = 3.7\text{e}^{-4} \text{ days}^{-1} \pm <1\text{e}^{-5}$, pMCMC < 0.001). Likewise, lizards in high density enclosures were predicted to reach larger maximum sizes (L_{inf} , grams) than lizards in low density enclosures (*L. delicata*: $\Delta [\text{High} - \text{Low}] = 0.005 \text{ g} \pm 3\text{e}^{-5}$, pMCMC < 0.001; *L. guichenoti*: $\Delta [\text{High} - \text{Low}] = 0.004 \text{ g} \pm 2\text{e}^{-5}$, pMCMC < 0.001).

Female lizards gain faster than male lizards

During model selection, sex was excluded as a covariate in main analyses of the effects of CORT and incubation temperature, for both *L. delicata* and *L. guichenoti* (see *Supplementary Document – Model Comparisons*). However, additional exploration of raw data suggested potential sex differences in growth for *L. guichenoti*, but did not indicate sex differences in growth for *L. delicata*. In a secondary analysis of *L. guichenoti* growth, including population density and sex as independent main effects, growth models predicted that females grow at faster rates (K , days⁻¹; $\Delta [\text{Female} - \text{Male}] = 5.4\text{e}^{-4} \text{ days}^{-1} \pm <1\text{e}^{-5}$, pMCMC < 0.001) and reach larger body sizes than males (L_{inf} , grams; $\Delta [\text{Female} - \text{Male}] = 0.093 \text{ g} \pm 1.2\text{e}^{-4}$, pMCMC < 0.001) (Supplemental Table 15).

CORT and warm temperatures induce more rapid structural growth

Developmental CORT exposure and incubation at 28°C similarly affected the growth of *L. delicata* and *L. guichenoti* in terms of body length as they did body mass (Figure 4, Supplemental Table 16). Lizards treated with CORT grew in SVL at higher growth rate coefficients (K) than Control-treated lizards (*L. delicata*: $\Delta [\text{CORT} - \text{Control}] = 7.3 \times 10^{-4} \text{ days}^{-1} \pm <1 \times 10^{-5}$; pMCMC < 0.001; *L. guichenoti*: $\Delta [\text{CORT} - \text{Control}] = 3.3 \times 10^{-4} \text{ days}^{-1} \pm <1 \times 10^{-5}$; pMCMC < 0.001). Lizards incubated at 28°C also gained SVL at faster rates than lizards incubated at 23°C (*L. delicata*: $\Delta [28^\circ\text{C} - 23^\circ\text{C}] = 2.7 \times 10^{-4} \text{ days}^{-1} \pm <1 \times 10^{-5}$, pMCMC < 0.001; *L. guichenoti*: $\Delta [28^\circ\text{C} - 23^\circ\text{C}] = 5 \times 10^{-5} \text{ days}^{-1} \pm <1 \times 10^{-5}$, pMCMC < 0.001).

Like growth models for mass, von Bertalanffy growth models of SVL also predicted that CORT-treated (*L. delicata*: $\Delta [\text{CORT} - \text{Control}] = 0.065 \text{ mm} \pm 2.4 \times 10^{-4}$; pMCMC < 0.001; *L. guichenoti*: $\Delta [\text{CORT} - \text{Control}] = 0.077 \text{ mm} \pm 2.8 \times 10^{-4}$; pMCMC < 0.001) and warm-incubated (*L. delicata*: $\Delta [28^\circ\text{C} - 23^\circ\text{C}] = 0.099 \text{ mm} \pm 3.6 \times 10^{-4}$, pMCMC < 0.001; *L. guichenoti*: $\Delta [28^\circ\text{C} - 23^\circ\text{C}] = 0.097 \text{ mm} \pm 3.4 \times 10^{-4}$, pMCMC < 0.001) lizards would reach larger asymptotic SVL (Linf) than Control and cool-incubated lizards. Growth models of SVL therefore predict that CORT and warm incubation induce *L. delicata* and *L. guichenoti* to reach adult SVL more rapidly, and to reach larger adult SVL assuming they continue growing at these same rates.

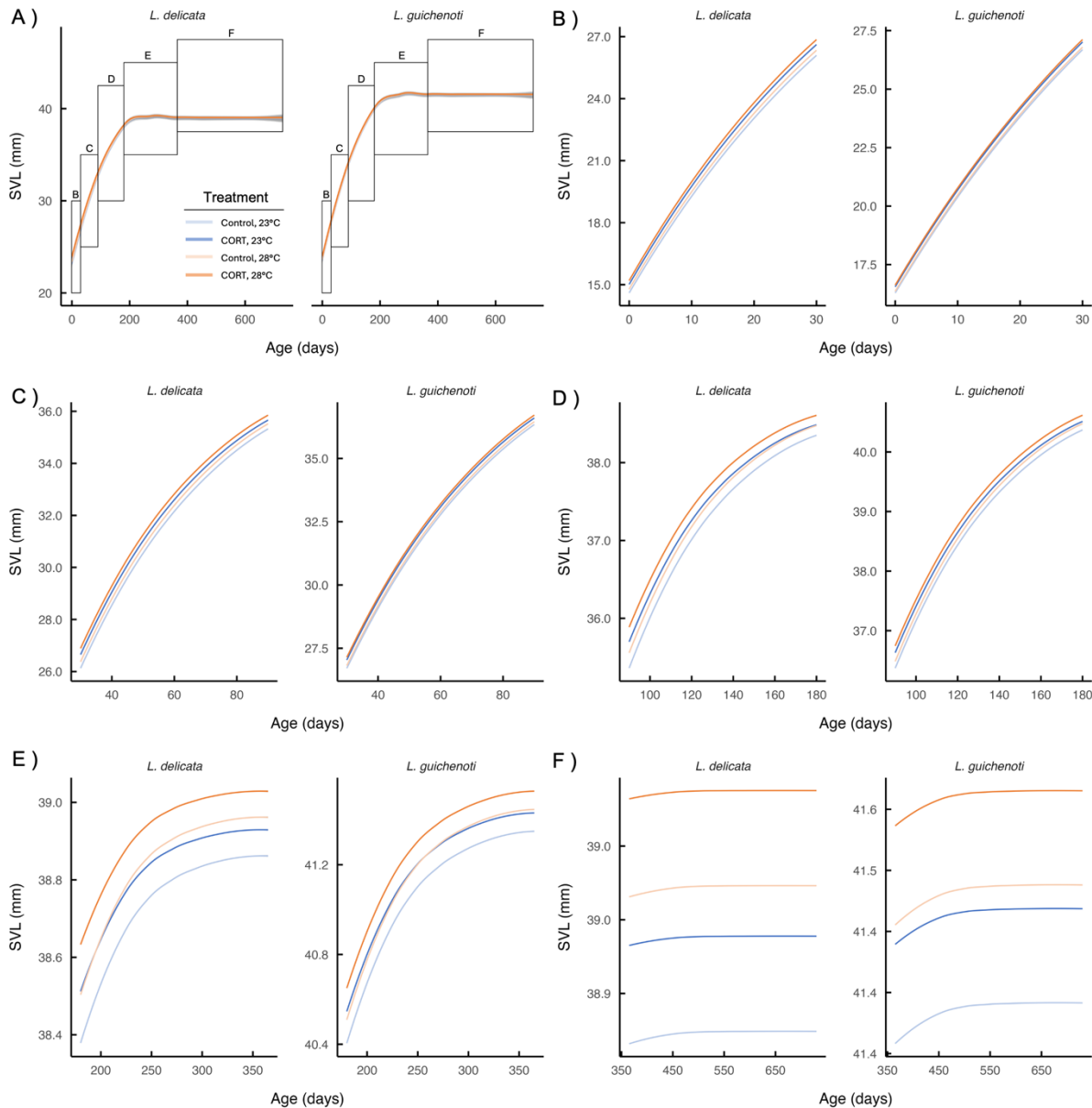


Figure 4. Effects of CORT treatment and incubation temperature on growth curves of *L. delicata* and *L. guichenoti* SVL. Growth curves are the snout-vent-length (SVL) of *L. delicata* and *L. guichenoti* throughout 730 days post-hatching (DPH) as predicted by Von Bertalanffy growth models (VBGM). VBGM used as input repeated measures of lizard SVL at 0 DPH, 30 DPH, 90 DPH, 270 DPH, 365 DPH, and 730 DPH. VBGM predicted the growth rate coefficient K (days^{-1}) and asymptotic body size L_{∞} (mm) for each developmental stress by incubation temperature treatment, which were then imputed into the Von Bertalanffy growth equation to predict SVL at any given age. A) is the full predicted growth curve from 0 DPH to 730 DPH. Rectangles represent periods of time between each measure, with the letters above each rectangle denoting the corresponding panel in the figure. B) through F) are, respectively, the growth curves from 0-30 DPH, 30-90 DPH, 90-180 DPH, 180-365 DPH, and 365-730 DPH.

Females gained body length more rapidly than males, but only in one species

Model selection supported the inclusion of sex as an independent predictor of growth in *L. guichenoti*, but did not support sex as a predictor of growth in *L. delicata* (see *Supplementary Document – Model Comparisons*). Exploration of the raw data also did not suggest sex-specific differences in growth trajectories between male and female *L. delicata*. For *L. guichenoti*, sex affected growth, with females predicted to grow at faster rates (K , days⁻¹; Δ [Female – Male] = $9e^{-5}$ days⁻¹, pMCMC < 0.001) and reach larger body lengths (Linf, mm; Δ [Female – Male] = $1.839 \text{ mm} \pm 0.001$, pMCMC < 0.001) than males (Supplemental Table 20).

High competition impairs body mass in adulthood

In addition to prenatal CORT and incubation temperature treatments, the mass of adult *L. delicata* and *L. guichenoti* depended on the population density in which they were held (*L. delicata*: Δ [High – Low] = $-0.164 \text{ g} \pm <0.001$, pMCMC < 0.001; *L. guichenoti*: Δ [High – Low] = $-0.309 \text{ g} \pm <0.001$, pMCMC < 0.001) (Supplemental Table 27). The influence of population density on adult mass was independent of CORT-treatment and incubation temperature, nor were there sex-specific effects of population density on mass. After accounting for developmental effects and any sex differences in mass, *L. delicata* from high density enclosures weighed ~14% less on average as adults than *L. delicata* from low density enclosures (Low Density: Estimate $\pm$ SE = $1.53 \text{ g} \pm 1.49e^{-4}$; High Density: Estimate $\pm$ SE = $1.22 \text{ g} \pm 1.01e^{-4}$). The effect of population density was even more pronounced in *L. guichenoti*, for which lizards from high density enclosures weighed ~20.3% less on average than lizards from low density enclosures (Low Density: Estimate $\pm$ SE = $1.20 \text{ g} \pm 1.29e^{-4}$; High Density: Estimate $\pm$ SE = $1.04 \text{ g} \pm 8.84e^{-5}$) (Supplemental Table 26).

Lizards show species-specific patterns of sexual dimorphism in mass at adulthood

Despite the exclusion of sex as a covariate in models of *L. delicata* growth, sex was included in models of adult size for both *L. delicata* and *L. guichenoti* (see *Supplementary Document – Model Comparisons*). In contrast to the expected pattern of female-biased sexual size dimorphism, male and female *L. guichenoti* did not differ in their mass at adulthood (Δ [Female – Male] = $-0.020 \text{ g} \pm <0.001$, pMCMC = 0.507). Adult mass in *L. delicata* also conflicted with expectations of female-biased sexual size dimorphism, with females weighing less at adulthood than males (Δ [Female – Male] = $-0.098 \text{ g} \pm <0.001$, pMCMC = 0.001) (Supplemental Table 27). It is noteworthy, however, that documentation of sexual size dimorphism in *L. delicata* and *L. guichenoti* often report dimorphism in metrics of body length rather than mass, and the degree of dimorphism varies among populations (Forsman and Shine, 1995; Qualls and Shine, 1997; Qualls and Shine, 1998; Qualls and Shine, 2000; Chapple et al., 2014).

Developmental CORT and incubation temperature have long-term effects on adult body length

Developmental CORT and incubation temperature influenced the SVL of adult *L. delicata* and *L. guichenoti* in the same manner as adult mass (Figure 5C, Supplemental Table 23). Lizards treated with CORT as eggs had shorter body lengths in adulthood than Control lizards (*L. delicata*: Δ [CORT – Control] = $-1.180 \text{ mm} \pm 0.001$, pMCMC < 0.001; *L. guichenoti*: Δ [CORT – Control] = $-0.655 \text{ mm} \pm 0.001$, pMCMC = 0.014). Lizards incubated at 28°C as eggs were also shorter as adults than lizards incubated at 23°C (*L. delicata*: Δ [28°C – 23°C] = $-1.791 \text{ mm} \pm 0.001$, pMCMC < 0.001; *L. guichenoti*: Δ [28°C – 23°C] = $-0.596 \text{ mm} \pm 0.001$, pMCMC = 0.05).

Also like adult mass, the effects of developmental CORT and incubation temperature on adult SVL were additive (Figure 5D, Supplemental Table 24). After accounting for sex-differences and population density effects on SVL, lizards receiving the Control treatment and incubated at 23°C ultimately reached the largest SVL as adults (*L. delicata*: $N_{\text{Control},23^{\circ}\text{C}} = 41$, Estimate $\pm$ SE = 43.657 mm $\pm$ 0.002; *L. guichenoti*: $N_{\text{Control},23^{\circ}\text{C}} = 51$, Estimate $\pm$ SE = 45.178 mm $\pm$ 0.002). Lizards treated with CORT but incubated at 23°C (*L. delicata*: $N_{\text{CORT},23^{\circ}\text{C}} = 37$, Estimate $\pm$ SE = 42.477 mm $\pm$ 0.002; *L. guichenoti*: $N_{\text{CORT},23^{\circ}\text{C}} = 48$, Estimate $\pm$ SE = 44.523 mm $\pm$ 0.002), and Control lizards incubated at 28°C were smaller (*L. delicata*: $N_{\text{Control},28^{\circ}\text{C}} = 40$, Estimate $\pm$ SE = 41.866 mm $\pm$ 0.002; *L. guichenoti*: $N_{\text{Control},28^{\circ}\text{C}} = 53$, Estimate $\pm$ SE = 44.582 mm $\pm$ 0.002). Lizards treated with CORT and incubated at 28°C had the smallest SVL in adulthood (*L. delicata*: $N_{\text{CORT},28^{\circ}\text{C}} = 40$, Estimate $\pm$ SE = 40.686 mm $\pm$ 0.002; *L. guichenoti*: $N_{\text{CORT},28^{\circ}\text{C}} = 55$, Estimate $\pm$ SE = 43.927 mm $\pm$ 0.002).

Body length differences in adulthood, like adult mass, were also more pronounced than they were at hatching. After accounting for sex-specific differences in body length and the effects of population density on SVL (described below), CORT-treated, warm-incubated *L. delicata* were ~2.971 mm (~7% of total SVL) smaller than Control, cool-incubated lizards. But CORT-treated, warm-incubated *L. guichenoti* were only ~1.251 mm (~2.8% of total SVL) smaller than Control, cool-incubated lizards. This disparity is likely driven by the relatively weaker effects of temperature on the SVL of *L. guichenoti* than *L. delicata*. The effect of incubation temperature on *L. guichenoti* adult SVL was only marginally non-significant in *L. guichenoti* (pMCMC = 0.05) but clearly significant in *L. guichenoti* (pMCMC < 0.001). Comparisons of effect sizes between *L. delicata* and *L. guichenoti* indicate that the negative effect of incubation temperature on adult SVL was greater in *L. delicata* than *L. guichenoti* ($\Delta [L. \text{delicata} - L. \text{guichenoti}] = -1.194$, pMCMC = 0.009) (Supplemental Table 26). This finding suggests *L. guichenoti* is relatively more robust to the long-term effects of incubation temperature on adult SVL than is *L. delicata*.

High competition reduces structural body size in adult lizards

The influence of population density on adult SVL was consistent with the effects of population density on adult mass (Supplemental Table 28). Lizards reared to adulthood in high density enclosures had shorter body lengths than lizards reared in low density enclosures (*L. delicata*: $\Delta [\text{High} - \text{Low}] = -1.511$ mm $\pm$ 0.002, pMCMC < 0.001; *L. guichenoti*: $\Delta [\text{High} - \text{Low}] = -2.664$ mm $\pm$ 0.002, pMCMC < 0.001). Averaging across developmental treatments and between sexes, *L. delicata* in high density enclosures were ~3.53% shorter as adults than *L. delicata* in low density enclosures (Low: Estimate $\pm$ SE = 42.6 mm $\pm$ 1.53e⁻³; High: Estimate $\pm$ SE = 41.1 mm $\pm$ 1.05e⁻³). The effect of population density on adult SVL was more pronounced in *L. guichenoti*, with *L. guichenoti* from high density enclosures ~5.93% shorter than *L. guichenoti* from low density enclosures (Low: Estimate $\pm$ SE = 45.6 mm $\pm$ 1.34e⁻³; High: Estimate $\pm$ SE = 42.9 mm $\pm$ 9.08e⁻⁴) (Supplemental Table 24).

Female lizards have larger body lengths than males

Consistent with previous work on *L. delicata* and *L. guichenoti*, which finds female-biased sexual size dimorphism based on body length, there were sex differences in SVL in *L. delicata* and *L. guichenoti* (*L. delicata*: $\Delta [\text{Female} - \text{Male}] = 0.812$ mm $\pm$ 0.002, pMCMC = 0.017; *L. guichenoti*: $\Delta [\text{Female} - \text{Male}] = 2.069$ mm $\pm$ 0.002, pMCMC < 0.001) (Supplemental Table 28). Sex-differences in body length were independent of developmental CORT treatment, incubation temperature, and population density, suggesting no sex-specific effects of the pre- or post-natal environment on adult body size. After accounting for developmental effects and the influence of population density, female *L. delicata* (Estimate $\pm$ SE = 42.2 mm $\pm$ 1.56e⁻³) were ~1.9% larger on average than male *L. delicata* (Estimate $\pm$ SE = 41.4 mm $\pm$ 1.09e⁻³). Sex differences in SVL were more pronounced in *L. guichenoti*, and female *L. guichenoti* (Estimate $\pm$ SE = 45.3 mm $\pm$ 1.05e⁻³) were ~4.9% larger at adulthood than male *L. guichenoti* (Estimate $\pm$ SE = 43.2 mm $\pm$ 9.08e⁻⁴). Taken alongside the effects of sex on mass we observed, *L. delicata* females are

920 both longer and lighter than are males, while *L. guichenoti* females are longer than but of equal weight
921 with males (Supplemental Table 24).

Appendix III.3: Supplemental Tables

1 Supplemental Table 1. Summary statistics for raw values of developmental length and hatchling size in *L. delicata* and *L. guichenoti*. For each combination of developmental
2 CORT treatment (CORT or Control) and incubation temperature (23°C or 28°C), we give the number of hatchlings measured as counts N, and the Mean $\pm$ SD for each of
3 development length, hatchling mass, and hatchling SVL.

Species	Trait	Control,23°C	CORT,23°C	Control,28°C	CORT,28°C
<i>L. delicata</i>	N	73	69	71	70
	Development Length (days)	49.918 $\pm$ 3.407	50.841 $\pm$ 2.779	31.479 $\pm$ 1.351	32.471 $\pm$ 1.878
	Hatchling Mass (g)	0.113 $\pm$ 0.011	0.112 $\pm$ 0.011	0.109 $\pm$ 0.012	0.108 $\pm$ 0.011
	Hatchling SVL (mm)	18.677 $\pm$ 0.773	18.477 $\pm$ 0.831	18.42 $\pm$ 0.946	18.299 $\pm$ 0.697
<i>L. guichenoti</i>	N	70	72	70	76
	Development Length (days)	53.614 $\pm$ 5.32	54.458 $\pm$ 3.599	33.757 $\pm$ 1.689	35.553 $\pm$ 4.603
	Hatchling Mass (g)	0.141 $\pm$ 0.015	0.142 $\pm$ 0.016	0.141 $\pm$ 0.016	0.136 $\pm$ 0.015
	Hatchling SVL (mm)	19.955 $\pm$ 0.661	19.892 $\pm$ 0.773	19.809 $\pm$ 0.885	19.653 $\pm$ 0.846

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5 Supplemental Table 2. Analysis of CORT and incubation temperature effects on development length. For the length of time to hatch for *L. delicata* and *L. guichenoti*, we
6 show the difference in mean between predicted posterior distributions (Estimate), the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95%
7 C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The
8 summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for
9 Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C). We also show the
10 predicted relationship between initial egg mass and development length, with the estimate representing the predicted change in time to hatch for a 1 gram increase in egg
11 mass.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Development Length (days)	Stress [CORT - Control]	0.878	0.001	0.409	1.348	<0.001
		Temperature [28°C - 23°C]	-18.348	0.001	-18.821	-17.874	<0.001
		Egg Mass (g)	-23.423	0.040	-37.169	-9.442	0.001
<i>L. guichenoti</i>		Stress [CORT - Control]	1.434	0.002	0.547	2.318	0.002
		Temperature [28°C - 23°C]	-19.336	0.002	-20.231	-18.440	<0.001
		Egg Mass (g)	-33.930	0.049	-51.282	-16.464	<0.001

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14 Supplemental Table 3. Summary of predicted posterior distributions of *L. delicata* and *L. guichenoti* development length (days from oviposition to hatching) for each CORT-
 15 treatment by incubation temperature combination. Predictive posterior distributions were generated by Bayesian GLMMs including CORT, incubation temperature, and initial
 16 egg mass as fixed effects. Clutch was included as a random effect to account for similarities among siblings. Models were run separately for each species. The mean of the
 17 predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Trait	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Development Length (days)	Control, 23°C	49.791	0.001	49.291	50.296
		Control, 28°C	31.443	0.001	30.935	31.952
		CORT, 23°C	50.669	0.001	50.164	51.171
		CORT, 28°C	32.321	0.001	31.815	32.823
	Egg Mass (g)		-23.423	0.040	-37.169	-9.442
<i>L. guichenoti</i>	Development Length (days)	Control, 23°C	53.272	0.002	52.449	54.093
		Control, 28°C	33.936	0.002	33.121	34.761
		CORT, 23°C	54.706	0.002	53.888	55.527
		CORT, 28°C	35.370	0.002	34.580	36.164
	Egg Mass (g)		-33.930	0.049	-51.282	-16.464

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Supplemental Table 4. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* development length. Data are the differences in predicted posterior distributions generated by Bayesian GLMMs including CORT treatment, incubation temperature, and initial egg mass as main effects. Clutch was included as a random effect to account for similarities among siblings. Contrasts are the differences in posterior distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Development Length (days)	[Control,23°C] - [Control,28°C]	18.348	0.001	17.874	18.821	<0.001
		[Control,23°C] - [CORT,23°C]	-0.878	0.001	-1.348	-0.409	<0.001
		[Control,23°C] - [CORT,28°C]	17.470	0.002	16.811	18.125	<0.001
		[Control,28°C] - [CORT,23°C]	-19.225	0.002	-19.901	-18.551	<0.001
		[Control,28°C] - [CORT,28°C]	-0.878	0.001	-1.348	-0.409	<0.001
		[CORT,23°C] - [CORT,28°C]	18.348	0.001	17.874	18.821	<0.001
<i>L. guichenoti</i>	Development Length (days)	[Control,23°C] - [Control,28°C]	19.336	0.003	18.440	20.231	<0.001
		[Control,23°C] - [CORT,23°C]	-1.434	0.003	-2.318	-0.547	0.002
		[Control,23°C] - [CORT,28°C]	17.902	0.004	16.642	19.152	<0.001
		[Control,28°C] - [CORT,23°C]	-20.770	0.004	-22.046	-19.491	<0.001
		[Control,28°C] - [CORT,28°C]	-1.434	0.003	-2.318	-0.547	0.002
		[CORT,23°C] - [CORT,28°C]	19.336	0.003	18.440	20.231	<0.001

27 Supplemental Table 5. Comparisons of CORT-treatment and incubation temperature effects on development length between *L. delicata* and *L. guichenoti*. Contrasts are the
 28 differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects
 29 on development length. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper
 30 (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Development Length (days)	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.556	0.002	-1.557	0.457	0.279
	Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.988	0.002	-0.026	2.007	0.057

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Supplemental Table 6. Analysis of CORT and incubation temperature effects on hatchling mass. For the mass of *L. delicata* and *L. guichenoti* at hatching, we show the difference in mean between predicted posterior distributions (Estimate), the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C). We also show the predicted relationship between initial egg mass and hatchling mass, with the estimate representing the predicted change in hatchling mass for a 1 gram increase in egg mass.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Hatchling Mass (g)	Stress [CORT - Control]	-0.002	<0.001	-0.003	0.000	0.011
		Temperature [28°C - 23°C]	-0.004	<0.001	-0.006	-0.003	<0.001
		Egg Mass (g)	0.273	<0.001	0.216	0.331	<0.001
<i>L. guichenoti</i>		Stress [CORT - Control]	-0.002	<0.001	-0.004	0.000	0.019
		Temperature [28°C - 23°C]	-0.003	<0.001	-0.006	-0.001	0.001
		Egg Mass (g)	0.291	<0.001	0.236	0.345	<0.001

Supplemental Table 7. Analysis of CORT and incubation temperature effects on hatchling SVL. For *L. delicata* and *L. guichenoti* SVL at hatching, we show the difference in mean between predicted posterior distributions (Estimate), the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C). We also show the predicted relationship between initial egg mass and hatchling SVL, with the estimate representing the predicted change in hatchling body length for a 1 gram increase in egg mass.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Hatchling SVL (mm)	Stress [CORT - Control]	-0.171	<0.001	-0.333	-0.005	0.044
		Temperature [28°C - 23°C]	-0.254	<0.001	-0.418	-0.090	0.003
		Egg Mass (g)	12.523	0.013	8.022	17.059	<0.001
<i>L. guichenoti</i>		Stress [CORT - Control]	-0.122	<0.001	-0.280	0.034	0.122
		Temperature [28°C - 23°C]	-0.227	<0.001	-0.385	-0.067	0.006
		Egg Mass (g)	12.194	0.009	8.970	15.445	<0.001

Supplemental Table 8. Summary of predicted posterior distributions of *L. delicata* and *L. guichenoti* hatchling size (mass and SVL) for each CORT-treatment by incubation temperature combination. Predictive posterior distributions were generated by multi-response Bayesian GLMMs including CORT, incubation temperature, and initial egg mass as fixed effects. Clutch was included as a random effect to account for similarities among siblings. Models were run separately for each species. The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Trait	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Hatchling Mass (g)	Control, 23°C	0.114	<0.001	0.111	0.116
		Control, 28°C	0.110	<0.001	0.107	0.112
		CORT, 23°C	0.111	<0.001	0.109	0.114
		CORT, 28°C	0.107	<0.001	0.105	0.110
	Hatchling SVL (mm)	Control, 23°C	18.673	0.001	18.485	18.858
		Control, 28°C	18.444	0.001	18.262	18.624
		CORT, 23°C	18.455	0.001	18.263	18.649
		CORT, 28°C	18.227	0.001	18.047	18.407
<i>L. guichenoti</i>	Hatchling Mass (g)	Control, 23°C	0.143	<0.001	0.140	0.146
		Control, 28°C	0.140	<0.001	0.137	0.143
		CORT, 23°C	0.142	<0.001	0.139	0.145
		CORT, 28°C	0.138	<0.001	0.135	0.141
	Hatchling SVL (mm)	Control, 23°C	19.948	<0.001	19.779	20.118
		Control, 28°C	19.745	<0.001	19.574	19.913
		CORT, 23°C	19.891	<0.001	19.719	20.064
		CORT, 28°C	19.688	<0.001	19.522	19.853

53 Supplemental Table 9. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* hatchling size (mass or SVL). Data are the differences in predicted
54 posterior distributions generated by Bayesian multi-response GLMMs including CORT treatment, incubation temperature, and initial egg mass as main effects. Clutch was
55 included as a random effect to account for similarities among siblings. Contrasts are the differences in posterior distributions between treatment groups. The mean of the
56 difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.
57 The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Hatchling Mass (g)	[Control,23°C] - [Control,28°C]	0.004	<0.001	0.002	0.006	<0.001
		[Control,23°C] - [CORT,23°C]	0.002	<0.001	0.000	0.004	0.044
		[Control,23°C] - [CORT,28°C]	0.006	<0.001	0.003	0.009	<0.001
		[Control,28°C] - [CORT,23°C]	-0.002	<0.001	-0.005	0.001	0.276
		[Control,28°C] - [CORT,28°C]	0.002	<0.001	0.000	0.004	0.044
		[CORT,23°C] - [CORT,28°C]	0.004	<0.001	0.002	0.006	<0.001
	Hatchling SVL (mm)	[Control,23°C] - [Control,28°C]	0.228	0.001	0.038	0.419	0.019
		[Control,23°C] - [CORT,23°C]	0.218	0.001	0.020	0.414	0.031
		[Control,23°C] - [CORT,28°C]	0.446	0.001	0.177	0.712	0.002
		[Control,28°C] - [CORT,23°C]	-0.010	0.001	-0.294	0.266	0.949
		[Control,28°C] - [CORT,28°C]	0.218	0.001	0.020	0.414	0.031
		[CORT,23°C] - [CORT,28°C]	0.228	0.001	0.038	0.419	0.019
<i>L. guichenoti</i>	Hatchling Mass (g)	[Control,23°C] - [Control,28°C]	0.003	<0.001	0.001	0.006	0.017
		[Control,23°C] - [CORT,23°C]	0.002	<0.001	-0.001	0.004	0.275
		[Control,23°C] - [CORT,28°C]	0.005	<0.001	0.001	0.009	0.012
		[Control,28°C] - [CORT,23°C]	-0.002	<0.001	-0.006	0.002	0.343
		[Control,28°C] - [CORT,28°C]	0.002	<0.001	-0.001	0.004	0.275
		[CORT,23°C] - [CORT,28°C]	0.003	<0.001	0.001	0.006	0.017

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
	Hatchling SVL (mm)	[Control,23°C] - [Control,28°C]	0.203	0.001	0.011	0.396	0.038
		[Control,23°C] - [CORT,23°C]	0.057	0.001	-0.130	0.247	0.555
		[Control,23°C] - [CORT,28°C]	0.260	0.001	-0.004	0.525	0.053
		[Control,28°C] - [CORT,23°C]	-0.146	0.001	-0.423	0.129	0.302
		[Control,28°C] - [CORT,28°C]	0.057	0.001	-0.130	0.247	0.555
		[CORT,23°C] - [CORT,28°C]	0.203	0.001	0.011	0.396	0.038

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Supplemental Table 10. Comparisons of CORT-treatment and incubation temperature effects on hatchling size (mass or SVL) between *L. delicata* and *L. guichenoti*. Contrasts are the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on hatchling size. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Hatchling Mass (g)	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.001	<0.001	-0.004	0.003	0.669
	Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.001	<0.001	-0.004	0.003	0.753
Hatchling SVL (mm)	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.161	0.001	-0.431	0.116	0.245
	Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.025	0.001	-0.295	0.246	0.852

Supplemental Table 11. Analysis of CORT and incubation temperature effects on growth trajectories of mass in *L. delicata* and *L. guichenoti*. For the growth rate coefficient (K) and asymptotic maximum mass (Linf) of *L. delicata* and *L. guichenoti*, we show the difference in mean between posterior distributions (Estimate) predicted by Bayesian von Bertalanffy growth models, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Growth Rate Coefficient (K; 1/days)	Stress [CORT - Control]	0.00021	<0.00001	0.00001	0.00061	<0.001
		Temperature [28°C - 23°C]	0.00022	<0.00001	0.00001	0.00065	<0.001
	Asymptotic Size (g)	Stress [CORT - Control]	0.00822	3e-05	0.00022	0.02877	<0.001
		Temperature [28°C - 23°C]	0.01571	5e-05	0.00050	0.04916	<0.001
<i>L. guichenoti</i>	Growth Rate Coefficient (K; 1/days)	Stress [CORT - Control]	0.00060	<0.00001	0.00005	0.00131	<0.001
		Temperature [28°C - 23°C]	0.00015	<0.00001	0.00000	0.00050	<0.001
	Asymptotic Size (g)	Stress [CORT - Control]	0.01205	4e-05	0.00031	0.04125	<0.001
		Temperature [28°C - 23°C]	0.03683	1e-04	0.00198	0.09152	<0.001

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73 Supplemental Table 12. Summary of predicted posterior distributions of *L. delicata* and *L. guichenoti* growth curves (mass) for each CORT-treatment by incubation
74 temperature combination. Predictive posterior distributions were generated by Bayesian von Bertalanffy growth models including CORT treatment and incubation
75 temperature as main effects. For *L. delicata*, population density was included as an independent main effect. Individual was included as a random effect to account for
76 repeated measurements. Models were run separately for each species. The mean of the predictive posterior distribution (Estimate), the standard error about the mean
77 (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Trait	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Growth Rate Coefficient (K; 1 / days)	Control, 23°C	0.00575	<0.00001	0.00524	0.00623
		Control, 28°C	0.00597	<0.00001	0.00547	0.00647
		CORT, 23°C	0.00595	<0.00001	0.00545	0.00646
		CORT, 28°C	0.00617	<0.00001	0.00568	0.00670
		Density	0.00041	<0.00001	0.00002	0.00097
	Asymptotic Maximum Size (Linf; grams)	Control, 23°C	1.13236	1e-04	1.09579	1.16757
		Control, 28°C	1.14807	1e-04	1.11256	1.18479
		CORT, 23°C	1.14058	1e-04	1.10446	1.17646
		CORT, 28°C	1.15629	1e-04	1.12121	1.19336
		Density	0.00536	3e-05	0.00015	0.01938
<i>L. guichenoti</i>	Growth Rate Coefficient (K; 1 / days)	Control, 23°C	0.00913	<0.00001	0.00860	0.00966
		Control, 28°C	0.00928	<0.00001	0.00876	0.00980
		CORT, 23°C	0.00973	<0.00001	0.00917	0.01035
		CORT, 28°C	0.00988	<0.00001	0.00932	0.01050
	Asymptotic Maximum Size (Linf; grams)	Control, 23°C	1.42403	0.00012	1.37984	1.46389
		Control, 28°C	1.46086	0.00012	1.42010	1.50352
		CORT, 23°C	1.43608	0.00012	1.39284	1.47711
		CORT, 28°C	1.47290	0.00012	1.43307	1.51541

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80 Supplemental Table 13. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* growth curves (mass). Data are the differences in predicted posterior
81 distributions generated by Bayesian von Bertalanffy growth models including CORT treatment and incubation temperature as main effects. For *L. delicata*, population density
82 was included as an independent main effect. Individual was included as a random effect to account for repeated measurements. Contrasts are the differences in posterior
83 distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower
84 (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from
85 zero.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Growth Rate Coefficient (K; 1/days)	[Control,23°C] - [Control,28°C]	-0.00022	<0.00001	-0.00065	-0.00001	<0.001
		[Control,23°C] - [CORT,23°C]	-0.00021	<0.00001	-0.00061	-0.00001	<0.001
		[Control,23°C] - [CORT,28°C]	-0.00043	<0.00001	-0.00100	-0.00007	<0.001
		[Control,28°C] - [CORT,23°C]	0.00002	<0.00001	-0.00047	0.00051	0.946
		[Control,28°C] - [CORT,28°C]	-0.00021	<0.00001	-0.00061	-0.00001	<0.001
		[CORT,23°C] - [CORT,28°C]	-0.00022	<0.00001	-0.00065	-0.00001	<0.001
	Asymptotic Size (g)	[Control,23°C] - [Control,28°C]	-0.01571	7e-05	-0.04916	-0.00050	<0.001
		[Control,23°C] - [CORT,23°C]	-0.00822	4e-05	-0.02877	-0.00022	<0.001
		[Control,23°C] - [CORT,28°C]	-0.02393	9e-05	-0.06131	-0.00307	<0.001
		[Control,28°C] - [CORT,23°C]	0.00749	9e-05	-0.01964	0.04250	0.628
		[Control,28°C] - [CORT,28°C]	-0.00822	4e-05	-0.02877	-0.00022	<0.001
		[CORT,23°C] - [CORT,28°C]	-0.01571	7e-05	-0.04916	-0.00050	<0.001
<i>L. guichenoti</i>	Growth Rate Coefficient (K; 1/days)	[Control,23°C] - [Control,28°C]	-0.00015	<0.00001	-0.00050	0.00000	<0.001
		[Control,23°C] - [CORT,23°C]	-0.00060	<0.00001	-0.00131	-0.00005	<0.001
		[Control,23°C] - [CORT,28°C]	-0.00075	<0.00001	-0.00152	-0.00014	<0.001
		[Control,28°C] - [CORT,23°C]	-0.00045	<0.00001	-0.00120	0.00018	0.186
		[Control,28°C] - [CORT,28°C]	-0.00060	<0.00001	-0.00131	-0.00005	<0.001

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
		[CORT,23°C] - [CORT,28°C]	-0.00015	<0.00001	-0.00050	0.00000	<0.001
	Asymptotic	[Control,23°C] - [Control,28°C]	-0.03683	0.00014	-0.09152	-0.00198	<0.001
	Size (g)	[Control,23°C] - [CORT,23°C]	-0.01205	6e-05	-0.04125	-0.00031	<0.001
		[Control,23°C] - [CORT,28°C]	-0.04887	0.00015	-0.10862	-0.00781	<0.001
		[Control,28°C] - [CORT,23°C]	0.02478	0.00015	-0.02190	0.08198	0.34
		[Control,28°C] - [CORT,28°C]	-0.01205	6e-05	-0.04125	-0.00031	<0.001
		[CORT,23°C] - [CORT,28°C]	-0.03683	0.00014	-0.09152	-0.00198	<0.001

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88 Supplemental Table 14. Comparisons of CORT-treatment and incubation temperature effects on growth curves (mass) between *L. delicata* and *L. guichenoti*. Contrasts are the
89 differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects
90 on growth parameters. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper
91 (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Growth Rate Coefficient (K; 1/days)	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.00040	<0.00001	-0.00116	0.00028	0.284
	Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.00007	<0.00001	-0.00036	0.00055	0.739
Asymptotic Size (g)	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.00383	5e-05	-0.03514	0.02175	0.795
	Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.02112	0.00011	-0.07982	0.02925	0.449

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Supplemental Table 15. Analysis of sex-differences and population density effects on the growth in mass of *L. delicata* and *L. guichenoti*. Estimates are the mean differences in posterior distributions between groups as predicted by Bayesian von Bertalanffy growth models. Est.Err. is the standard error about the resulting distribution. L95% C.I. and U95% C.I. are, respectively, the lower and upper 95% confidence intervals of the resulting distribution. The pMCMC value is a test statistic of whether the difference between distributions is different from zero. A pMCMC value of <0.05 indicates a significant effect. We tested for sex-differences in growth as the difference in distributions of females to males, and population density effects as the difference between distributions predicted for lizards in high-density enclosures and low-density enclosures. Only population density was included as an independent effect in our main analysis of developmental effects on *L. delicata*. Sex-differences and population density effects for *L. guichenoti* are from a secondary model including only sex and population density as main effects.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Growth Rate Coefficient (K; 1/days)	Population Density [High - Low]	0.00041	<0.00001	0.00002	0.00097	<0.001
	Asymptotic Size (g)	Population Density [High - Low]	0.00536	3e-05	0.00015	0.01938	<0.001
<i>L. guichenoti</i>	Growth Rate Coefficient (K; 1/days)	Sex [Female - Male]	0.00054	<0.00001	0.00004	0.00124	<0.001
		Population Density [High - Low]	0.00037	<0.00001	0.00002	0.00098	<0.001
	Asymptotic Size (g)	Sex [Female - Male]	0.09317	0.00012	0.03088	0.15558	<0.001
		Population Density [High - Low]	0.00417	2e-05	0.00011	0.01527	<0.001

Supplemental Table 16. Analysis of CORT and incubation temperature effects on growth trajectories for body length. For the growth rate coefficient (K) and asymptotic maximum mass (Linf) of *L. delicata* and *L. guichenoti*, we show the difference in mean between posterior distributions (Estimate) predicted by Bayesian von Bertalanffy growth models, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C). Additionally, for *L. guichenoti*, we test for sex-differences in growth trajectories as the difference between distributions predicted for females and males.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Growth Rate Coefficient (K; 1/days)	Stress [CORT - Control]	0.00073	<0.00001	0.00004	0.00181	<0.001
		Temperature [28°C - 23°C]	0.00027	<0.00001	0.00001	0.00089	<0.001
	Asymptotic Size (mm)	Stress [CORT - Control]	0.06457	0.00024	0.00170	0.22871	<0.001
		Temperature [28°C - 23°C]	0.09879	0.00036	0.00290	0.34203	<0.001
<i>L. guichenoti</i>	Growth Rate Coefficient (K; 1/days)	Stress [CORT - Control]	0.00033	<0.00001	0.00001	0.00088	<0.001
		Temperature [28°C - 23°C]	0.00005	<0.00001	0.00000	0.00018	<0.001
	Asymptotic Size (mm)	Stress [CORT - Control]	0.07710	0.00028	0.00201	0.26496	<0.001
		Temperature [28°C - 23°C]	0.09654	0.00034	0.00281	0.32065	<0.001

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Supplemental Table 17. Summary of predicted posterior distributions of *L. delicata* and *L. guichenoti* growth curves (SVL) for each CORT-treatment by incubation temperature combination. Predictive posterior distributions were generated by Bayesian von Bertalanffy growth models including CORT treatment and incubation temperature as main effects. For *L. guichenoti*, sex was included as an independent main effect. Individual was included as a random effect to account for repeated measurements. Models were run separately for each species. The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Trait	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Growth Rate Coefficient (K; 1 / days)	Control, 23°C	0.02141	<0.00001	0.02025	0.02264
		Control, 28°C	0.02168	<0.00001	0.02049	0.02295
		CORT, 23°C	0.02214	<0.00001	0.02085	0.02357
		CORT, 28°C	0.02241	<0.00001	0.02108	0.02388
	Asymptotic Maximum Size (Linf; millimeters)	Control, 23°C	38.87434	0.00099	38.51937	39.21847
		Control, 28°C	38.97313	0.00099	38.62361	39.32177
		CORT, 23°C	38.93891	0.00099	38.58598	39.28469
		CORT, 28°C	39.03770	0.00099	38.69030	39.38887
<i>L. guichenoti</i>	Growth Rate Coefficient (K; 1 / days)	Control, 23°C	0.01784	<0.00001	0.01700	0.01872
		Control, 28°C	0.01789	<0.00001	0.01705	0.01878
		CORT, 23°C	0.01817	<0.00001	0.01730	0.01909
		CORT, 28°C	0.01822	<0.00001	0.01736	0.01915
	Asymptotic Maximum Size (Linf; millimeters)	Sex	0.00009	<0.00001	0.00000	0.00032
		Control, 23°C	41.39172	0.00107	41.01817	41.76216
		Control, 28°C	41.48826	0.0011	41.10771	41.87510
		CORT, 23°C	41.46882	0.00107	41.09923	41.84270
		CORT, 28°C	41.56536	0.00109	41.18879	41.95549
		Sex	1.83995	0.00134	1.37036	2.30941

116 Supplemental Table 18. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* growth curves (SVL). Data are the differences in predicted posterior
117 distributions generated by Bayesian von Bertalanffy growth models including CORT treatment and incubation temperature as main effects. For *L. guichenoti*, sex was
118 included as an independent main effect. Individual was included as a random effect to account for repeated measurements. Contrasts are the differences in posterior
119 distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower
120 (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from
121 zero.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Growth Rate Coefficient (K; 1/days)	[Control,23°C] - [Control,28°C]	-0.00027	<0.00001	-0.00089	-0.00001	<0.001
		[Control,23°C] - [CORT,23°C]	-0.00073	<0.00001	-0.00181	-0.00004	<0.001
		[Control,23°C] - [CORT,28°C]	-0.00100	<0.00001	-0.00222	-0.00016	<0.001
		[Control,28°C] - [CORT,23°C]	-0.00046	<0.00001	-0.00160	0.00048	0.381
		[Control,28°C] - [CORT,28°C]	-0.00073	<0.00001	-0.00181	-0.00004	<0.001
		[CORT,23°C] - [CORT,28°C]	-0.00027	<0.00001	-0.00089	-0.00001	<0.001
	Asymptotic Size (mm)	[Control,23°C] - [Control,28°C]	-0.09879	0.00051	-0.34203	-0.00290	<0.001
		[Control,23°C] - [CORT,23°C]	-0.06457	0.00034	-0.22871	-0.00170	<0.001
		[Control,23°C] - [CORT,28°C]	-0.16336	0.00062	-0.43711	-0.02104	<0.001
		[Control,28°C] - [CORT,23°C]	0.03423	0.00062	-0.16872	0.29299	0.776
		[Control,28°C] - [CORT,28°C]	-0.06457	0.00034	-0.22871	-0.00170	<0.001
		[CORT,23°C] - [CORT,28°C]	-0.09879	0.00051	-0.34203	-0.00290	<0.001
	Growth Rate Coefficient (K; 1/days)	[Control,23°C] - [Control,28°C]	-0.00005	<0.00001	-0.00018	0.00000	<0.001
		[Control,23°C] - [CORT,23°C]	-0.00033	<0.00001	-0.00088	-0.00001	<0.001
		[Control,23°C] - [CORT,28°C]	-0.00038	<0.00001	-0.00094	-0.00004	<0.001
		[Control,28°C] - [CORT,23°C]	-0.00028	<0.00001	-0.00084	0.00006	0.17
		[Control,28°C] - [CORT,28°C]	-0.00033	<0.00001	-0.00088	-0.00001	<0.001
<i>L. guichenoti</i>	Growth Rate Coefficient (K; 1/days)	[Control,23°C] - [Control,28°C]	-0.00005	<0.00001	-0.00018	0.00000	<0.001
		[Control,23°C] - [CORT,23°C]	-0.00033	<0.00001	-0.00088	-0.00001	<0.001
		[Control,23°C] - [CORT,28°C]	-0.00038	<0.00001	-0.00094	-0.00004	<0.001
		[Control,28°C] - [CORT,23°C]	-0.00028	<0.00001	-0.00084	0.00006	0.17
		[Control,28°C] - [CORT,28°C]	-0.00033	<0.00001	-0.00088	-0.00001	<0.001

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
		[CORT,23°C] - [CORT,28°C]	-0.00005	<0.00001	-0.00018	0.00000	<0.001
	Asymptotic Size (mm)	[Control,23°C] - [Control,28°C]	-0.09654	0.00049	-0.32065	-0.00281	<0.001
		[Control,23°C] - [CORT,23°C]	-0.07710	4e-04	-0.26496	-0.00201	<0.001
		[Control,23°C] - [CORT,28°C]	-0.17364	0.00063	-0.44622	-0.02290	<0.001
		[Control,28°C] - [CORT,23°C]	0.01945	0.00063	-0.20486	0.26903	0.873
		[Control,28°C] - [CORT,28°C]	-0.07710	4e-04	-0.26496	-0.00201	<0.001
		[CORT,23°C] - [CORT,28°C]	-0.09654	0.00049	-0.32065	-0.00281	<0.001

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Supplemental Table 19. Comparisons of CORT-treatment and incubation temperature effects on growth curves (SVL) between *L. delicata* and *L. guichenoti*. Contrasts are the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on growth parameters. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Growth Rate Coefficient (K; 1/days)	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.00040	<0.00001	-0.00052	0.00157	0.467
	Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.00022	<0.00001	-0.00008	0.00085	0.278
Asymptotic Size (mm)	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.01253	0.00037	-0.22116	0.17798	0.904
	Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.00225	5e-04	-0.25458	0.27132	0.999

Supplemental Table 20. Analysis of sex-differences in the growth trajectories for body length of *L. guichenoti*. For the growth rate coefficient (K) and asymptotic maximum mass (Linf) of *L. delicata* and *L. guichenoti*, we show the difference in mean between posterior distributions (Estimate) predicted by Bayesian von Bertalanffy growth models, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. We tested for sex-differences in growth as the difference in distributions of female *L. guichenoti* to male *L. guichenoti*. Sex was only included as an independent effect in our main analysis of CORT and incubation temperature on *L. guichenoti*, and not *L. delicata*. Exploration of raw data did not support an independent effect of sex on the growth of *L. delicata*. Exploration also did not support independent effects of population density on growth in *L. delicata* or *L. guichenoti*.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. guichenoti</i>	Growth Rate Coefficient (K; 1/days)	Sex [Female - Male]	0.00009	<0.00001	0.00000	0.00032	<0.001
	Asymptotic Size (mm)	Sex [Female - Male]	1.83995	0.00134	1.37036	2.30941	<0.001

139 Supplemental Table 21. Summary statistics of raw adult body size for *L. delicata* and *L. guichenoti*. Data are body size measurements from lizards captured during the final
140 remeasurement period in longitudinal studies, with an average age of 760 (± 21) days for both *L. delicata* and *L. guichenoti*. N is the number of animals measured for each
141 developmental CORT (Control or CORT) by incubation temperature (23°C or 28°C) treatment. Means $\pm$ SD for mass (grams) and SVL (mm) are the population-level
142 averages, not accounting for sex-differences or population density.

Species	Trait	Control,23°C	CORT,23°C	Control,28°C	CORT,28°C
<i>L. delicata</i>	N	41	37	40	40
	Adult Mass (g)	1.146 $\pm$ 0.217	1.079 $\pm$ 0.216	1.156 $\pm$ 0.214	1.058 $\pm$ 0.202
	Adult SVL (mm)	42.268 $\pm$ 2.48	41 $\pm$ 2.404	41.949 $\pm$ 1.877	40.475 $\pm$ 2.242
<i>L. guichenoti</i>	N	51	48	53	55
	Adult Mass (g)	1.343 $\pm$ 0.269	1.268 $\pm$ 0.235	1.372 $\pm$ 0.285	1.29 $\pm$ 0.284
	Adult SVL (mm)	43.569 $\pm$ 2.532	43.188 $\pm$ 2.639	44.774 $\pm$ 2.965	43.782 $\pm$ 2.587

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145 Supplemental Table 22. Analysis of CORT and incubation temperature effects on adult mass in *L. delicata* and *L. guichenoti*. Data are predicted posterior distributions
 146 generated by Bayesian GLMMs including both adult mass and adult SVL as response variables. For the mass of adult *L. delicata* and *L. guichenoti*, we show the difference in
 147 mean between predicted posterior distributions (Estimate), the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence
 148 intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown
 149 for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and
 150 incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Adult Mass (g)	Stress [CORT - Control]	-0.084	<0.001	-0.135	-0.034	0.001
		Temperature [28°C - 23°C]	-0.128	<0.001	-0.186	-0.071	<0.001
<i>L. guichenoti</i>		Stress [CORT - Control]	-0.074	<0.001	-0.132	-0.015	0.014
		Temperature [28°C - 23°C]	-0.092	<0.001	-0.158	-0.026	0.006

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Supplemental Table 23. Analysis of CORT and incubation temperature effects on adult mass in *L. delicata* and *L. guichenoti*. Data are predicted posterior distributions generated by Bayesian GLMMs including both adult mass and adult SVL as response variables. For the mass of adult *L. delicata* and *L. guichenoti*, we show the difference in mean between predicted posterior distributions (Estimate), the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Adult SVL (mm)	Stress [CORT - Control]	-1.180	0.001	-1.782	-0.580	<0.001
		Temperature [28°C - 23°C]	-1.791	0.001	-2.469	-1.106	<0.001
<i>L. guichenoti</i>		Stress [CORT - Control]	-0.655	0.001	-1.177	-0.130	0.014
		Temperature [28°C - 23°C]	-0.596	0.001	-1.179	0.001	0.05

159 Supplemental Table 24. Summary of predicted posterior distributions of *L. delicata* and *L. guichenoti* adult size (mass or SVL) for each CORT-treatment by incubation
160 temperature combination. Predictive posterior distributions were generated by multi-response Bayesian GLMMs models including CORT treatment and incubation
161 temperature as main effects. Sex and population density were included as independent main effects. Age (centred about the mean) was included as a covariate to account for
162 the effects of variation in age on body size. Models were run separately for each species. The mean of the predictive posterior distribution (Estimate), the standard error about
163 the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Trait	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Adult Mass (g)	Control,23°C	1.361	0.000	1.300	1.421
		Control,28°C	1.233	0.000	1.173	1.293
		CORT,23°C	1.276	0.000	1.215	1.338
		CORT,28°C	1.148	0.000	1.090	1.206
		Sex	-0.098	0.000	-0.154	-0.042
		Population Density	-0.164	0.000	-0.217	-0.110
	Adult SVL (mm)	Control,23°C	43.657	0.002	42.946	44.372
		Control,28°C	41.866	0.002	41.160	42.585
		CORT,23°C	42.477	0.002	41.743	43.200
		CORT,28°C	40.686	0.002	39.990	41.374
		Sex	0.812	0.002	0.148	1.473
		Population Density	-1.511	0.002	-2.137	-0.876
<i>L. guichenoti</i>	Adult Mass (g)	Control,23°C	1.625	0.000	1.548	1.704
		Control,28°C	1.533	0.000	1.456	1.610
		CORT,23°C	1.552	0.000	1.473	1.631
		CORT,28°C	1.459	0.000	1.382	1.536
		Sex	-0.020	0.000	-0.080	0.040
		Population Density	-0.309	0.000	-0.373	-0.246

Species	Trait	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
	Adult SVL (mm)	Control,23°C	45.178	0.002	44.486	45.884
		Control,28°C	44.582	0.002	43.889	45.277
		CORT,23°C	44.523	0.002	43.820	45.229
		CORT,28°C	43.927	0.002	43.242	44.610
		Sex	2.069	0.002	1.526	2.613
		Population Density	-2.664	0.002	-3.240	-2.089

Supplemental Table 25. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* adult size (mass or SVL). Data are the differences in predicted posterior distributions generated by multi-response Bayesian GLMMs including CORT treatment and incubation temperature as main effects. Sex and population density were included as independent main effects. Age (centred about the mean) was included as a covariate to account for the effects of variation in age on body size. Contrasts are the differences in posterior distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Adult Mass (g)	[Control,23°C] - [Control,28°C]	0.128	<0.001	0.071	0.186	<0.001
		[Control,23°C] - [CORT,23°C]	0.084	<0.001	0.034	0.135	0.001
		[Control,23°C] - [CORT,28°C]	0.213	<0.001	0.138	0.287	<0.001
		[Control,28°C] - [CORT,23°C]	-0.044	<0.001	-0.122	0.036	0.276
		[Control,28°C] - [CORT,28°C]	0.084	<0.001	0.034	0.135	0.001
		[CORT,23°C] - [CORT,28°C]	0.128	<0.001	0.071	0.186	<0.001
	Adult SVL (mm)	[Control,23°C] - [Control,28°C]	1.791	0.002	1.106	2.469	<0.001
		[Control,23°C] - [CORT,23°C]	1.180	0.002	0.580	1.782	<0.001
		[Control,23°C] - [CORT,28°C]	2.971	0.003	2.085	3.857	<0.001
		[Control,28°C] - [CORT,23°C]	-0.610	0.003	-1.538	0.321	0.2
		[Control,28°C] - [CORT,28°C]	1.180	0.002	0.580	1.782	<0.001
		[CORT,23°C] - [CORT,28°C]	1.791	0.002	1.106	2.469	<0.001
<i>L. guichenoti</i>	Adult Mass (g)	[Control,23°C] - [Control,28°C]	0.092	<0.001	0.026	0.158	0.006
		[Control,23°C] - [CORT,23°C]	0.074	<0.001	0.015	0.132	0.014
		[Control,23°C] - [CORT,28°C]	0.166	<0.001	0.080	0.252	<0.001
		[Control,28°C] - [CORT,23°C]	-0.019	<0.001	-0.109	0.070	0.684
		[Control,28°C] - [CORT,28°C]	0.074	<0.001	0.015	0.132	0.014

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
		[CORT,23°C] - [CORT,28°C]	0.092	<0.001	0.026	0.158	0.006
	Adult SVL (mm)	[Control,23°C] - [Control,28°C]	0.596	0.002	-0.001	1.179	0.05
		[Control,23°C] - [CORT,23°C]	0.655	0.001	0.130	1.177	0.014
		[Control,23°C] - [CORT,28°C]	1.251	0.002	0.481	2.033	0.001
		[Control,28°C] - [CORT,23°C]	0.058	0.002	-0.730	0.864	0.893
		[Control,28°C] - [CORT,28°C]	0.655	0.001	0.130	1.177	0.014
		[CORT,23°C] - [CORT,28°C]	0.596	0.002	-0.001	1.179	0.05

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173 Supplemental Table 26. Comparisons of CORT-treatment and incubation temperature effects on adult size (mass or SVL) between *L. delicata* and *L. guichenoti*. Contrasts are
 174 the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C)
 175 effects on body size. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper
 176 (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Adult Mass (g)	Stress	-0.011	<0.001	-0.088	0.067	0.779
	[<i>L. delicata</i> - <i>L. guichenoti</i>]					
	Temperature	-0.036	<0.001	-0.123	0.053	0.423
	[<i>L. delicata</i> - <i>L. guichenoti</i>]					
Adult SVL (mm)	Stress	-0.526	0.002	-1.321	0.273	0.193
	[<i>L. delicata</i> - <i>L. guichenoti</i>]					
	Temperature	-1.194	0.002	-2.101	-0.291	0.009
	[<i>L. delicata</i> - <i>L. guichenoti</i>]					

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178 Supplemental Table 27. Analysis of sex-differences and population density effects on the mass of adult *L. delicata* and *L. guichenoti*. Estimates are the mean differences in
179 posterior distributions between groups as predicted by multi-response Bayesian GLMMs including both mass and SVL as response variables. Est.Err. is the standard error
180 about the resulting distribution. L95% C.I. and U95% C.I. are, respectively, the lower and upper 95% confidence intervals of the resulting distribution. The pMCMC value is
181 a test statistic of whether the difference between distributions is different from zero. A pMCMC value of <0.05 indicates a significant effect. We tested for sex-differences in
182 growth as the difference in distributions of females to males, and population density effects as the difference between distributions predicted for lizards in high-density
183 enclosures and low-density enclosures. Population density and sex were included as independent effects in the main analysis of CORT and incubation temperature effects on
184 adult mass for both species.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Adult Mass (g)	Sex [Female - Male]	-0.098	<0.001	-0.154	-0.042	0.001
		Population Density [High - Low]	-0.164	<0.001	-0.154	-0.110	<0.001
<i>L. guichenoti</i>		Sex [Female - Male]	-0.020	<0.001	-0.080	0.040	0.507
		Population Density [High - Low]	-0.309	<0.001	-0.080	-0.246	<0.001

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186 Supplemental Table 28. Analysis of sex-differences and population density effects on the SVL of adult *L. delicata* and *L. guichenoti*. Estimates are the mean differences in
187 posterior distributions between groups as predicted by multi-response Bayesian GLMMs including both mass and SVL as response variables. Est.Err. is the standard error
188 about the resulting distribution. L95% C.I. and U95% C.I. are, respectively, the lower and upper 95% confidence intervals of the resulting distribution. The pMCMC value is
189 a test statistic of whether the difference between distributions is different from zero. A pMCMC value of <0.05 indicates a significant effect. We tested for sex-differences in
190 growth as the difference in distributions of females to males, and population density effects as the difference between distributions predicted for lizards in high-density
191 enclosures and low-density enclosures. Population density and sex were included as independent effects in the main analysis of CORT and incubation temperature effects on
192 adult mass for both species.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
L. delicata	Adult SVL (mm)	Sex [Female - Male]	0.812	0.002	0.148	1.473	0.017
		Population Density [High - Low]	-1.511	0.002	0.148	-0.876	<0.001
L. guichenoti		Sex [Female - Male]	2.069	0.002	1.526	2.613	<0.001
		Population Density [High - Low]	-2.664	0.002	1.526	-2.089	<0.001

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Supplemental Table 29. Summary of raw survival data from *L. delicata* and *L. guichenoti* in naturalistic outdoor enclosures. Data are derived from counts of animals that were not recaptured in either of the last two remeasurement periods, when lizards were ~500 or ~760 days old. We give the total number of lizards assigned to each developmental CORT (Control or CORT) by incubation temperature (23°C or 28°C) treatment, the number that were considered to have survived or died, and the percentage of the total that survived or died.

Species	Trait	Control,23°C	CORT,23°C	Control,28°C	CORT,28°C
<i>L. delicata</i>	N (Total)	59	57	61	60
	N (Survived)	52	48	53	50
	N (Died)	7	9	8	10
	Survival (%)	88.1%	84.2%	86.9%	83.3%
	Mortality (%)	11.9%	15.8%	13.1%	16.7%
<i>L. guichenoti</i>	N (Total)	67	67	65	69
	N (Survived)	61	61	62	66
	N (Died)	6	6	3	3
	Survival (%)	91%	91%	95.4%	95.7%
	Mortality (%)	9%	9%	4.6%	4.3%

199 Supplemental Table 30. Analysis of CORT and incubation temperature effects on the probability of mortality in late adulthood in *L. delicata* and *L. guichenoti*. Data are the
 200 predicted posterior distributions of a Bayesian GLMM on the ‘logit’ scale in which mortality included CORT-treatment and incubation temperature as fixed effects, and
 201 enclosure as a random intercept to account for enclosure-specific mortality rates. For mortality probabilities in *L. delicata* and *L. guichenoti*, we show the difference in mean
 202 between posterior distributions (Estimate) predicted by Bayesian von Bertalanffy growth models, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and
 203 upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from
 204 zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the
 205 distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Mortality Probability	Stress [CORT - Control]	0.442	0.002	-0.404	1.297	0.307
		Temperature [28°C - 23°C]	0.142	0.002	-0.713	1.003	0.741
<i>L. guichenoti</i>		Stress [CORT - Control]	-0.034	0.002	-1.029	0.962	0.947
		Temperature [28°C - 23°C]	-0.778	0.002	-1.844	0.215	0.127

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207 Supplemental Table 31. Summary of predicted posterior distributions of *L. delicata* and *L. guichenoti* mortality probabilities for each CORT-treatment by incubation
 208 temperature combination. Predictive posterior distributions were generated by Bayesian GLMMs including CORT treatment and incubation temperature as main effects. For
 209 *L. delicata*, sex was included as an independent main effect. Enclosure was included as a random effect to account for enclosure-specific mortality events. Models were run
 210 separately for each species. The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper
 211 (U95% C.I.) values are provided. Estimate, L95% C.I., and U95% C.I. values are on a logit scale.

Species	Trait	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Mortality Probability	Control,23°C	-3.825	0.004	-5.332	-2.570
		Control,28°C	-3.682	0.004	-5.140	-2.471
		CORT,23°C	-3.383	0.004	-4.804	-2.195
		CORT,28°C	-3.241	0.004	-4.615	-2.097
<i>L. guichenoti</i>		Control,23°C	-2.461	0.003	-3.425	-1.652
		Control,28°C	-3.239	0.003	-4.379	-2.276
		CORT,23°C	-2.495	0.003	-3.460	-1.685
		CORT,28°C	-3.273	0.003	-4.419	-2.317

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Supplemental Table 32. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* mortality probabilities. Data are the differences in predicted posterior distributions generated by Bayesian GLMMs including CORT treatment and incubation temperature as main effects. For *L. delicata*, sex was included as an independent main effect. Enclosure was included as a random effect to account for enclosure-specific mortality events. Models were run separately for each species. Contrasts are the differences in posterior distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. Estimate, L95% C.I., and U95% C.I. values are on a logit scale. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Mortality Probability	[Control,23°C] - [Control,28°C]	-0.1425	0.0024	-1.0025	0.7127	0.7411
		[Control,23°C] - [CORT,23°C]	-0.4416	0.0024	-1.2973	0.4041	0.3067
		[Control,23°C] - [CORT,28°C]	-0.5841	0.0035	-1.8153	0.6243	0.3425
		[Control,28°C] - [CORT,23°C]	-0.2991	0.0034	-1.4963	0.9007	0.6249
		[Control,28°C] - [CORT,28°C]	-0.4416	0.0024	-1.2973	0.4041	0.3067
		[CORT,23°C] - [CORT,28°C]	-0.1425	0.0024	-1.0025	0.7127	0.7411
<i>L. guichenoti</i>		[Control,23°C] - [Control,28°C]	0.7782	0.0029	-0.2153	1.8442	0.1271
		[Control,23°C] - [CORT,23°C]	0.0340	0.0028	-0.9622	1.0294	0.9465
		[Control,23°C] - [CORT,28°C]	0.8122	0.0041	-0.5954	2.2573	0.2586
		[Control,28°C] - [CORT,23°C]	-0.7441	0.0041	-2.1934	0.6636	0.3048
		[Control,28°C] - [CORT,28°C]	0.0340	0.0028	-0.9622	1.0294	0.9465
		[CORT,23°C] - [CORT,28°C]	0.7782	0.0029	-0.2153	1.8442	0.1271

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220 Supplemental Table 33. Comparisons of CORT-treatment and incubation temperature effects on mortality probabilities between *L. delicata* and *L. guichenoti*. Contrasts are
221 the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C)
222 effects on mortality probabilities. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.)
223 and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Mortality Probability	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.4756	0.0026	-0.8363	1.7725	0.4728
	Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.9207	0.0027	-0.3933	2.2849	0.1736

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Supplemental Table 34. Analysis of developmental CORT and incubation temperature effects on resting metabolic rates of oxygen consumption (RMR VO₂) and carbon dioxide production (RMR VCO₂) in juvenile *L. delicata* and *L. guichenoti*. Data are derived from gas exchange measurements acquired via push-mode flow respirometry. Data were log-transformed before modelling. Data were modelled as a multi-response Bayesian GLMMs including CORT-treatment and incubation temperature as fixed effects, with the logarithmic mass of each lizard included as a covariate to account for mass-scaling of metabolic rate, and date included as a random intercept. Residual correlations between VO₂ and VCO₂ were accounted for in the multi-response GLMM. Predicted posterior distributions were exponentiated back into the original units of gas exchange (mL • min⁻¹) for reporting. We show the difference in mean between posterior distributions (Estimate) predicted by Bayesian GLMM, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	RMR VO ₂ (mL O ₂ • min ⁻¹)	Stress [CORT - Control]	-0.0005	<0.0001	-0.0014	0.0005	0.302
		Temperature [28°C - 23°C]	0.0001	<0.0001	-0.0008	0.0010	0.852
	RMR VCO ₂ (mL CO ₂ • min ⁻¹)	Stress [CORT - Control]	-0.0004	<0.0001	-0.0011	0.0005	0.371
		Temperature [28°C - 23°C]	0.0001	<0.0001	-0.0006	0.0009	0.724
<i>L. guichenoti</i>	RMR VO ₂ (mL O ₂ • min ⁻¹)	Stress [CORT - Control]	0.0003	<0.0001	-0.0005	0.0011	0.470
		Temperature [28°C - 23°C]	-0.0008	<0.0001	-0.0016	0.0000	0.050
	RMR VCO ₂ (mL CO ₂ • min ⁻¹)	Stress [CORT - Control]	0.0005	<0.0001	-0.0002	0.0012	0.186
		Temperature [28°C - 23°C]	-0.0006	<0.0001	-0.0013	0.0001	0.113

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Supplemental Table 35. Summary of predicted posterior distributions of *L. delicata* and *L. guichenoti* resting metabolic rates of gas exchange (VO₂ or VCO₂) for each CORT-treatment by incubation temperature combination. Predictive posterior distributions were generated by multi-response Bayesian GLMMs including CORT treatment and incubation temperature as main effects. Log-transformed mass was included as a covariate to account for the mass-scaling of metabolic rates. Trial date was included as a random effect to account for any unexplained sources of environmental or machine variation throughout the experiment. Models were run separately for each species. The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Trait	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	RMR VO ₂ (mL O ₂ / min)	Control,23°C	0.0064	<0.0001	0.0057	0.0072
		Control,28°C	0.0065	<0.0001	0.0058	0.0073
		CORT,23°C	0.0059	<0.0001	0.0052	0.0067
		CORT,28°C	0.0060	<0.0001	0.0052	0.0069
	RMR VCO ₂ (mL CO ₂ / min)	Control,23°C	0.0071	<0.0001	0.0063	0.0080
		Control,28°C	0.0073	<0.0001	0.0065	0.0082
		CORT,23°C	0.0068	<0.0001	0.0060	0.0077
		CORT,28°C	0.0069	<0.0001	0.0060	0.0079
<i>L. guichenoti</i>	RMR VO ₂ (mL O ₂ / min)	Control,23°C	0.0067	<0.0001	0.0059	0.0074
		Control,28°C	0.0059	<0.0001	0.0053	0.0065
		CORT,23°C	0.0070	<0.0001	0.0062	0.0078
		CORT,28°C	0.0062	<0.0001	0.0055	0.0069
	RMR VCO ₂ (mL CO ₂ / min)	Control,23°C	0.0071	<0.0001	0.0063	0.0079
		Control,28°C	0.0065	<0.0001	0.0059	0.0073
		CORT,23°C	0.0076	<0.0001	0.0067	0.0085
		CORT,28°C	0.0070	<0.0001	0.0062	0.0078

244 Supplemental Table 36. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* resting metabolic rates of gas exchange (VO₂ or VCO₂). Data are the
245 differences in predicted posterior distributions generated by multi-response Bayesian GLMMs including CORT treatment and incubation temperature as main effects. Log-
246 transformed mass was included as a covariate to account for the mass-scaling of metabolic rates. Trial date was included as a random effect to account for any unexplained
247 sources of environmental or machine variation throughout the experiment. Models were run separately for each species. Contrasts are the differences in posterior distributions
248 between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.)
249 and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	RMR VO ₂ (mL O ₂ / min)	[Control,23°C] - [Control,28°C]	-0.0001	<0.0001	-0.001	8e-04	0.8524
		[Control,23°C] - [CORT,23°C]	0.0005	<0.0001	-5e-04	0.0014	0.3021
		[Control,23°C] - [CORT,28°C]	0.0004	<0.0001	-0.001	0.0017	0.5628
		[Control,28°C] - [CORT,23°C]	0.0006	<0.0001	-6e-04	0.0017	0.3352
		[Control,28°C] - [CORT,28°C]	0.0005	<0.0001	-5e-04	0.0014	0.3021
		[CORT,23°C] - [CORT,28°C]	-0.0001	<0.0001	-9e-04	7e-04	0.8524
	RMR VCO ₂ (mL CO ₂ / min)	[Control,23°C] - [Control,28°C]	-0.0001	<0.0001	-9e-04	6e-04	0.7239
		[Control,23°C] - [CORT,23°C]	0.0004	<0.0001	-5e-04	0.0011	0.3710
		[Control,23°C] - [CORT,28°C]	0.0002	<0.0001	-0.001	0.0014	0.7083
		[Control,28°C] - [CORT,23°C]	0.0005	<0.0001	-5e-04	0.0015	0.3271
		[Control,28°C] - [CORT,28°C]	0.0004	<0.0001	-5e-04	0.0011	0.3710
		[CORT,23°C] - [CORT,28°C]	-0.0001	<0.0001	-9e-04	6e-04	0.7239
<i>L. guichenoti</i>	RMR VO ₂ (mL O ₂ / min)	[Control,23°C] - [Control,28°C]	0.0008	<0.0001	<0.0001	0.0015	0.0499
		[Control,23°C] - [CORT,23°C]	-0.0003	<0.0001	-0.0012	5e-04	0.4696
		[Control,23°C] - [CORT,28°C]	0.0005	<0.0001	-6e-04	0.0016	0.3901
		[Control,28°C] - [CORT,23°C]	-0.0011	<0.0001	-0.0022	<0.0001	0.0539
		[Control,28°C] - [CORT,28°C]	-0.0003	<0.0001	-0.001	5e-04	0.4696

Species	Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
		[CORT,23°C] - [CORT,28°C]	0.0008	<0.0001	<0.0001	0.0016	0.0499
	RMR VCO ₂ (mL CO ₂ / min)	[Control,23°C] - [Control,28°C]	0.0005	<0.0001	-1e-04	0.0012	0.1134
		[Control,23°C] - [CORT,23°C]	-0.0005	<0.0001	-0.0012	2e-04	0.1856
		[Control,23°C] - [CORT,28°C]	0.0001	<0.0001	-9e-04	0.0011	0.8565
		[Control,28°C] - [CORT,23°C]	-0.0010	<0.0001	-0.0021	-1e-04	0.0379
		[Control,28°C] - [CORT,28°C]	-0.0005	<0.0001	-0.0012	2e-04	0.1856
		[CORT,23°C] - [CORT,28°C]	0.0006	<0.0001	-2e-04	0.0013	0.1134

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252 Supplemental Table 37. Comparisons of CORT-treatment and incubation temperature effects on resting metabolic rates of gas exchange (VO₂ or VCO₂) between *L. delicata*
 253 and *L. guichenoti*. Contrasts are the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation
 254 temperature (28°C – 23°C) effects on metabolic rate. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the
 255 lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different
 256 from zero.

Trait	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
RMR VO ₂ (mL O ₂ / min)	Stress	-0.0008	<0.0001	-0.0020	0.0005	0.2114
	[<i>L. delicata</i> - <i>L. guichenoti</i>]					
	Temperature	0.0009	<0.0001	-0.0003	0.0020	0.1438
	[<i>L. delicata</i> - <i>L. guichenoti</i>]					
RMR VCO ₂ (mL CO ₂ / min)	Stress	-0.0008	<0.0001	-0.0019	0.0002	0.1253
	[<i>L. delicata</i> - <i>L. guichenoti</i>]					
	Temperature	0.0007	<0.0001	-0.0003	0.0018	0.1788
	[<i>L. delicata</i> - <i>L. guichenoti</i>]					

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Supplemental Table 38. Analysis of main effects of developmental CORT treatment and incubation temperature on cellular morphology in the livers of juvenile and adult *L. delicata* and *L. guichenoti*. Data are the predictive posterior distributions derived from a multi-response Bayesian GLMM including log-transformed fluorescence values of cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, and oxidative DNA damage as responses and accounting for residual correlations among each parameter. For each parameter, CORT treatment and incubation temperature were included as fixed effects, and plate number was included as a random intercept. Mitochondrial mass included cell size as an independent covariate to account for the influence of cell size variation on the quantity of mitochondria in a cell. We show the difference in mean between posterior distributions (Estimate) predicted by Bayesian GLMM, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Liver	Cell Size (MFI)	Juvenile	Stress [CORT - Control]	-0.021	<0.001	-0.087	0.044	0.520
				Temperature [28°C - 23°C]	-0.045	<0.001	-0.110	0.019	0.167
			Adult	Stress [CORT - Control]	0.009	<0.001	-0.024	0.042	0.582
				Temperature [28°C - 23°C]	-0.006	<0.001	-0.035	0.024	0.707
		Mitochondrial Mass (MFI)	Juvenile	Stress [CORT - Control]	0.031	<0.001	-0.040	0.102	0.389
				Temperature [28°C - 23°C]	-0.055	<0.001	-0.125	0.015	0.122
			Adult	Stress [CORT - Control]	-0.040	<0.001	-0.151	0.075	0.483
				Temperature [28°C - 23°C]	0.083	<0.001	-0.015	0.184	0.098
<i>L. guichenoti</i>		Cell Size (MFI)	Juvenile	Stress [CORT - Control]	-0.046	<0.001	-0.116	0.024	0.205

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
		Mitochondrial Mass (MFI)	Adult	Temperature [28°C - 23°C]	0.056	<0.001	-0.013	0.125	0.108
				Stress [CORT - Control]	-0.020	<0.001	-0.062	0.021	0.333
			Juvenile	Temperature [28°C - 23°C]	-0.017	<0.001	-0.059	0.024	0.404
				Stress [CORT - Control]	0.027	<0.001	-0.029	0.083	0.335
			Adult	Temperature [28°C - 23°C]	0.010	<0.001	-0.045	0.065	0.721
				Stress [CORT - Control]	0.027	<0.001	-0.066	0.121	0.565
				Temperature [28°C - 23°C]	0.031	<0.001	-0.065	0.128	0.512

Supplemental Table 39. Summary of predicted posterior distributions of juvenile and adult *L. delicata* and *L. guichenoti* cellular morphology (cell size or mitochondrial mass) in liver cells for each CORT-treatment by incubation temperature combination. Predictive posterior distributions were generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Liver	Cell Size (MFI)	Juvenile	Control,23°C	0.0349	0.0003	-0.0829	0.1505
				Control,28°C	-0.0099	0.0003	-0.1288	0.1073
				CORT,23°C	0.0136	0.0003	-0.1059	0.1327
				CORT,28°C	-0.0312	0.0004	-0.1584	0.0964
			Adult	Control,23°C	-0.0244	0.0003	-0.1169	0.0670
				Control,28°C	-0.0299	0.0003	-0.1232	0.0624
				CORT,23°C	-0.0154	0.0003	-0.1086	0.0762
				CORT,28°C	-0.0209	0.0003	-0.1143	0.0707
		Mitochondrial Mass (MFI)	Juvenile	Control,23°C	0.0040	0.0006	-0.2110	0.2147
				Control,28°C	-0.0505	0.0006	-0.2661	0.1610
				CORT,23°C	0.0347	0.0006	-0.1814	0.2477
				CORT,28°C	-0.0199	0.0006	-0.2411	0.2005
			Adult	Control,23°C	-0.0174	0.0005	-0.2056	0.1754
				Control,28°C	0.0659	0.0005	-0.1256	0.2568
				CORT,23°C	-0.0571	0.0005	-0.2515	0.1304
				CORT,28°C	0.0262	0.0005	-0.1720	0.2125

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. guichenoti</i>		Cell Size (MFI)	Juvenile	Control,23°C	-0.0125	0.0003	-0.1268	0.1050
				Control,28°C	0.0438	0.0003	-0.0687	0.1556
				CORT,23°C	-0.0581	0.0003	-0.1715	0.0566
				CORT,28°C	-0.0017	0.0003	-0.1160	0.1112
			Adult	Control,23°C	0.0308	0.0003	-0.0782	0.1395
				Control,28°C	0.0135	0.0003	-0.0942	0.1213
				CORT,23°C	0.0105	0.0003	-0.0986	0.1208
				CORT,28°C	-0.0069	0.0003	-0.1148	0.1026
		Mitochondrial Mass (MFI)	Juvenile	Control,23°C	-0.0147	0.0005	-0.2105	0.1792
				Control,28°C	-0.0049	0.0005	-0.1991	0.1881
				CORT,23°C	0.0125	0.0006	-0.1829	0.2085
				CORT,28°C	0.0224	0.0005	-0.1719	0.2178
			Adult	Control,23°C	0.0081	0.0007	-0.2317	0.2474
				Control,28°C	0.0395	0.0007	-0.1995	0.2730
				CORT,23°C	0.0355	0.0007	-0.2027	0.2758
				CORT,28°C	0.0668	0.0007	-0.1744	0.3006

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Supplemental Table 40. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* cellular morphology (cell size or mitochondrial mass) in liver cells. Data are the differences in predicted posterior distributions generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). Contrasts are the differences in posterior distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Cell Size (MFI)	Juvenile	Liver	[Control, 23°C] - [Control, 28°C]	0.045	0	-0.019	0.110	0.167
				[Control, 23°C] - [CORT, 23°C]	0.021	0	-0.044	0.087	0.520
				[Control, 23°C] - [CORT, 28°C]	0.066	0	-0.034	0.168	0.192
				[Control, 28°C] - [CORT, 23°C]	-0.023	0	-0.107	0.060	0.572
				[Control, 28°C] - [CORT, 28°C]	0.021	0	-0.044	0.087	0.520
				[CORT, 23°C] - [CORT, 28°C]	0.045	0	-0.019	0.110	0.167
		Adult	Liver	[Control, 23°C] - [Control, 28°C]	0.006	0	-0.024	0.035	0.707
				[Control, 23°C] - [CORT, 23°C]	-0.009	0	-0.042	0.024	0.582
				[Control, 23°C] - [CORT, 28°C]	-0.004	0	-0.048	0.041	0.871
				[Control, 28°C] - [CORT, 23°C]	-0.015	0	-0.059	0.031	0.516
				[Control, 28°C] - [CORT, 28°C]	-0.009	0	-0.042	0.024	0.582
				[CORT, 23°C] - [CORT, 28°C]	0.006	0	-0.024	0.035	0.707
	Mitochondrial Mass (MFI)	Juvenile	Liver	[Control, 23°C] - [Control, 28°C]	0.055	0	-0.015	0.125	0.122
				[Control, 23°C] - [CORT, 23°C]	-0.031	0	-0.102	0.040	0.389
				[Control, 23°C] - [CORT, 28°C]	0.024	0	-0.087	0.134	0.662

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. guichenoti</i>	Cell Size (MFI)	Adult		[Control, 28°C] - [CORT, 23°C]	-0.085	0	-0.173	0.002	0.056
				[Control, 28°C] - [CORT, 28°C]	-0.031	0	-0.102	0.040	0.389
				[CORT, 23°C] - [CORT, 28°C]	0.055	0	-0.015	0.125	0.122
				[Control, 23°C] - [Control, 28°C]	-0.083	0	-0.184	0.015	0.098
				[Control, 23°C] - [CORT, 23°C]	0.040	0	-0.075	0.151	0.483
				[Control, 23°C] - [CORT, 28°C]	-0.044	0	-0.196	0.106	0.567
				[Control, 28°C] - [CORT, 23°C]	0.123	0	-0.030	0.274	0.113
		[Control, 28°C] - [CORT, 28°C]		0.040	0	-0.075	0.151	0.483	
		[CORT, 23°C] - [CORT, 28°C]		-0.083	0	-0.184	0.015	0.098	
		Juvenile		[Control, 23°C] - [Control, 28°C]	-0.056	0	-0.125	0.013	0.108
				[Control, 23°C] - [CORT, 23°C]	0.046	0	-0.024	0.116	0.205
				[Control, 23°C] - [CORT, 28°C]	-0.011	0	-0.113	0.093	0.833
				[Control, 28°C] - [CORT, 23°C]	0.102	0	0.007	0.198	0.037
				[Control, 28°C] - [CORT, 28°C]	0.046	0	-0.024	0.116	0.205
				[CORT, 23°C] - [CORT, 28°C]	-0.056	0	-0.125	0.013	0.108
	Adult			[Control, 23°C] - [Control, 28°C]	0.017	0	-0.024	0.059	0.404
		[Control, 23°C] - [CORT, 23°C]		0.020	0	-0.021	0.062	0.333	
		[Control, 23°C] - [CORT, 28°C]		0.038	0	-0.021	0.097	0.207	
		[Control, 28°C] - [CORT, 23°C]		0.003	0	-0.056	0.062	0.922	
		[Control, 28°C] - [CORT, 28°C]		0.020	0	-0.021	0.062	0.333	
		[CORT, 23°C] - [CORT, 28°C]		0.017	0	-0.024	0.059	0.404	
		Mitochondrial Mass (MFI)		Juvenile	[Control, 23°C] - [Control, 28°C]	-0.010	0	-0.065	0.045

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
				[Control, 23°C] - [CORT, 23°C]	-0.027	0	-0.083	0.029	0.335
				[Control, 23°C] - [CORT, 28°C]	-0.037	0	-0.116	0.041	0.354
				[Control, 28°C] - [CORT, 23°C]	-0.017	0	-0.097	0.061	0.660
				[Control, 28°C] - [CORT, 28°C]	-0.027	0	-0.083	0.029	0.335
				[CORT, 23°C] - [CORT, 28°C]	-0.010	0	-0.065	0.045	0.721
		Adult		[Control, 23°C] - [Control, 28°C]	-0.031	0	-0.128	0.065	0.512
				[Control, 23°C] - [CORT, 23°C]	-0.027	0	-0.121	0.066	0.565
				[Control, 23°C] - [CORT, 28°C]	-0.059	0	-0.195	0.078	0.388
				[Control, 28°C] - [CORT, 23°C]	0.004	0	-0.128	0.135	0.947
				[Control, 28°C] - [CORT, 28°C]	-0.027	0	-0.121	0.066	0.565
				[CORT, 23°C] - [CORT, 28°C]	-0.031	0	-0.128	0.065	0.512

Supplemental Table 41. Comparisons of CORT-treatment and incubation temperature effects on cellular morphology (cell size or mitochondrial mass) in liver cells between *L. delicata* and *L. guichenoti*. Contrasts are the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Cell Size (MFI)	Juvenile	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.024	0	-0.073	0.119	0.619
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.101	0	-0.196	-0.007	0.036
	Adult		Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.029	0	-0.024	0.083	0.271
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.012	0	-0.039	0.063	0.651
Mitochondrial Mass (MFI)	Juvenile		Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.003	0	-0.086	0.093	0.944
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.064	0	-0.153	0.024	0.151
	Adult		Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.067	0	-0.213	0.082	0.361
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.052	0	-0.088	0.191	0.457

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Supplemental Table 42. Comparisons of CORT-treatment and incubation temperature effects on cellular morphology (cell size or mitochondrial mass) in liver cells between juveniles and adults of *L. delicata* and *L. guichenoti*. Contrasts are the differences between adult and juvenile lizards in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Tissue	Species	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Cell Size (MFI)	Liver	<i>L. delicata</i>	Stress [Adult - Juvenile]	0.030	0	-0.043	0.105	0.417
			Temperature [Adult - Juvenile]	0.039	0	-0.032	0.110	0.273
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	0.025	0	-0.057	0.108	0.548
			Temperature [Adult - Juvenile]	-0.074	0	-0.154	0.007	0.072
Mitochondrial Mass (MFI)		<i>L. delicata</i>	Stress [Adult - Juvenile]	-0.070	0	-0.202	0.063	0.298
			Temperature [Adult - Juvenile]	0.138	0	0.016	0.260	0.025
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	0.000	0	-0.108	0.108	0.996
			Temperature [Adult - Juvenile]	0.021	0	-0.089	0.132	0.702

Supplemental Table 43. Analysis of main effects of developmental CORT treatment and incubation temperature on cellular morphology in the hearts of juvenile and adult *L. delicata* and *L. guichenoti*. Data are the predictive posterior distributions derived from a multi-response Bayesian GLMM including log-transformed fluorescence values of cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, and oxidative DNA damage as responses and accounting for residual correlations among each parameter. For each parameter, CORT treatment and incubation temperature were included as fixed effects, and plate number was included as a random intercept. Mitochondrial mass included cell size as an independent covariate to account for the influence of cell size variation on the quantity of mitochondria in a cell. We show the difference in mean between posterior distributions (Estimate) predicted by Bayesian GLMM, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Heart	Cell Size (MFI)	Juvenile	Stress [CORT - Control]	-0.003	<0.001	-0.043	0.037	0.888
				Temperature [28°C - 23°C]	-0.002	<0.001	-0.041	0.037	0.904
			Adult	Stress [CORT - Control]	0.013	<0.001	-0.075	0.100	0.755
				Temperature [28°C - 23°C]	-0.006	<0.001	-0.081	0.070	0.881
		Mitochondrial Mass (MFI)	Juvenile	Stress [CORT - Control]	-0.008	<0.001	-0.068	0.052	0.778
				Temperature [28°C - 23°C]	-0.022	<0.001	-0.080	0.036	0.443
			Adult	Stress [CORT - Control]	0.165	<0.001	0.042	0.286	0.010
				Temperature [28°C - 23°C]	-0.088	<0.001	-0.189	0.018	0.095
<i>L. guichenoti</i>		Cell Size (MFI)	Juvenile	Stress [CORT - Control]	-0.003	<0.001	-0.030	0.024	0.833

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
				Temperature [28°C - 23°C]	0.007	<0.001	-0.020	0.033	0.596
			Adult	Stress [CORT - Control]	0.022	<0.001	-0.024	0.068	0.343
				Temperature [28°C - 23°C]	0.009	<0.001	-0.037	0.055	0.685
		Mitochondrial Mass (MFI)	Juvenile	Stress [CORT - Control]	0.022	<0.001	-0.023	0.067	0.326
				Temperature [28°C - 23°C]	-0.025	<0.001	-0.070	0.020	0.260
			Adult	Stress [CORT - Control]	-0.040	<0.001	-0.148	0.067	0.454
				Temperature [28°C - 23°C]	-0.027	<0.001	-0.135	0.080	0.614

Supplemental Table 44. Summary of predicted posterior distributions of juvenile and adult *L. delicata* and *L. guichenoti* cellular morphology (cell size or mitochondrial mass) in heart cells for each CORT-treatment by incubation temperature combination. Predictive posterior distributions were generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Heart	Cell Size (MFI)	Juvenile	Control,23°C	0.0005	0.0001	-0.0532	0.0533
				Control,28°C	-0.0017	0.0002	-0.0575	0.0524
				CORT,23°C	-0.0022	0.0002	-0.0589	0.0526
				CORT,28°C	-0.0045	0.0002	-0.0656	0.0555
		Mitochondrial Mass (MFI)	Adult	Control,23°C	0.0029	0.0006	-0.1896	0.2000
				Control,28°C	-0.0026	0.0006	-0.1981	0.1897
				CORT,23°C	0.0160	0.0006	-0.1728	0.2175
				CORT,28°C	0.0105	0.0006	-0.1862	0.2117
	Heart	Cell Size (MFI)	Juvenile	Control,23°C	-0.0050	0.0004	-0.1611	0.1519
				Control,28°C	-0.0272	0.0004	-0.1841	0.1297
				CORT,23°C	-0.0134	0.0005	-0.1734	0.1448
				CORT,28°C	-0.0356	0.0005	-0.2003	0.1257
		Mitochondrial Mass (MFI)	Adult	Control,23°C	-0.0792	0.0014	-0.5738	0.4187
				Control,28°C	-0.1668	0.0014	-0.6643	0.3271
				CORT,23°C	0.0854	0.0014	-0.4066	0.5864
				CORT,28°C	-0.0022	0.0014	-0.4987	0.5003

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. guichenoti</i>		Cell Size (MFI)	Juvenile	Control,23°C	-0.0010	0.0001	-0.0389	0.0359
				Control,28°C	0.0059	0.0001	-0.0297	0.0407
				CORT,23°C	-0.0037	0.0001	-0.0402	0.0331
				CORT,28°C	0.0031	0.0001	-0.0321	0.0394
		Mitochondrial Mass (MFI)	Adult	Control,23°C	-0.0480	0.0008	-0.3446	0.2439
				Control,28°C	-0.0386	0.0008	-0.3347	0.2544
				CORT,23°C	-0.0262	0.0008	-0.3207	0.2650
				CORT,28°C	-0.0169	0.0008	-0.3102	0.2745
		Cell Size (MFI)	Juvenile	Control,23°C	0.0050	0.0004	-0.1444	0.1565
				Control,28°C	-0.0205	0.0004	-0.1681	0.1267
				CORT,23°C	0.0272	0.0004	-0.1208	0.1768
				CORT,28°C	0.0017	0.0004	-0.1455	0.1485
		Mitochondrial Mass (MFI)	Adult	Control,23°C	0.0066	0.0014	-0.5232	0.5169
				Control,28°C	-0.0202	0.0014	-0.5499	0.4948
				CORT,23°C	-0.0334	0.0014	-0.5562	0.4741
				CORT,28°C	-0.0602	0.0014	-0.5814	0.4504

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Supplemental Table 45. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* cellular morphology (cell size or mitochondrial mass) in heart cells. Data are the differences in predicted posterior distributions generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). Contrasts are the differences in posterior distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Cell Size (MFI)	Juvenile	Heart	[Control, 23°C] - [Control, 28°C]	0.002	0	-0.037	0.041	0.904
				[Control, 23°C] - [CORT, 23°C]	0.003	0	-0.037	0.043	0.888
				[Control, 23°C] - [CORT, 28°C]	0.005	0	-0.055	0.064	0.864
				[Control, 28°C] - [CORT, 23°C]	0.000	0	-0.051	0.051	0.984
				[Control, 28°C] - [CORT, 28°C]	0.003	0	-0.037	0.043	0.888
				[CORT, 23°C] - [CORT, 28°C]	0.002	0	-0.037	0.041	0.904
		Adult	Heart	[Control, 23°C] - [Control, 28°C]	0.006	0	-0.070	0.081	0.881
				[Control, 23°C] - [CORT, 23°C]	-0.013	0	-0.100	0.075	0.755
				[Control, 23°C] - [CORT, 28°C]	-0.008	0	-0.130	0.118	0.892
				[Control, 28°C] - [CORT, 23°C]	-0.019	0	-0.123	0.089	0.706
				[Control, 28°C] - [CORT, 28°C]	-0.013	0	-0.100	0.075	0.755
				[CORT, 23°C] - [CORT, 28°C]	0.006	0	-0.070	0.081	0.881
	Mitochondrial Mass (MFI)	Juvenile	Heart	[Control, 23°C] - [Control, 28°C]	0.022	0	-0.036	0.080	0.443
				[Control, 23°C] - [CORT, 23°C]	0.008	0	-0.052	0.068	0.778
				[Control, 23°C] - [CORT, 28°C]	0.031	0	-0.060	0.121	0.501

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. guichenoti</i>	Cell Size (MFI)	Adult		[Control, 28°C] - [CORT, 23°C]	-0.014	0	-0.090	0.062	0.718
				[Control, 28°C] - [CORT, 28°C]	0.008	0	-0.052	0.068	0.778
				[CORT, 23°C] - [CORT, 28°C]	0.022	0	-0.036	0.080	0.443
				[Control, 23°C] - [Control, 28°C]	0.088	0	-0.018	0.189	0.095
				[Control, 23°C] - [CORT, 23°C]	-0.165	0	-0.286	-0.042	0.010
				[Control, 23°C] - [CORT, 28°C]	-0.077	0	-0.249	0.092	0.357
				[Control, 28°C] - [CORT, 23°C]	-0.252	0	-0.398	-0.103	0.003
		Juvenile		[Control, 28°C] - [CORT, 28°C]	-0.165	0	-0.286	-0.042	0.010
				[CORT, 23°C] - [CORT, 28°C]	0.088	0	-0.018	0.189	0.095
				[Control, 23°C] - [Control, 28°C]	-0.007	0	-0.033	0.020	0.596
				[Control, 23°C] - [CORT, 23°C]	0.003	0	-0.024	0.030	0.833
				[Control, 23°C] - [CORT, 28°C]	-0.004	0	-0.042	0.035	0.829
				[Control, 28°C] - [CORT, 23°C]	0.010	0	-0.026	0.045	0.585
				[Control, 28°C] - [CORT, 28°C]	0.003	0	-0.024	0.030	0.833
		Adult		[CORT, 23°C] - [CORT, 28°C]	-0.007	0	-0.033	0.020	0.596
				[Control, 23°C] - [Control, 28°C]	-0.009	0	-0.055	0.037	0.685
				[Control, 23°C] - [CORT, 23°C]	-0.022	0	-0.068	0.024	0.343
				[Control, 23°C] - [CORT, 28°C]	-0.031	0	-0.092	0.030	0.303
				[Control, 28°C] - [CORT, 23°C]	-0.012	0	-0.081	0.057	0.717
				[Control, 28°C] - [CORT, 28°C]	-0.022	0	-0.068	0.024	0.343
				[CORT, 23°C] - [CORT, 28°C]	-0.009	0	-0.055	0.037	0.685
	Mitochondrial Mass (MFI)	Juvenile		[Control, 23°C] - [Control, 28°C]	0.025	0	-0.020	0.070	0.260

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
				[Control, 23°C] - [CORT, 23°C]	-0.022	0	-0.067	0.023	0.326
				[Control, 23°C] - [CORT, 28°C]	0.003	0	-0.063	0.069	0.915
				[Control, 28°C] - [CORT, 23°C]	-0.048	0	-0.109	0.014	0.127
				[Control, 28°C] - [CORT, 28°C]	-0.022	0	-0.067	0.023	0.326
				[CORT, 23°C] - [CORT, 28°C]	0.025	0	-0.020	0.070	0.260
		Adult		[Control, 23°C] - [Control, 28°C]	0.027	0	-0.080	0.135	0.614
				[Control, 23°C] - [CORT, 23°C]	0.040	0	-0.067	0.148	0.454
				[Control, 23°C] - [CORT, 28°C]	0.067	0	-0.077	0.212	0.345
				[Control, 28°C] - [CORT, 23°C]	0.013	0	-0.148	0.175	0.867
				[Control, 28°C] - [CORT, 28°C]	0.040	0	-0.067	0.148	0.454
				[CORT, 23°C] - [CORT, 28°C]	0.027	0	-0.080	0.135	0.614

Supplemental Table 46. Comparisons of CORT-treatment and incubation temperature effects on cellular morphology (cell size or mitochondrial mass) in heart cells between *L. delicata* and *L. guichenoti*. Contrasts are the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Cell Size (MFI)	Juvenile	Heart	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.000	0	-0.048	0.048	0.999
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.009	0	-0.055	0.038	0.696
	Adult		Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.009	0	-0.108	0.090	0.860
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.015	0	-0.104	0.073	0.732
Mitochondrial Mass (MFI)	Juvenile		Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.031	0	-0.106	0.044	0.420
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.003	0	-0.069	0.076	0.931
	Adult		Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.205	0	0.042	0.367	0.016
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.061	0	-0.207	0.088	0.413

Supplemental Table 47. Comparisons of CORT-treatment and incubation temperature effects on cellular morphology (cell size or mitochondrial mass) in heart cells between juveniles and adults of *L. delicata* and *L. guichenoti*. Contrasts are the differences between adult and juvenile lizards in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Tissue	Species	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Cell Size (MFI)	Heart	<i>L. delicata</i>	Stress [Adult - Juvenile]	0.016	0	-0.080	0.111	0.732
			Temperature [Adult - Juvenile]	-0.003	0	-0.089	0.082	0.939
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	0.025	0	-0.028	0.078	0.360
			Temperature [Adult - Juvenile]	0.002	0	-0.050	0.055	0.928
Mitochondrial Mass (MFI)		<i>L. delicata</i>	Stress [Adult - Juvenile]	0.173	0	0.037	0.309	0.014
			Temperature [Adult - Juvenile]	-0.065	0	-0.182	0.053	0.267
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	-0.062	0	-0.179	0.055	0.287
			Temperature [Adult - Juvenile]	-0.001	0	-0.117	0.114	0.987

349 Supplemental Table 48. Comparisons of CORT-treatment and incubation temperature effects on cellular morphology (cell size or mitochondrial mass) between liver and heart
350 cells in *L. delicata* and *L. guichenoti*. Contrasts are the differences between liver and heart cells in effect size distributions for the developmental stress (CORT – Control) or
351 incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean
352 (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC <
353 0.05) different from zero.

Species	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Cell Size (MFI)	Adult	Stress [Liver - Heart]	-0.004	0	-0.097	0.089	0.928
			Temperature [Liver - Heart]	0.000	0	-0.081	0.082	0.999
		Juvenile	Stress [Liver - Heart]	-0.019	0	-0.096	0.058	0.634
			Temperature [Liver - Heart]	-0.043	0	-0.117	0.032	0.266
	Mitochondrial Mass (MFI)	Adult	Stress [Liver - Heart]	-0.204	0	-0.368	-0.039	0.020
			Temperature [Liver - Heart]	0.171	0	0.027	0.313	0.020
		Juvenile	Stress [Liver - Heart]	0.039	0	-0.054	0.133	0.409
			Temperature [Liver - Heart]	-0.032	0	-0.124	0.058	0.483
<i>L. guichenoti</i>	Cell Size (MFI)	Adult	Stress [Liver - Heart]	-0.042	0	-0.104	0.020	0.177
			Temperature [Liver - Heart]	-0.027	0	-0.089	0.035	0.389
		Juvenile	Stress [Liver - Heart]	-0.043	0	-0.118	0.033	0.262

Species	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
	Mitochondrial Mass (MFI)	Adult	Temperature [Liver - Heart]	0.049	0	-0.025	0.124	0.186
			Stress [Liver - Heart]	0.067	0	-0.074	0.210	0.351
		Juvenile	Temperature [Liver - Heart]	0.058	0	-0.085	0.203	0.423
			Stress [Liver - Heart]	0.005	0	-0.067	0.078	0.887
			Temperature [Liver - Heart]	0.035	0	-0.036	0.106	0.325

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Supplemental Table 49. Analysis of main effects of developmental CORT treatment and incubation temperature on mitochondrial function in the livers of juvenile and adult *L. delicata* and *L. guichenoti*. Data are the predictive posterior distributions derived from a multi-response Bayesian GLMM including log-transformed fluorescence values of cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, and oxidative DNA damage as responses and accounting for residual correlations among each parameter. For each parameter, CORT treatment and incubation temperature were included as fixed effects, and plate number was included as a random intercept. Mitochondrial membrane potential was included as a covariate in models of mitochondrial superoxide to allow for estimation of superoxide production under the same levels of respiration. We show the difference in mean between posterior distributions (Estimate) predicted by Bayesian GLMM, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Liver	Mitochondrial Membrane Potential (MFI)	Juvenile	Stress [CORT - Control]	0.050	<0.001	-0.038	0.137	0.254
				Temperature [28°C - 23°C]	0.005	<0.001	-0.080	0.088	0.912
			Adult	Stress [CORT - Control]	0.030	<0.001	-0.046	0.106	0.437
				Temperature [28°C - 23°C]	-0.001	<0.001	-0.068	0.067	0.977
		Mitochondrial Superoxide (MFI)	Juvenile	Stress [CORT - Control]	-0.044	<0.001	-0.103	0.015	0.138
				Temperature [28°C - 23°C]	0.024	<0.001	-0.032	0.080	0.401
			Adult	Stress [CORT - Control]	-0.005	<0.001	-0.050	0.038	0.807
				Temperature [28°C - 23°C]	0.015	<0.001	-0.023	0.054	0.425
<i>L. guichenoti</i>		Mitochondrial Membrane Potential (MFI)	Juvenile	Stress [CORT - Control]	-0.011	<0.001	-0.063	0.041	0.680

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
		Mitochondrial Superoxide (MFI)	Adult	Temperature [28°C - 23°C]	0.006	<0.001	-0.045	0.057	0.817
				Stress [CORT - Control]	-0.011	<0.001	-0.080	0.058	0.753
			Juvenile	Temperature [28°C - 23°C]	-0.002	<0.001	-0.072	0.068	0.964
				Stress [CORT - Control]	-0.004	<0.001	-0.060	0.051	0.876
			Adult	Temperature [28°C - 23°C]	0.014	<0.001	-0.041	0.070	0.614
				Stress [CORT - Control]	-0.013	<0.001	-0.081	0.054	0.716
				Temperature [28°C - 23°C]	0.039	<0.001	-0.030	0.108	0.256

Supplemental Table 50. Summary of predicted posterior distributions of juvenile and adult *L. delicata* and *L. guichenoti* mitochondrial function (mitochondrial membrane potential or mitochondrial superoxide) in liver cells for each CORT-treatment by incubation temperature combination. Predictive posterior distributions were generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Liver	Mitochondrial Membrane Potential (MFI)	Juvenile	Control,23°C	-0.0103	0.0005	-0.2047	0.1837
				Control,28°C	-0.0057	0.0005	-0.2011	0.1891
				CORT,23°C	0.0395	0.0006	-0.1564	0.2375
				CORT,28°C	0.0442	0.0006	-0.1589	0.2486
			Adult	Control,23°C	-0.0791	0.0006	-0.2767	0.1196
				Control,28°C	-0.0799	0.0006	-0.2788	0.1156
				CORT,23°C	-0.0495	0.0006	-0.2486	0.1520
				CORT,28°C	-0.0504	0.0006	-0.2502	0.1500
		Mitochondrial Superoxide (MFI)	Juvenile	Control,23°C	0.0246	0.0006	-0.1785	0.2271
				Control,28°C	0.0486	0.0006	-0.1543	0.2530
				CORT,23°C	-0.0195	0.0006	-0.2223	0.1873
				CORT,28°C	0.0046	0.0006	-0.2015	0.2156
			Adult	Control,23°C	-0.0323	0.0004	-0.1702	0.1016
				Control,28°C	-0.0170	0.0004	-0.1557	0.1169
				CORT,23°C	-0.0377	0.0004	-0.1750	0.0967
				CORT,28°C	-0.0224	0.0004	-0.1603	0.1110

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. guichenoti</i>	Mitochondrial Membrane Potential (MFI)		Juvenile	Control,23°C	0.0070	0.0004	-0.1501	0.1638
				Control,28°C	0.0130	0.0004	-0.1432	0.1700
				CORT,23°C	-0.0037	0.0004	-0.1605	0.1511
				CORT,28°C	0.0023	0.0004	-0.1544	0.1585
			Adult	Control,23°C	-0.0133	0.0005	-0.1841	0.1573
				Control,28°C	-0.0150	0.0005	-0.1843	0.1559
				CORT,23°C	-0.0240	0.0005	-0.1946	0.1481
				CORT,28°C	-0.0257	0.0005	-0.1945	0.1461
	Mitochondrial Superoxide (MFI)		Juvenile	Control,23°C	-0.0045	0.0005	-0.1829	0.1702
				Control,28°C	0.0095	0.0005	-0.1686	0.1843
				CORT,23°C	-0.0089	0.0005	-0.1858	0.1667
				CORT,28°C	0.0050	0.0005	-0.1720	0.1802
			Adult	Control,23°C	-0.0115	0.0004	-0.1664	0.1416
				Control,28°C	0.0275	0.0004	-0.1267	0.1807
				CORT,23°C	-0.0240	0.0004	-0.1784	0.1335
				CORT,28°C	0.0150	0.0004	-0.1390	0.1719

Supplemental Table 51. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* mitochondrial function (mitochondrial membrane potential or mitochondrial superoxide) in liver cells. Data are the differences in predicted posterior distributions generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). Contrasts are the differences in posterior distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Mitochondrial Membrane Potential (MFI)	Juvenile	Liver	[Control, 23°C] - [Control, 28°C]	-0.005	0	-0.088	0.080	0.912
				[Control, 23°C] - [CORT, 23°C]	-0.050	0	-0.137	0.038	0.254
				[Control, 23°C] - [CORT, 28°C]	-0.054	0	-0.186	0.079	0.416
				[Control, 28°C] - [CORT, 23°C]	-0.045	0	-0.154	0.063	0.405
				[Control, 28°C] - [CORT, 28°C]	-0.050	0	-0.137	0.038	0.254
				[CORT, 23°C] - [CORT, 28°C]	-0.005	0	-0.088	0.080	0.912
		Adult	Liver	[Control, 23°C] - [Control, 28°C]	0.001	0	-0.067	0.068	0.977
				[Control, 23°C] - [CORT, 23°C]	-0.030	0	-0.106	0.046	0.437
				[Control, 23°C] - [CORT, 28°C]	-0.029	0	-0.131	0.072	0.577
				[Control, 28°C] - [CORT, 23°C]	-0.030	0	-0.133	0.071	0.543
				[Control, 28°C] - [CORT, 28°C]	-0.030	0	-0.106	0.046	0.437
				[CORT, 23°C] - [CORT, 28°C]	0.001	0	-0.067	0.068	0.977
	Mitochondrial Superoxide (MFI)	Juvenile	Liver	[Control, 23°C] - [Control, 28°C]	-0.024	0	-0.080	0.032	0.401
				[Control, 23°C] - [CORT, 23°C]	0.044	0	-0.015	0.103	0.138
				[Control, 23°C] - [CORT, 28°C]	0.020	0	-0.069	0.111	0.658

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. guichenoti</i>	Mitochondrial Membrane Potential (MFI)	Adult		[Control, 28°C] - [CORT, 23°C]	0.068	0	-0.005	0.141	0.068
				[Control, 28°C] - [CORT, 28°C]	0.044	0	-0.015	0.103	0.138
				[CORT, 23°C] - [CORT, 28°C]	-0.024	0	-0.080	0.032	0.401
				[Control, 23°C] - [Control, 28°C]	-0.015	0	-0.054	0.023	0.425
				[Control, 23°C] - [CORT, 23°C]	0.005	0	-0.038	0.050	0.807
				[Control, 23°C] - [CORT, 28°C]	-0.010	0	-0.068	0.048	0.731
				[Control, 28°C] - [CORT, 23°C]	0.021	0	-0.038	0.080	0.485
				[Control, 28°C] - [CORT, 28°C]	0.005	0	-0.038	0.050	0.807
				[CORT, 23°C] - [CORT, 28°C]	-0.015	0	-0.054	0.023	0.425
		Juvenile		[Control, 23°C] - [Control, 28°C]	-0.006	0	-0.057	0.045	0.817
				[Control, 23°C] - [CORT, 23°C]	0.011	0	-0.041	0.063	0.680
				[Control, 23°C] - [CORT, 28°C]	0.005	0	-0.069	0.080	0.901
				[Control, 28°C] - [CORT, 23°C]	0.017	0	-0.053	0.087	0.637
				[Control, 28°C] - [CORT, 28°C]	0.011	0	-0.041	0.063	0.680
				[CORT, 23°C] - [CORT, 28°C]	-0.006	0	-0.057	0.045	0.817
	Mitochondrial Superoxide (MFI)	Adult		[Control, 23°C] - [Control, 28°C]	0.002	0	-0.068	0.072	0.964
				[Control, 23°C] - [CORT, 23°C]	0.011	0	-0.058	0.080	0.753
				[Control, 23°C] - [CORT, 28°C]	0.012	0	-0.086	0.111	0.806
				[Control, 28°C] - [CORT, 23°C]	0.009	0	-0.089	0.106	0.854
				[Control, 28°C] - [CORT, 28°C]	0.011	0	-0.058	0.080	0.753
				[CORT, 23°C] - [CORT, 28°C]	0.002	0	-0.068	0.072	0.964
		Juvenile		[Control, 23°C] - [Control, 28°C]	-0.014	0	-0.070	0.041	0.614

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
				[Control, 23°C] - [CORT, 23°C]	0.004	0	-0.051	0.060	0.876
				[Control, 23°C] - [CORT, 28°C]	-0.010	0	-0.090	0.072	0.808
				[Control, 28°C] - [CORT, 23°C]	0.018	0	-0.058	0.095	0.629
				[Control, 28°C] - [CORT, 28°C]	0.004	0	-0.051	0.060	0.876
				[CORT, 23°C] - [CORT, 28°C]	-0.014	0	-0.070	0.041	0.614
		Adult		[Control, 23°C] - [Control, 28°C]	-0.039	0	-0.108	0.030	0.256
				[Control, 23°C] - [CORT, 23°C]	0.013	0	-0.054	0.081	0.716
				[Control, 23°C] - [CORT, 28°C]	-0.026	0	-0.123	0.070	0.585
				[Control, 28°C] - [CORT, 23°C]	0.051	0	-0.044	0.148	0.283
				[Control, 28°C] - [CORT, 28°C]	0.013	0	-0.054	0.081	0.716
				[CORT, 23°C] - [CORT, 28°C]	-0.039	0	-0.108	0.030	0.256

Supplemental Table 52. Comparisons of CORT-treatment and incubation temperature effects on mitochondrial function (mitochondrial membrane potential or mitochondrial superoxide) in liver cells between *L. delicata* and *L. guichenoti*. Contrasts are the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Mitochondrial Membrane Potential (MFI)	Juvenile	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.061	0	-0.041	0.161	0.240
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.001	0	-0.100	0.096	0.980
	Adult	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.040	0	-0.061	0.141	0.433
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.001	0	-0.097	0.099	0.984
Mitochondrial Superoxide (MFI)	Juvenile	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.040	0	-0.121	0.041	0.333
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.010	0	-0.070	0.088	0.797
	Adult	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.007	0	-0.073	0.088	0.866
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.024	0	-0.102	0.055	0.550

Supplemental Table 53. Comparisons of CORT-treatment and incubation temperature effects on mitochondrial function (mitochondrial membrane potential or mitochondrial superoxide) in liver cells between juveniles and adults of *L. delicata* and *L. guichenoti*. Contrasts are the differences between adult and juvenile lizards in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Tissue	Species	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Mitochondrial Membrane Potential (MFI)	Liver	<i>L. delicata</i>	Stress [Adult - Juvenile]	-0.020	0	-0.135	0.094	0.726
			Temperature [Adult - Juvenile]	-0.005	0	-0.113	0.103	0.919
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	0.000	0	-0.085	0.087	1.000
			Temperature [Adult - Juvenile]	-0.008	0	-0.094	0.079	0.857
Mitochondrial Superoxide (MFI)		<i>L. delicata</i>	Stress [Adult - Juvenile]	0.039	0	-0.035	0.113	0.297
			Temperature [Adult - Juvenile]	-0.009	0	-0.076	0.060	0.794
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	-0.008	0	-0.096	0.079	0.853
			Temperature [Adult - Juvenile]	0.025	0	-0.063	0.113	0.571

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Supplemental Table 54. Analysis of main effects of developmental CORT treatment and incubation temperature on mitochondrial function in the hearts of juvenile and adult *L. delicata* and *L. guichenoti*. Data are the predictive posterior distributions derived from a multi-response Bayesian GLMM including log-transformed fluorescence values of cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, and oxidative DNA damage as responses and accounting for residual correlations among each parameter. For each parameter, CORT treatment and incubation temperature were included as fixed effects, and plate number was included as a random intercept. Mitochondrial membrane potential was included as a covariate in models of mitochondrial superoxide to allow for estimation of superoxide production under the same levels of respiration. We show the difference in mean between posterior distributions (Estimate) predicted by Bayesian GLMM, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Heart	Mitochondrial Membrane Potential (MFI)	Juvenile	Stress [CORT - Control]	0.045	0	-0.017	0.106	0.155
				Temperature [28°C - 23°C]	-0.013	0	-0.072	0.047	0.664
			Adult	Stress [CORT - Control]	-0.106	0	-0.240	0.028	0.113
				Temperature [28°C - 23°C]	-0.039	0	-0.153	0.079	0.485
		Mitochondrial Superoxide (MFI)	Juvenile	Stress [CORT - Control]	0.027	0	-0.031	0.083	0.357
				Temperature [28°C - 23°C]	0.020	0	-0.034	0.075	0.469
			Adult	Stress [CORT - Control]	0.009	0	-0.057	0.075	0.776
				Temperature [28°C - 23°C]	0.001	0	-0.053	0.055	0.959
<i>L. guichenoti</i>		Mitochondrial Membrane Potential (MFI)	Juvenile	Stress [CORT - Control]	-0.017	0	-0.055	0.022	0.387
				Temperature [28°C - 23°C]	-0.015	0	-0.054	0.023	0.428

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
			Adult	Stress [CORT - Control]	0.016	0	-0.052	0.085	0.632
				Temperature [28°C - 23°C]	0.010	0	-0.060	0.078	0.775
		Mitochondrial Superoxide (MFI)	Juvenile	Stress [CORT - Control]	0.027	0	-0.023	0.078	0.280
				Temperature [28°C - 23°C]	-0.014	0	-0.065	0.036	0.585
			Adult	Stress [CORT - Control]	-0.004	0	-0.038	0.029	0.792
				Temperature [28°C - 23°C]	0.004	0	-0.029	0.037	0.828

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Supplemental Table 55. Summary of predicted posterior distributions of juvenile and adult *L. delicata* and *L. guichenoti* mitochondrial function (mitochondrial membrane potential or mitochondrial superoxide) in heart cells for each CORT-treatment by incubation temperature combination. Predictive posterior distributions were generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Heart	Mitochondrial Membrane Potential (MFI)	Juvenile	Control,23°C	0.0003	0.0006	-0.1982	0.1967
				Control,28°C	-0.0126	0.0006	-0.2109	0.1830
				CORT,23°C	0.0449	0.0006	-0.1570	0.2450
				CORT,28°C	0.0320	0.0006	-0.1725	0.2333
			Adult	Control,23°C	0.0823	0.0008	-0.2154	0.3820
				Control,28°C	0.0431	0.0008	-0.2534	0.3360
				CORT,23°C	-0.0236	0.0008	-0.3177	0.2799
				CORT,28°C	-0.0628	0.0009	-0.3640	0.2431
		Mitochondrial Superoxide (MFI)	Juvenile	Control,23°C	-0.0184	0.0003	-0.1342	0.0973
				Control,28°C	0.0013	0.0003	-0.1154	0.1179
				CORT,23°C	0.0082	0.0003	-0.1098	0.1273
				CORT,28°C	0.0279	0.0003	-0.0952	0.1519
			Adult	Control,23°C	-0.0113	0.0005	-0.1843	0.1618
				Control,28°C	-0.0101	0.0005	-0.1801	0.1656
				CORT,23°C	-0.0023	0.0005	-0.1732	0.1709
				CORT,28°C	-0.0012	0.0005	-0.1721	0.1773

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. guichenoti</i>	Mitochondrial Membrane Potential (MFI)		Juvenile	Control,23°C	0.0212	0.0006	-0.1814	0.2249
				Control,28°C	0.0058	0.0006	-0.1952	0.2105
				CORT,23°C	0.0045	0.0006	-0.1982	0.2085
				CORT,28°C	-0.0108	0.0006	-0.2123	0.1952
			Adult	Control,23°C	-0.0525	0.0012	-0.4633	0.3571
				Control,28°C	-0.0429	0.0012	-0.4529	0.3694
				CORT,23°C	-0.0362	0.0012	-0.4474	0.3705
				CORT,28°C	-0.0266	0.0012	-0.4349	0.3796
	Mitochondrial Superoxide (MFI)		Juvenile	Control,23°C	0.0045	0.0003	-0.1174	0.1271
				Control,28°C	-0.0096	0.0003	-0.1300	0.1117
				CORT,23°C	0.0318	0.0003	-0.0900	0.1548
				CORT,28°C	0.0177	0.0003	-0.1038	0.1396
			Adult	Control,23°C	0.0113	0.0003	-0.1017	0.1289
				Control,28°C	0.0149	0.0003	-0.0996	0.1319
				CORT,23°C	0.0069	0.0003	-0.1060	0.1230
				CORT,28°C	0.0105	0.0003	-0.1029	0.1264

Supplemental Table 56. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* mitochondrial function (mitochondrial membrane potential or mitochondrial superoxide) in heart cells. Data are the differences in predicted posterior distributions generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). Contrasts are the differences in posterior distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Mitochondrial Membrane Potential (MFI)	Juvenile	Heart	[Control, 23°C] - [Control, 28°C]	0.013	0.000	-0.047	0.072	0.664
				[Control, 23°C] - [CORT, 23°C]	-0.045	0.000	-0.106	0.017	0.155
				[Control, 23°C] - [CORT, 28°C]	-0.032	0.000	-0.125	0.061	0.495
				[Control, 28°C] - [CORT, 23°C]	-0.058	0.000	-0.136	0.020	0.146
				[Control, 28°C] - [CORT, 28°C]	-0.045	0.000	-0.106	0.017	0.155
				[CORT, 23°C] - [CORT, 28°C]	0.013	0.000	-0.047	0.072	0.664
		Adult	Heart	[Control, 23°C] - [Control, 28°C]	0.039	0.000	-0.079	0.153	0.485
				[Control, 23°C] - [CORT, 23°C]	0.106	0.000	-0.028	0.240	0.113
				[Control, 23°C] - [CORT, 28°C]	0.145	0.001	-0.042	0.331	0.118
				[Control, 28°C] - [CORT, 23°C]	0.067	0.000	-0.097	0.234	0.411
				[Control, 28°C] - [CORT, 28°C]	0.106	0.000	-0.028	0.240	0.113
				[CORT, 23°C] - [CORT, 28°C]	0.039	0.000	-0.079	0.153	0.485
	Mitochondrial Superoxide (MFI)	Juvenile	Heart	[Control, 23°C] - [Control, 28°C]	-0.020	0.000	-0.075	0.034	0.469
				[Control, 23°C] - [CORT, 23°C]	-0.027	0.000	-0.083	0.031	0.357
				[Control, 23°C] - [CORT, 28°C]	-0.046	0.000	-0.131	0.039	0.276

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. guichenoti</i>	Mitochondrial Membrane Potential (MFI)	Adult		[Control, 28°C] - [CORT, 23°C]	-0.007	0.000	-0.079	0.066	0.838
				[Control, 28°C] - [CORT, 28°C]	-0.027	0.000	-0.083	0.031	0.357
				[CORT, 23°C] - [CORT, 28°C]	-0.020	0.000	-0.075	0.034	0.469
				[Control, 23°C] - [Control, 28°C]	-0.001	0.000	-0.055	0.053	0.959
				[Control, 23°C] - [CORT, 23°C]	-0.009	0.000	-0.075	0.057	0.776
				[Control, 23°C] - [CORT, 28°C]	-0.010	0.000	-0.103	0.084	0.824
				[Control, 28°C] - [CORT, 23°C]	-0.008	0.000	-0.085	0.068	0.840
				[Control, 28°C] - [CORT, 28°C]	-0.009	0.000	-0.075	0.057	0.776
				[CORT, 23°C] - [CORT, 28°C]	-0.001	0.000	-0.055	0.053	0.959
		Juvenile		[Control, 23°C] - [Control, 28°C]	0.015	0.000	-0.023	0.054	0.428
				[Control, 23°C] - [CORT, 23°C]	0.017	0.000	-0.022	0.055	0.387
				[Control, 23°C] - [CORT, 28°C]	0.032	0.000	-0.024	0.088	0.256
				[Control, 28°C] - [CORT, 23°C]	0.001	0.000	-0.051	0.053	0.952
				[Control, 28°C] - [CORT, 28°C]	0.017	0.000	-0.022	0.055	0.387
				[CORT, 23°C] - [CORT, 28°C]	0.015	0.000	-0.023	0.054	0.428
	Mitochondrial Superoxide (MFI)	Adult		[Control, 23°C] - [Control, 28°C]	-0.010	0.000	-0.078	0.060	0.775
				[Control, 23°C] - [CORT, 23°C]	-0.016	0.000	-0.085	0.052	0.632
				[Control, 23°C] - [CORT, 28°C]	-0.026	0.000	-0.117	0.067	0.559
				[Control, 28°C] - [CORT, 23°C]	-0.007	0.000	-0.111	0.097	0.893
				[Control, 28°C] - [CORT, 28°C]	-0.016	0.000	-0.085	0.052	0.632
				[CORT, 23°C] - [CORT, 28°C]	-0.010	0.000	-0.078	0.060	0.775
		Juvenile		[Control, 23°C] - [Control, 28°C]	0.014	0.000	-0.036	0.065	0.585

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
				[Control, 23°C] - [CORT, 23°C]	-0.027	0.000	-0.078	0.023	0.280
				[Control, 23°C] - [CORT, 28°C]	-0.013	0.000	-0.088	0.060	0.720
				[Control, 28°C] - [CORT, 23°C]	-0.041	0.000	-0.111	0.027	0.234
				[Control, 28°C] - [CORT, 28°C]	-0.027	0.000	-0.078	0.023	0.280
				[CORT, 23°C] - [CORT, 28°C]	0.014	0.000	-0.036	0.065	0.585
		Adult		[Control, 23°C] - [Control, 28°C]	-0.004	0.000	-0.037	0.029	0.828
				[Control, 23°C] - [CORT, 23°C]	0.004	0.000	-0.029	0.038	0.792
				[Control, 23°C] - [CORT, 28°C]	0.001	0.000	-0.044	0.045	0.968
				[Control, 28°C] - [CORT, 23°C]	0.008	0.000	-0.042	0.058	0.744
				[Control, 28°C] - [CORT, 28°C]	0.004	0.000	-0.029	0.038	0.792
				[CORT, 23°C] - [CORT, 28°C]	-0.004	0.000	-0.037	0.029	0.828

Supplemental Table 57. Comparisons of CORT-treatment and incubation temperature effects on mitochondrial function (mitochondrial membrane potential or mitochondrial superoxide) in heart cells between *L. delicata* and *L. guichenoti*. Contrasts are the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Mitochondrial Membrane Potential (MFI)	Juvenile	Heart	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.061	0	-0.011	0.134	0.096
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.002	0	-0.069	0.073	0.947
	Adult	Heart	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.122	0	-0.272	0.026	0.104
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.049	0	-0.181	0.086	0.467
Mitochondrial Superoxide (MFI)	Juvenile	Heart	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.001	0	-0.077	0.076	0.990
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.034	0	-0.040	0.108	0.364
	Adult	Heart	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.013	0	-0.060	0.086	0.712
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.002	0	-0.066	0.061	0.941

Supplemental Table 58. Comparisons of CORT-treatment and incubation temperature effects on mitochondrial function (mitochondrial membrane potential or mitochondrial superoxide) in heart cells between juveniles and adults of *L. delicata* and *L. guichenoti*. Contrasts are the differences between adult and juvenile lizards in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Tissue	Species	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Mitochondrial Membrane Potential (MFI)	Heart	<i>L. delicata</i>	Stress [Adult - Juvenile]	-0.151	0	-0.298	-0.004	0.045
			Temperature [Adult - Juvenile]	-0.026	0	-0.154	0.105	0.680
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	0.033	0	-0.046	0.112	0.405
			Temperature [Adult - Juvenile]	0.025	0	-0.054	0.104	0.522
Mitochondrial Superoxide (MFI)		<i>L. delicata</i>	Stress [Adult - Juvenile]	-0.018	0	-0.103	0.069	0.687
			Temperature [Adult - Juvenile]	-0.019	0	-0.096	0.058	0.635
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	-0.032	0	-0.092	0.029	0.298
			Temperature [Adult - Juvenile]	0.018	0	-0.042	0.078	0.572

Supplemental Table 57. Comparisons of CORT-treatment and incubation temperature effects on mitochondrial function (mitochondrial membrane potential or mitochondrial superoxide) between liver and heart cells in *L. delicata* and *L. guichenoti*. Contrasts are the differences between liver and heart cells in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Mitochondrial Membrane Potential (MFI)	Adult	Stress [Liver - Heart]	0.135	0	-0.018	0.289	0.082
			Temperature [Liver - Heart]	0.038	0	-0.096	0.170	0.556
		Juvenile	Stress [Liver - Heart]	0.005	0	-0.102	0.112	0.921
			Temperature [Liver - Heart]	0.018	0	-0.086	0.120	0.739
	Mitochondrial Superoxide (MFI)	Adult	Stress [Liver - Heart]	-0.014	0	-0.094	0.065	0.719
			Temperature [Liver - Heart]	0.014	0	-0.052	0.082	0.674
		Juvenile	Stress [Liver - Heart]	-0.071	0	-0.153	0.011	0.091
			Temperature [Liver - Heart]	0.004	0	-0.075	0.082	0.909
<i>L. guichenoti</i>	Mitochondrial Membrane Potential (MFI)	Adult	Stress [Liver - Heart]	-0.027	0	-0.124	0.069	0.578
			Temperature [Liver - Heart]	-0.011	0	-0.110	0.088	0.822
		Juvenile	Stress [Liver - Heart]	0.006	0	-0.058	0.070	0.858
			Temperature [Liver - Heart]	0.021	0	-0.042	0.085	0.505

Species	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
	Mitochondrial Superoxide (MFI)	Adult	Stress [Liver - Heart]	-0.008	0	-0.084	0.067	0.829
			Temperature [Liver - Heart]	0.035	0	-0.041	0.111	0.350
		Juvenile	Stress [Liver - Heart]	-0.032	0	-0.107	0.043	0.406
			Temperature [Liver - Heart]	0.028	0	-0.047	0.104	0.460

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Supplemental Table 59. Analysis of main effects of developmental CORT treatment and incubation temperature on oxidative damage in the livers of juvenile and adult *L. delicata* and *L. guichenoti*. Data are the predictive posterior distributions derived from a multi-response Bayesian GLMM including log-transformed fluorescence values of cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, and oxidative DNA damage as responses and accounting for residual correlations among each parameter. For each parameter, CORT treatment and incubation temperature were included as fixed effects, and plate number was included as a random intercept. We show the difference in mean between posterior distributions (Estimate) predicted by Bayesian GLMM, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Liver	Lipid Peroxidation (MFI)	Juvenile	Stress [CORT - Control]	0.022	<0.001	-0.082	0.124	0.676
				Temperature [28°C - 23°C]	0.015	<0.001	-0.085	0.117	0.757
			Adult	Stress [CORT - Control]	0.038	<0.001	-0.066	0.144	0.468
				Temperature [28°C - 23°C]	0.002	<0.001	-0.092	0.095	0.961
		Oxidative DNA Damage (MFI)	Juvenile	Stress [CORT - Control]	0.086	<0.001	-0.009	0.180	0.074
				Temperature [28°C - 23°C]	0.002	<0.001	-0.089	0.094	0.972
			Adult	Stress [CORT - Control]	0.005	<0.001	-0.103	0.112	0.931
				Temperature [28°C - 23°C]	-0.031	<0.001	-0.127	0.066	0.528
<i>L. guichenoti</i>		Lipid Peroxidation (MFI)	Juvenile	Stress [CORT - Control]	-0.021	<0.001	-0.108	0.067	0.635
				Temperature [28°C - 23°C]	-0.045	<0.001	-0.131	0.042	0.303

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
		Oxidative DNA Damage (MFI)	Adult	Stress [CORT - Control]	-0.009	<0.001	-0.090	0.070	0.818
				Temperature [28°C - 23°C]	0.012	<0.001	-0.071	0.095	0.763
			Juvenile	Stress [CORT - Control]	0.140	<0.001	-0.013	0.293	0.073
				Temperature [28°C - 23°C]	-0.023	<0.001	-0.171	0.124	0.753
			Adult	Stress [CORT - Control]	0.031	<0.001	-0.068	0.127	0.522
				Temperature [28°C - 23°C]	-0.044	<0.001	-0.143	0.056	0.386

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Supplemental Table 60. Summary of predicted posterior distributions of juvenile and adult *L. delicata* and *L. guichenoti* oxidative damage (lipid peroxidation or oxidative DNA damage) in liver cells for each CORT-treatment by incubation temperature combination. Predictive posterior distributions were generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Liver	Lipid Peroxidation (MFI)	Juvenile	Control,23°C	-0.0250	0.0006	-0.2389	0.1841
				Control,28°C	-0.0096	0.0006	-0.2227	0.1983
				CORT,23°C	-0.0033	0.0006	-0.2192	0.2076
				CORT,28°C	0.0121	0.0006	-0.2140	0.2313
			Adult	Control,23°C	-0.0411	0.0006	-0.2446	0.1656
				Control,28°C	-0.0390	0.0006	-0.2412	0.1681
				CORT,23°C	-0.0032	0.0006	-0.2109	0.1976
				CORT,28°C	-0.0012	0.0006	-0.2081	0.2014
		Oxidative DNA Damage (MFI)	Juvenile	Control,23°C	-0.0365	0.0005	-0.2080	0.1398
				Control,28°C	-0.0349	0.0005	-0.2092	0.1422
				CORT,23°C	0.0490	0.0005	-0.1305	0.2291
				CORT,28°C	0.0507	0.0005	-0.1385	0.2374
			Adult	Control,23°C	0.0427	0.0008	-0.2616	0.3458
				Control,28°C	0.0122	0.0009	-0.2931	0.3146
				CORT,23°C	0.0473	0.0009	-0.2592	0.3497
				CORT,28°C	0.0167	0.0009	-0.2910	0.3187

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. guichenoti</i>		Lipid Peroxidation (MFI)	Juvenile	Control,23°C	0.0392	0.0006	-0.1745	0.2545
				Control,28°C	-0.0055	0.0006	-0.2147	0.2110
				CORT,23°C	0.0184	0.0006	-0.1959	0.2314
				CORT,28°C	-0.0262	0.0006	-0.2370	0.1886
			Adult	Control,23°C	-0.0410	0.0005	-0.2225	0.1426
				Control,28°C	-0.0287	0.0005	-0.2087	0.1554
				CORT,23°C	-0.0504	0.0005	-0.2312	0.1325
				CORT,28°C	-0.0381	0.0005	-0.2198	0.1468
		Oxidative DNA Damage (MFI)	Juvenile	Control,23°C	-0.0468	0.0007	-0.2944	0.2024
				Control,28°C	-0.0702	0.0007	-0.3120	0.1707
				CORT,23°C	0.0936	0.0007	-0.1534	0.3411
				CORT,28°C	0.0702	0.0007	-0.1746	0.3177
			Adult	Control,23°C	-0.0059	0.0008	-0.3052	0.2924
				Control,28°C	-0.0498	0.0008	-0.3476	0.2503
				CORT,23°C	0.0250	0.0008	-0.2767	0.3246
				CORT,28°C	-0.0190	0.0008	-0.3172	0.2836

Supplemental Table 61. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* oxidative damage (lipid peroxidation or oxidative DNA damage) in liver cells. Data are the differences in predicted posterior distributions generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). Contrasts are the differences in posterior distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Lipid Peroxidation (MFI)	Juvenile	Liver	[Control, 23°C] - [Control, 28°C]	-0.015	0.000	-0.117	0.085	0.757
				[Control, 23°C] - [CORT, 23°C]	-0.022	0.000	-0.124	0.082	0.676
				[Control, 23°C] - [CORT, 28°C]	-0.037	0.000	-0.197	0.120	0.647
				[Control, 28°C] - [CORT, 23°C]	-0.006	0.000	-0.137	0.122	0.920
				[Control, 28°C] - [CORT, 28°C]	-0.022	0.000	-0.124	0.082	0.676
				[CORT, 23°C] - [CORT, 28°C]	-0.015	0.000	-0.117	0.085	0.757
		Adult	Liver	[Control, 23°C] - [Control, 28°C]	-0.002	0.000	-0.095	0.092	0.961
				[Control, 23°C] - [CORT, 23°C]	-0.038	0.000	-0.144	0.066	0.468
				[Control, 23°C] - [CORT, 28°C]	-0.040	0.000	-0.177	0.097	0.561
				[Control, 28°C] - [CORT, 23°C]	-0.036	0.000	-0.181	0.105	0.622
				[Control, 28°C] - [CORT, 28°C]	-0.038	0.000	-0.144	0.066	0.468
				[CORT, 23°C] - [CORT, 28°C]	-0.002	0.000	-0.095	0.092	0.961
	Oxidative DNA Damage (MFI)	Juvenile	Liver	[Control, 23°C] - [Control, 28°C]	-0.002	0.000	-0.094	0.089	0.972
				[Control, 23°C] - [CORT, 23°C]	-0.086	0.000	-0.180	0.009	0.074
				[Control, 23°C] - [CORT, 28°C]	-0.087	0.000	-0.232	0.057	0.232

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. guichenoti</i>	Lipid Peroxidation (MFI)	Adult		[Control, 28°C] - [CORT, 23°C]	-0.084	0.000	-0.201	0.034	0.156
				[Control, 28°C] - [CORT, 28°C]	-0.086	0.000	-0.180	0.009	0.074
				[CORT, 23°C] - [CORT, 28°C]	-0.002	0.000	-0.094	0.089	0.972
				[Control, 23°C] - [Control, 28°C]	0.031	0.000	-0.066	0.127	0.528
				[Control, 23°C] - [CORT, 23°C]	-0.005	0.000	-0.112	0.103	0.931
				[Control, 23°C] - [CORT, 28°C]	0.026	0.000	-0.115	0.167	0.718
				[Control, 28°C] - [CORT, 23°C]	-0.035	0.000	-0.182	0.111	0.632
				[Control, 28°C] - [CORT, 28°C]	-0.005	0.000	-0.112	0.103	0.931
				[CORT, 23°C] - [CORT, 28°C]	0.031	0.000	-0.066	0.127	0.528
		Juvenile		[Control, 23°C] - [Control, 28°C]	0.045	0.000	-0.042	0.131	0.303
				[Control, 23°C] - [CORT, 23°C]	0.021	0.000	-0.067	0.108	0.635
				[Control, 23°C] - [CORT, 28°C]	0.065	0.000	-0.061	0.192	0.310
				[Control, 28°C] - [CORT, 23°C]	-0.024	0.000	-0.143	0.095	0.691
				[Control, 28°C] - [CORT, 28°C]	0.021	0.000	-0.067	0.108	0.635
				[CORT, 23°C] - [CORT, 28°C]	0.045	0.000	-0.042	0.131	0.303
	Oxidative DNA Damage (MFI)	Adult		[Control, 23°C] - [Control, 28°C]	-0.012	0.000	-0.095	0.071	0.763
				[Control, 23°C] - [CORT, 23°C]	0.009	0.000	-0.070	0.090	0.818
				[Control, 23°C] - [CORT, 28°C]	-0.003	0.000	-0.116	0.112	0.959
				[Control, 28°C] - [CORT, 23°C]	0.022	0.000	-0.094	0.138	0.710
				[Control, 28°C] - [CORT, 28°C]	0.009	0.000	-0.070	0.090	0.818
				[CORT, 23°C] - [CORT, 28°C]	-0.012	0.000	-0.095	0.071	0.763
		Juvenile		[Control, 23°C] - [Control, 28°C]	0.023	0.000	-0.124	0.171	0.753

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
				[Control, 23°C] - [CORT, 23°C]	-0.140	0.000	-0.293	0.013	0.073
				[Control, 23°C] - [CORT, 28°C]	-0.117	0.001	-0.334	0.102	0.289
				[Control, 28°C] - [CORT, 23°C]	-0.164	0.001	-0.369	0.041	0.114
				[Control, 28°C] - [CORT, 28°C]	-0.140	0.000	-0.293	0.013	0.073
				[CORT, 23°C] - [CORT, 28°C]	0.023	0.000	-0.124	0.171	0.753
		Adult		[Control, 23°C] - [Control, 28°C]	0.044	0.000	-0.056	0.143	0.386
				[Control, 23°C] - [CORT, 23°C]	-0.031	0.000	-0.127	0.068	0.522
				[Control, 23°C] - [CORT, 28°C]	0.013	0.000	-0.126	0.151	0.845
				[Control, 28°C] - [CORT, 23°C]	-0.075	0.000	-0.214	0.066	0.294
				[Control, 28°C] - [CORT, 28°C]	-0.031	0.000	-0.127	0.068	0.522
				[CORT, 23°C] - [CORT, 28°C]	0.044	0.000	-0.056	0.143	0.386

Supplemental Table 62. Comparisons of CORT-treatment and incubation temperature effects on oxidative damage (lipid peroxidation or oxidative DNA damage) in liver cells between *L. delicata* and *L. guichenoti*. Contrasts are the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Lipid Peroxidation (MFI)	Juvenile	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.042	0	-0.091	0.178	0.541
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.060	0	-0.073	0.194	0.371
	Adult	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.047	0	-0.084	0.181	0.474
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.010	0	-0.135	0.113	0.866
Oxidative DNA Damage (MFI)	Juvenile	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.055	0	-0.233	0.123	0.541
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.025	0	-0.149	0.200	0.774
	Adult	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.026	0	-0.171	0.120	0.720
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.013	0	-0.126	0.151	0.853

497 Supplemental Table 63. Comparisons of CORT-treatment and incubation temperature effects on oxidative damage (lipid peroxidation or oxidative DNA damage)
 498 in liver cells between juveniles and adults of *L. delicata* and *L. guichenoti*. Contrasts are the differences between adult and juvenile lizards in effect size
 499 distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference
 500 in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The
 501 pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Tissue	Species	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Lipid Peroxidation (MFI)	Liver	<i>L. delicata</i>	Stress [Adult - Juvenile]	0.016	0	-0.131	0.164	0.827
			Temperature [Adult - Juvenile]	-0.013	0	-0.150	0.124	0.850
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	0.011	0	-0.107	0.131	0.843
			Temperature [Adult - Juvenile]	0.057	0	-0.063	0.177	0.349
Oxidative DNA Damage (MFI)		<i>L. delicata</i>	Stress [Adult - Juvenile]	-0.081	0	-0.224	0.062	0.263
			Temperature [Adult - Juvenile]	-0.032	0	-0.165	0.100	0.635
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	-0.110	0	-0.290	0.070	0.226
			Temperature [Adult - Juvenile]	-0.021	0	-0.200	0.157	0.820

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Supplemental Table 64. Analysis of main effects of developmental CORT treatment and incubation temperature on oxidative damage in the hearts of juvenile and adult *L. delicata* and *L. guichenoti*. Data are the predictive posterior distributions derived from a multi-response Bayesian GLMM including log-transformed fluorescence values of cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, and oxidative DNA damage as responses and accounting for residual correlations among each parameter. For each parameter, CORT treatment and incubation temperature were included as fixed effects, and plate number was included as a random intercept. We show the difference in mean between posterior distributions (Estimate) predicted by Bayesian GLMM, the standard error about the Estimate (Est.Err.), the lower (L95% C.I.) and upper (U95% C.I.) 95% confidence intervals of the resulting difference in distributions, and a pMCMC value that tests whether the difference in distributions differs from zero. The summary statistics are shown for the difference in distributions in main effects of CORT-treatment (predicted distribution for CORT treated animals minus the distribution for Control treated animals) and incubation temperature (distribution for animals incubated at 28°C minus the distribution for animals incubated at 23°C).

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Heart	Lipid Peroxidation (MFI)	Juvenile	Stress [CORT - Control]	-0.125	<0.001	-0.279	0.025	0.105
				Temperature [28°C - 23°C]	-0.028	<0.001	-0.170	0.116	0.702
			Adult	Stress [CORT - Control]	0.047	<0.001	-0.065	0.163	0.402
				Temperature [28°C - 23°C]	0.085	<0.001	-0.015	0.182	0.089
		Oxidative DNA Damage (MFI)	Juvenile	Stress [CORT - Control]	-0.212	<0.001	-0.449	0.030	0.082
				Temperature [28°C - 23°C]	-0.089	<0.001	-0.319	0.138	0.432
			Adult	Stress [CORT - Control]	0.093	<0.001	-0.083	0.272	0.286
				Temperature [28°C - 23°C]	0.153	<0.001	0.005	0.303	0.043
<i>L. guichenoti</i>		Lipid Peroxidation (MFI)	Juvenile	Stress [CORT - Control]	-0.052	<0.001	-0.179	0.073	0.399
				Temperature [28°C - 23°C]	0.048	<0.001	-0.084	0.181	0.470

Species	Tissue	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
			Adult	Stress [CORT - Control]	0.009	<0.001	-0.082	0.101	0.834
				Temperature [28°C - 23°C]	-0.044	<0.001	-0.136	0.047	0.325
		Oxidative DNA Damage (MFI)	Juvenile	Stress [CORT - Control]	0.069	<0.001	-0.117	0.254	0.448
				Temperature [28°C - 23°C]	0.109	<0.001	-0.086	0.301	0.262
			Adult	Stress [CORT - Control]	-0.022	<0.001	-0.181	0.135	0.781
				Temperature [28°C - 23°C]	-0.127	<0.001	-0.285	0.029	0.107

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Supplemental Table 65. Summary of predicted posterior distributions of juvenile and adult *L. delicata* and *L. guichenoti* oxidative damage (lipid peroxidation or oxidative DNA damage) in heart cells for each CORT-treatment by incubation temperature combination. Predictive posterior distributions were generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). The mean of the predictive posterior distribution (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided.

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. delicata</i>	Heart	Lipid Peroxidation (MFI)	Juvenile	Control,23°C	0.0771	0.0005	-0.0858	0.2434
				Control,28°C	0.0496	0.0005	-0.1173	0.2168
				CORT,23°C	-0.0483	0.0005	-0.2234	0.1401
				CORT,28°C	-0.0758	0.0005	-0.2635	0.1240
			Adult	Control,23°C	-0.0825	0.0009	-0.4119	0.2432
				Control,28°C	0.0024	0.0009	-0.3309	0.3289
				CORT,23°C	-0.0355	0.0009	-0.3720	0.2870
				CORT,28°C	0.0493	0.0009	-0.2910	0.3785
	Heart	Oxidative DNA Damage (MFI)	Juvenile	Control,23°C	0.0569	0.0010	-0.2864	0.3916
				Control,28°C	-0.0319	0.0010	-0.3704	0.3012
				CORT,23°C	-0.1549	0.0010	-0.5256	0.1893
				CORT,28°C	-0.2437	0.0011	-0.6250	0.1172
			Adult	Control,23°C	-0.1837	0.0024	-1.0621	0.6914
				Control,28°C	-0.0303	0.0024	-0.9102	0.8487
				CORT,23°C	-0.0909	0.0025	-0.9809	0.7840
				CORT,28°C	0.0626	0.0025	-0.8304	0.9398

Species	Tissue	Trait	Age Class	Treatment	Estimate	Est.Err.	L95% C.I.	U95% C.I.
<i>L. guichenoti</i>		Lipid Peroxidation (MFI)	Juvenile	Control,23°C	0.0497	0.0007	-0.1809	0.2901
				Control,28°C	0.0975	0.0006	-0.1243	0.3323
				CORT,23°C	-0.0027	0.0006	-0.2282	0.2310
				CORT,28°C	0.0451	0.0006	-0.1792	0.2810
			Adult	Control,23°C	0.0240	0.0007	-0.2410	0.2890
				Control,28°C	-0.0202	0.0007	-0.2866	0.2476
				CORT,23°C	0.0335	0.0007	-0.2300	0.3012
				CORT,28°C	-0.0108	0.0007	-0.2714	0.2567
		Oxidative DNA Damage (MFI)	Juvenile	Control,23°C	-0.1724	0.0010	-0.5234	0.1533
				Control,28°C	-0.0637	0.0009	-0.3941	0.2581
				CORT,23°C	-0.1033	0.0009	-0.4407	0.2207
				CORT,28°C	0.0054	0.0009	-0.3281	0.3361
			Adult	Control,23°C	0.0980	0.0023	-0.7192	0.9164
				Control,28°C	-0.0295	0.0023	-0.8521	0.7915
				CORT,23°C	0.0761	0.0023	-0.7472	0.8986
				CORT,28°C	-0.0514	0.0023	-0.8726	0.7718

Supplemental Table 66. Pairwise comparisons among treatment groups for *L. delicata* and *L. guichenoti* oxidative damage (lipid peroxidation or oxidative DNA damage) in heart cells. Data are the differences in predicted posterior distributions generated by multi-response Bayesian GLMMs including six parameters of cellular physiology (cell size, mitochondrial mass, mitochondrial membrane potential, mitochondrial superoxide, lipid peroxidation, oxidative DNA damage). Each parameter included CORT treatment and incubation temperature as main effects. Cell size was included as a covariate in estimates of mitochondrial mass to account for larger cells having generally more mitochondria. Mitochondrial membrane potential was included as a covariate in estimates of mitochondrial superoxide to allow for estimation of oxidative stress under similar rates of respiration. Plate was included as a random effect to account for any unexplained sources of experimental or machine variation among trial dates. Individual was included as a random effect to account for triplicate samples for each tissue from each lizard. Models were run separately for each tissue (liver or heart), each age class (juvenile or adult), and each species (*L. delicata* or *L. guichenoti*). Contrasts are the differences in posterior distributions between treatment groups. The mean of the difference in predictive posterior distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Lipid Peroxidation (MFI)	Juvenile	Heart	[Control, 23°C] - [Control, 28°C]	0.028	0.000	-0.116	0.170	0.702
				[Control, 23°C] - [CORT, 23°C]	0.125	0.000	-0.025	0.279	0.105
				[Control, 23°C] - [CORT, 28°C]	0.153	0.001	-0.062	0.371	0.161
				[Control, 28°C] - [CORT, 23°C]	0.098	0.001	-0.100	0.301	0.322
				[Control, 28°C] - [CORT, 28°C]	0.125	0.000	-0.025	0.279	0.105
				[CORT, 23°C] - [CORT, 28°C]	0.028	0.000	-0.116	0.170	0.702
		Adult	Heart	[Control, 23°C] - [Control, 28°C]	-0.085	0.000	-0.182	0.015	0.089
				[Control, 23°C] - [CORT, 23°C]	-0.047	0.000	-0.163	0.065	0.402
				[Control, 23°C] - [CORT, 28°C]	-0.132	0.000	-0.284	0.018	0.086
				[Control, 28°C] - [CORT, 23°C]	0.038	0.000	-0.110	0.184	0.604
				[Control, 28°C] - [CORT, 28°C]	-0.047	0.000	-0.163	0.065	0.402
				[CORT, 23°C] - [CORT, 28°C]	-0.085	0.000	-0.182	0.015	0.089
	Oxidative DNA Damage (MFI)	Juvenile	Heart	[Control, 23°C] - [Control, 28°C]	0.089	0.001	-0.138	0.319	0.432
				[Control, 23°C] - [CORT, 23°C]	0.212	0.001	-0.030	0.449	0.082
				[Control, 23°C] - [CORT, 28°C]	0.301	0.001	-0.051	0.647	0.090

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. guichenoti</i>	Lipid Peroxidation (MFI)	Adult		[Control, 28°C] - [CORT, 23°C]	0.123	0.001	-0.198	0.433	0.422
				[Control, 28°C] - [CORT, 28°C]	0.212	0.001	-0.030	0.449	0.082
				[CORT, 23°C] - [CORT, 28°C]	0.089	0.001	-0.138	0.319	0.432
				[Control, 23°C] - [Control, 28°C]	-0.153	0.000	-0.303	-0.005	0.043
				[Control, 23°C] - [CORT, 23°C]	-0.093	0.000	-0.272	0.083	0.286
				[Control, 23°C] - [CORT, 28°C]	-0.246	0.001	-0.485	-0.011	0.043
				[Control, 28°C] - [CORT, 23°C]	0.061	0.001	-0.166	0.284	0.586
				[Control, 28°C] - [CORT, 28°C]	-0.093	0.000	-0.272	0.083	0.286
				[CORT, 23°C] - [CORT, 28°C]	-0.153	0.000	-0.303	-0.005	0.043
		Juvenile		[Control, 23°C] - [Control, 28°C]	-0.048	0.000	-0.181	0.084	0.470
				[Control, 23°C] - [CORT, 23°C]	0.052	0.000	-0.073	0.179	0.399
				[Control, 23°C] - [CORT, 28°C]	0.005	0.001	-0.189	0.199	0.967
				[Control, 28°C] - [CORT, 23°C]	0.100	0.000	-0.069	0.270	0.232
				[Control, 28°C] - [CORT, 28°C]	0.052	0.000	-0.073	0.179	0.399
				[CORT, 23°C] - [CORT, 28°C]	-0.048	0.000	-0.181	0.084	0.470
	Oxidative DNA Damage (MFI)	Adult		[Control, 23°C] - [Control, 28°C]	0.044	0.000	-0.047	0.136	0.325
				[Control, 23°C] - [CORT, 23°C]	-0.009	0.000	-0.101	0.082	0.834
				[Control, 23°C] - [CORT, 28°C]	0.035	0.000	-0.085	0.155	0.562
				[Control, 28°C] - [CORT, 23°C]	-0.054	0.000	-0.190	0.083	0.429
				[Control, 28°C] - [CORT, 28°C]	-0.009	0.000	-0.101	0.082	0.834
				[CORT, 23°C] - [CORT, 28°C]	0.044	0.000	-0.047	0.136	0.325
		Juvenile		[Control, 23°C] - [Control, 28°C]	-0.109	0.001	-0.301	0.086	0.262

Species	Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
				[Control, 23°C] - [CORT, 23°C]	-0.069	0.001	-0.254	0.117	0.448
				[Control, 23°C] - [CORT, 28°C]	-0.178	0.001	-0.464	0.109	0.222
				[Control, 28°C] - [CORT, 23°C]	0.040	0.001	-0.205	0.284	0.743
				[Control, 28°C] - [CORT, 28°C]	-0.069	0.001	-0.254	0.117	0.448
				[CORT, 23°C] - [CORT, 28°C]	-0.109	0.001	-0.301	0.086	0.262
		Adult		[Control, 23°C] - [Control, 28°C]	0.127	0.000	-0.029	0.285	0.107
				[Control, 23°C] - [CORT, 23°C]	0.022	0.000	-0.135	0.181	0.781
				[Control, 23°C] - [CORT, 28°C]	0.149	0.001	-0.054	0.357	0.146
				[Control, 28°C] - [CORT, 23°C]	-0.106	0.001	-0.340	0.132	0.369
				[Control, 28°C] - [CORT, 28°C]	0.022	0.000	-0.135	0.181	0.781
				[CORT, 23°C] - [CORT, 28°C]	0.127	0.000	-0.029	0.285	0.107

Supplemental Table 67. Comparisons of CORT-treatment and incubation temperature effects on oxidative damage (lipid peroxidation or oxidative DNA damage) in heart cells between *L. delicata* and *L. guichenoti*. Contrasts are the differences between *L. delicata* and *L. guichenoti* in effect size distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Age Class	Tissue	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Lipid Peroxidation (MFI)	Juvenile	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.042	0	-0.091	0.178	0.541
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.060	0	-0.073	0.194	0.371
	Adult	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.047	0	-0.084	0.181	0.474
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.010	0	-0.135	0.113	0.866
Oxidative DNA Damage (MFI)	Juvenile	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.055	0	-0.233	0.123	0.541
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.025	0	-0.149	0.200	0.774
	Adult	Liver	Stress [<i>L. delicata</i> - <i>L. guichenoti</i>]	-0.026	0	-0.171	0.120	0.720
			Temperature [<i>L. delicata</i> - <i>L. guichenoti</i>]	0.013	0	-0.126	0.151	0.853

543 Supplemental Table 68. Comparisons of CORT-treatment and incubation temperature effects on oxidative damage (lipid peroxidation or oxidative DNA damage)
544 in heart cells between juveniles and adults of *L. delicata* and *L. guichenoti*. Contrasts are the differences between adult and juvenile lizards in effect size
545 distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference
546 in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The
547 pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Trait	Tissue	Species	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
Lipid Peroxidation (MFI)	Heart	<i>L. delicata</i>	Stress [Adult - Juvenile]	0.172	0.000	-0.015	0.363	0.074
			Temperature [Adult - Juvenile]	0.112	0.000	-0.061	0.286	0.197
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	0.062	0.000	-0.091	0.219	0.425
			Temperature [Adult - Juvenile]	-0.092	0.000	-0.254	0.067	0.255
Oxidative DNA Damage (MFI)		<i>L. delicata</i>	Stress [Adult - Juvenile]	0.305	0.001	0.005	0.599	0.047
			Temperature [Adult - Juvenile]	0.242	0.001	-0.031	0.518	0.079
		<i>L. guichenoti</i>	Stress [Adult - Juvenile]	-0.091	0.000	-0.332	0.151	0.453
			Temperature [Adult - Juvenile]	-0.236	0.000	-0.484	0.010	0.060

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549

550 Supplemental Table 69. Comparisons of CORT-treatment and incubation temperature effects on mitochondrial function (mitochondrial membrane potential or
551 mitochondrial superoxide) between liver and heart cells in *L. delicata* and *L. guichenoti*. Contrasts are the differences between liver and heart cells in effect size
552 distributions for the developmental stress (CORT – Control) or incubation temperature (28°C – 23°C) effects on cellular physiology. The mean of the difference
553 in effect size distributions (Estimate), the standard error about the mean (Est.Err.), and the lower (L95% C.I.) and upper (U95% C.I.) values are provided. The
554 pMCMC value tests whether the difference in distributions is significantly (pMCMC < 0.05) different from zero.

Species	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
<i>L. delicata</i>	Lipid Peroxidation (MFI)	Adult	Stress [Liver - Heart]	-0.009	0.000	-0.165	0.144	0.909
			Temperature [Liver - Heart]	-0.083	0.000	-0.219	0.053	0.230
		Juvenile	Stress [Liver - Heart]	0.147	0.000	-0.035	0.332	0.114
			Temperature [Liver - Heart]	0.043	0.000	-0.130	0.217	0.629
	Oxidative DNA Damage (MFI)	Adult	Stress [Liver - Heart]	-0.088	0.000	-0.296	0.115	0.394
			Temperature [Liver - Heart]	-0.184	0.000	-0.362	-0.006	0.042
		Juvenile	Stress [Liver - Heart]	0.297	0.001	0.038	0.552	0.027
			Temperature [Liver - Heart]	0.090	0.000	-0.151	0.341	0.459
<i>L. guichenoti</i>	Lipid Peroxidation (MFI)	Adult	Stress [Liver - Heart]	-0.019	0.000	-0.140	0.103	0.753
			Temperature [Liver - Heart]	0.057	0.000	-0.066	0.180	0.360
		Juvenile	Stress [Liver - Heart]	0.032	0.000	-0.121	0.186	0.681
			Temperature [Liver - Heart]	-0.093	0.000	-0.251	0.066	0.246

Species	Trait	Age Class	Contrast	Estimate	Est.Err.	L95% C.I.	U95% C.I.	pMCMC
	Oxidative DNA Damage (MFI)	Adult	Stress [Liver - Heart]	0.053	0.000	-0.132	0.238	0.568
			Temperature [Liver - Heart]	0.084	0.000	-0.102	0.268	0.365
		Juvenile	Stress [Liver - Heart]	0.071	0.000	-0.167	0.312	0.553
			Temperature [Liver - Heart]	-0.132	0.000	-0.377	0.112	0.284

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Appendix IV

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Supplemental Material for Chapter 4

559

Appendix IV.1: Supplementary Methods

Methods 1: Animal husbandry

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

Eggs collection and incubation - Between mid-November 2023 to mid-January 2024, we placed a small box (12.5 L x 8.3 W x 5 H cm) with moist vermiculite in one side of the communal enclosures to provide females with a place to lay the eggs. These boxes were checked three days a week. After egg collection, we measured length and width with a digital caliper to the nearest 0.1 mm and weighted the eggs with a digital scale ± 0.001 g error. Then eggs were treated with CORT or vehicle (see CORT and temperature manipulation below) and were placed in individual cups (80 mL) with moist vermiculite (12 parts water to 4 parts vermiculite). The cups were covered with cling wrap to retain moisture and left in two incubators at two different temperatures (see CORT and temperature manipulation below) until hatching.

Hatchlings - Incubators were checked three times a week for hatchlings. Lizards were measured and weighed immediately after hatching. snout-vent length (SVL) and tail length (TL) were measured to the nearest millimeter, and weight was recorded using a digital scale with an accuracy of ± 0.001 g. Hatchlings were then placed in individual enclosures (18.7L x 13.2W x 6.3H cm) with nonstick matting and a small water dish. Watering, feeding, and temperature conditions were maintained as for adults (see above).

587 *Methods 2: flow cytometry*

588 *Brain mitochondrial activity*

589 **Homogenates:** After the medial cortex was extracted, it was transferred immediately to 1.5mL
590 centrifuge tubes containing 100µL of cold 1X PBS and kept on ice until further processing. The
591 tissue was mechanically homogenised by placing the tissue in the well of a 100µm mesh filter
592 (pluriStrainer) affixed atop a 1.5mL centrifuge tube, then mashed with the rubber end of an
593 insulin syringe stopper. The resulting homogenate was then rinsed through the filter with
594 1000µL of cold 1X PBS to prepare a homogenate suspension. Following homogenisation, we
595 centrifuged each sample at 1000 RCF for 10 minutes to pellet cells, then removed the
596 supernatant (hereafter, this process referred to as ‘washing’) and resuspended the cells in 500µL
597 1x PBS. This step was performed to remove cellular debris from homogenates.

598 From each 500µL suspension of homogenate collected on a given trial day, we first added 100µL
599 of homogenate to a pooled sample of each tissue type to use for single-colour controls, and the
600 remaining 400µL of homogenate was split among two 200µL aliquots. One aliquot was used
601 fresh to measure mitochondrial function (mitochondrial density, membrane potential, ROS), one
602 aliquot was cryopreserved for later measurements of oxidative damage (8-OHdG, lipid
603 peroxidation), and the third aliquot was cryopreserved for a different experiment.

604 **Staining fresh samples:** From fresh homogenate suspensions, we loaded the wells of a 96-well
605 flat-bottom plate (Nunc) with 50µL of homogenate in duplicates (2 wells per homogenate).
606 To each replicate well, we added 5µL of a fluorescent probe mix containing equal parts 5µM
607 MitoTracker Deep Red FM, 2.5µM MitoTracker Orange CMTMRos, and 50µM MitoSOX Red.
608 We used these fluorescent probes as indicators of mitochondrial density, mitochondrial
609 membrane potential, and superoxide (ROS) production, respectively. We then added 5µL of 10
610 µg/mL Hoechst 33342 Nuclear Viability Dye to each sample, which we used to distinguish live,
611 viable, intact cells from cellular debris. We then loaded 6 wells with 50µL of homogenate taken
612 from each pooled homogenate suspension (12 wells total), which were to be negative and single-
613 colour controls. One well was left unstained as a negative control, one was stained with all the
614 probes to be a positive control, and the remaining four wells were treated with 5µL of one of
615 5µM MitoTracker Deep Red FM, 2.5µM MitoTracker Orange CMTMRos, 50µM MitoSOX
616 Red, or 10 µg/mL Hoechst 33342 Nuclear Viability Dye. Any remaining pooled homogenate
617 was fixed and frozen as previously described. We incubated the loaded plate at 32 °C for 30
618 minutes to stain and then diluted the samples with 50µL cold 1x PBS to halt the staining process.
619 Upon the completion of staining, samples were immediately transferred to flow cytometry
620 facilities for data collection and were sampled within 2 hours. Samples prepared this way
621 remained viable for flow cytometry for approximately 5 hours post-staining at room temperature
622 (~19°C) before cells began rapidly degrading (DCL, personal observation).

623 The aliquots destined to the analysis of oxidative damage were stained with 20µL (10µL for
624 controls) of 10 µg/mL Hoechst 33342 Nuclear Viability Dye and 20µL of 100µM BODIPY
625 665/676 Lipid Peroxidation Sensor and incubated at 32°C for 20 minutes. Following staining, we
626 washed cells to prevent further binding of unbound fluorescent probes, then resuspended the
627 pellet and continue with cryopreservation.

628 **Cryopreservation:** Aliquots were fixed first by adding the samples into a 1mL solution of 1%
629 Neutral-Buffered Formalin (as a fixative agent) and incubating them at 32°C for 20 min. Then

washed the samples and resuspended the cells in 1mL cold 1X Tris-EDTA (chelates metals that can damage DNA during freezing) and 10% DMSO (a cryoprotectant). Samples were stored at -20°C until oxidative damage assays.

Staining cryopreserved samples: Assays of oxidative damage from cryopreserved samples were performed within 6 months of the initial processing and analysis of fresh samples. On the day of oxidative damage assays, we rapidly thawed frozen samples by briefly (1-2 minutes) submerging them in hot water. We washed each thawed sample twice, the first time resuspending the pelleted cells in 1000µL warm 1X Tris-EDTA, and the second time in 200µL warm 1X PBS containing 20µM digitonin. We incubated the samples at 32°C for 20 minutes to permeabilise the cell membrane, after which we washed the homogenate and resuspended the pelleted cells in 200µL 1X PBS. We added 20µL of 70µM 8-OHdG Polyclonal Antibody to each sample, and we left the homogenate overnight (~12 hours) for the antibody to bind to 8-OHdG, a marker of oxidative damage on DNA. The following day we counterstained the cells with 20µL of 100 µg/mL H+G Goat Anti-Rabbit Conjugate Antibody with Alexa-Fluor 488 at 32°C for 20 minutes. After the cells had been tagged with 8-OHdG antibodies and counterstained, we washed the cells once more and resuspended the pellet in 400µL of 1X PBS. Unstained and single-colour controls were treated identically to samples, but stained with only up to one of BODIPY 665/676, Hoechst 33342, 8-OHdG antibody, or Alexa-Fluor 488 conjugate. Additionally, one control was stained with both 8-OHdG antibody and the Alexa-Fluor 488 conjugate. We then loaded a 96-well plate with 100µL of each single-colour control and 100µL in triplicate of each sample. We performed all flow cytometry assays on samples within 48-hours of thawing the samples.

Flow cytometry: All flow cytometry assays were performed using a flow cytometer with 5-lasers (blue, red, yellow-green, violet, and ultraviolet), 20 detectors, and a high-throughput plate reader (Becton Dickson LSRFortessa X-20) using the default wavelength filters on detectors. Immediately prior to all assays, we performed a quality-control check and laser alignment using the CS&T function of BD FACSDiva (v. 8.0.1) and BD CS&T fluorescent beads (Lot No. 30664) diluted at 1 drop to 150µL 1X PBS. During data collection, data for single-colour controls was filtered using a liberal threshold of 200 on the FSC (roughly, cell size) detector, while data from samples was filtered using a threshold of 200 on the BUV-496 (Hoechst 33342) detector. These thresholds were chosen to filter small debris or inviable or non-intact cells from our observations. The detectors and voltage settings used in data acquisition for each assay type (mitochondrial function, oxidative damage) were determined during pilot trials prior to assays and were not changed during assays to allow for comparison among different plates and samples throughout the experiment. Voltages were chosen to center the distribution of observations in each channel at 10^3 fluorescent intensity and reduce observations of off-scale ($<10^1$ or $>10^5$) events. For the mitochondrial function assay, we recorded data from the following channels (in brackets: voltage; parameter): FSC (44; forward scatter), SSC (180; side scatter), Alexa-Fluor 488 (544; autofluorescence), BUV-496 (450; Hoechst 33342), APC (647; MitoTracker Deep Red FM), PE (522; MitoTracker Orange CMTMRos), and PerCP-Cy5-5 (592; MitoSOX Red) channels. For the oxidative damage assay, we recorded data from the following channels: FSC (425; forward scatter), SSC (300; side scatter), Alexa-Fluor 488 (275; 8-OHdG Antibody + Alexa-Fluor 488 conjugate), BUV-496 (525; Hoechst 33342), and PE-Cy5 (850; BODIPY 665/676). Fluorescent intensity data was collected via the BD FACSDiva (v. 8.0.1) software, with no compensation applied during data collection, and all on a linear scale (detectable range

of 0-252166). We recorded data for both the area and height of the fluorescent signal, but only used the area in downstream analyses, with height being recorded for the sake of quality control. Data was exported from BD FACSDiva as individual *.fcs (“flow cytometry standard”) files for each sample, then imported into FlowJo (v. 10.1) for processing.

Data processing: In FlowJo v. 10.1 we first transformed all fluorescent data to a logarithmic base 10 scale, then applied a basic gating process across all channels by filtering to observations within the detectable range (10^1 - 10^5) to remove any off-scale events. We then used a backgating process wherein we aimed to identify the approximate FSC (cell size) and SSC (cell complexity) range of viable cells that were positive for all stains. We primarily used the BUV-496 channel (Hoechst 33342) in the backgating process to identify intact, nucleated cells ($\text{BUV-496} > 10^3$). For the mitochondrial function assay, we aimed to identify populations of viable cells containing mitochondria ($\text{APC} > 10^3$) and actively respiring (PE and $\text{PerCP-Cy5-5} > 10^3$). For the oxidative damage assay, we aimed to identify populations of cells exhibiting both DNA damage ($\text{Alexa-Fluor 488} > 10^3$) and lipid peroxidation ($\text{PerCP} > 10^3$). When backgating was done, we filtered the data to the FSC by SSC range that captured the ideal population. We used the backgated population for compensation of fluorescent spillover between different fluorescent probes. To account for fluorescent spillover, we used a traditional compensation matrix using the compensation function of FlowJo v.10.1. We identified the “positive” population for each channel as the brightest ~2.5% of the distribution of observations in the respective single-colour control for that channel and used unstained controls as a universal negative. We visually inspected the compensation matrix and its effects on population distributions for under- and over-compensation, whereupon we changed the compensation matrix manually until data was properly compensated. We applied the compensation matrix to all samples for downstream processing. Following compensation, we again gated the data following the same process as for backgating but using the compensated parameters for each channel. Following gating, we exported the geometric mean (mean fluorescent intensity; MFI) and robust confidence-values for each channel for each sample. For analysis, we exported summary statistics of only the area of the fluorescent signal. Although we exported robust confidence values for checking repeatability between replicate samples, we used the geometric means for each individual as our main response variables in analyses.

Appendix IV.2: Supplemental Figures and Tables

Results of the final models

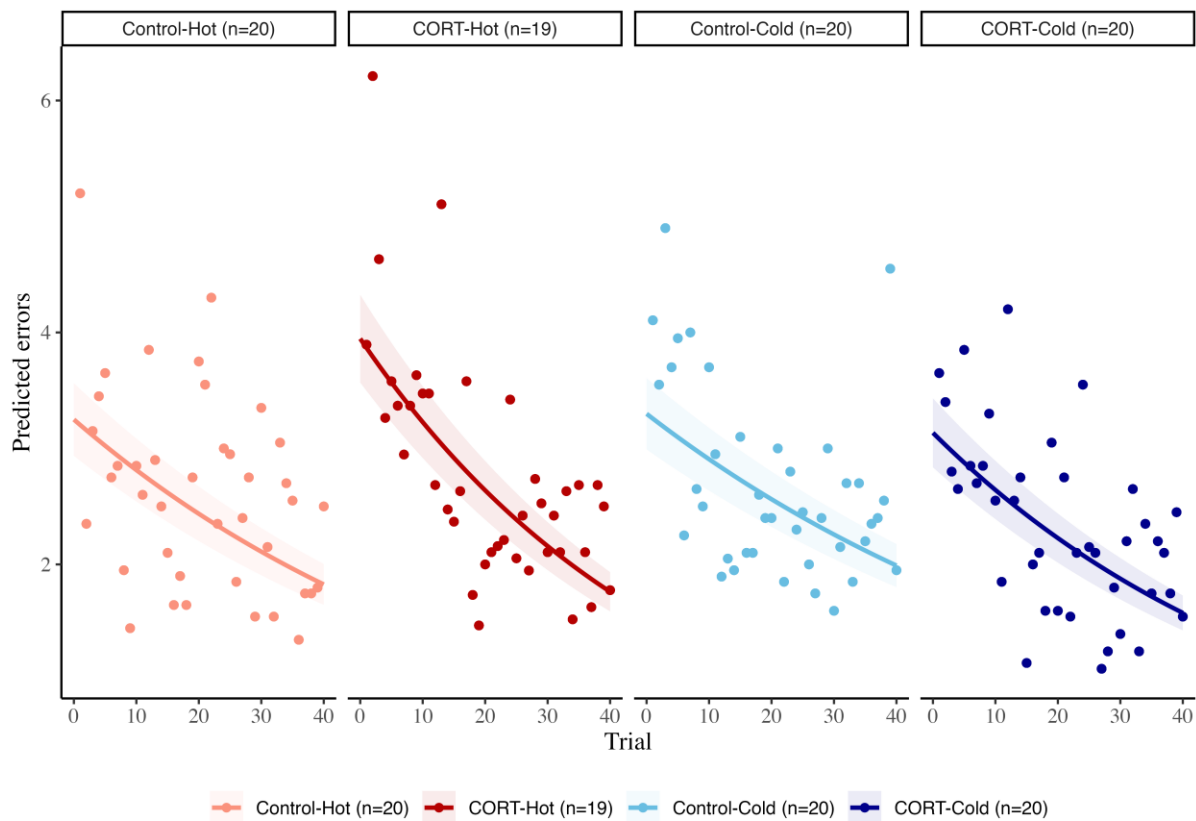


Fig. S1. Raw data of the learning task. Each point represents the mean number of errors per day. The lines represent the modeled data.

713 *Table S1. Estimates of the learning slopes per treatment, the 95% CI, and pMCMC values testing*
714 *the hypothesis that slope is different from 0. In bold, the values that are significant at pMCMC <*
715 *0.05.*

Treatment	Estimated slope	95 CI	pMCMC
CORT-Cold	-0.017	[-0.026, -0.008]	< 0.001
CORT-Hot	-0.020	[-0.029, -0.011]	< 0.001
Control-Cold	-0.013	[-0.021, -0.004]	< 0.05
Control-Hot	-0.014	[-0.023, -0.006]	< 0.05

716

717

718 *Table S2. Estimate of the contrasts between treatments for all the variables analysed, and*
719 *pMCMC values testing the hypothesis that contrast is different from 0. In bold, the values that*
720 *are significant at pMCMC < 0.05.*

Variable	Predictor	Contrast	pMCMC contrast
Mit density	Temperature	-0.018	0.917
	CORT	0.134	0.471
	Interaction	0.176	0.437
Metabolic capacity	Temperature	-0.036	0.839
	CORT	0.162	0.364
	Interaction	0.135	0.559
ROS	Temperature	-0.032	0.875
	CORT	0.148	0.450
	Interaction	0.199	0.382
DNA damage	Temperature	0.007	0.970
	CORT	0.217	0.394
	Interaction	-0.128	0.706
Lipid peroxidation	Temperature	-0.601	0.106
	CORT	0.093	0.701
	Interaction	-0.037	0.909
Learning slopes	Temperature	-0.002	0.701
	CORT	0.005	0.416
	Interaction	0.001	0.881

721 Contrasts were done by:

722 - *Temperature*: $\beta_{\text{Hot}} - \beta_{\text{Cold}}$

723 - *CORT*: $\beta_{\text{CORT}} - \beta_{\text{Control}}$

724 - *Interaction*: $(\beta_{\text{Control-Hot}} - \beta_{\text{CORT-Hot}}) - (\beta_{\text{Control-Cold}} - \beta_{\text{CORT-Cold}})$

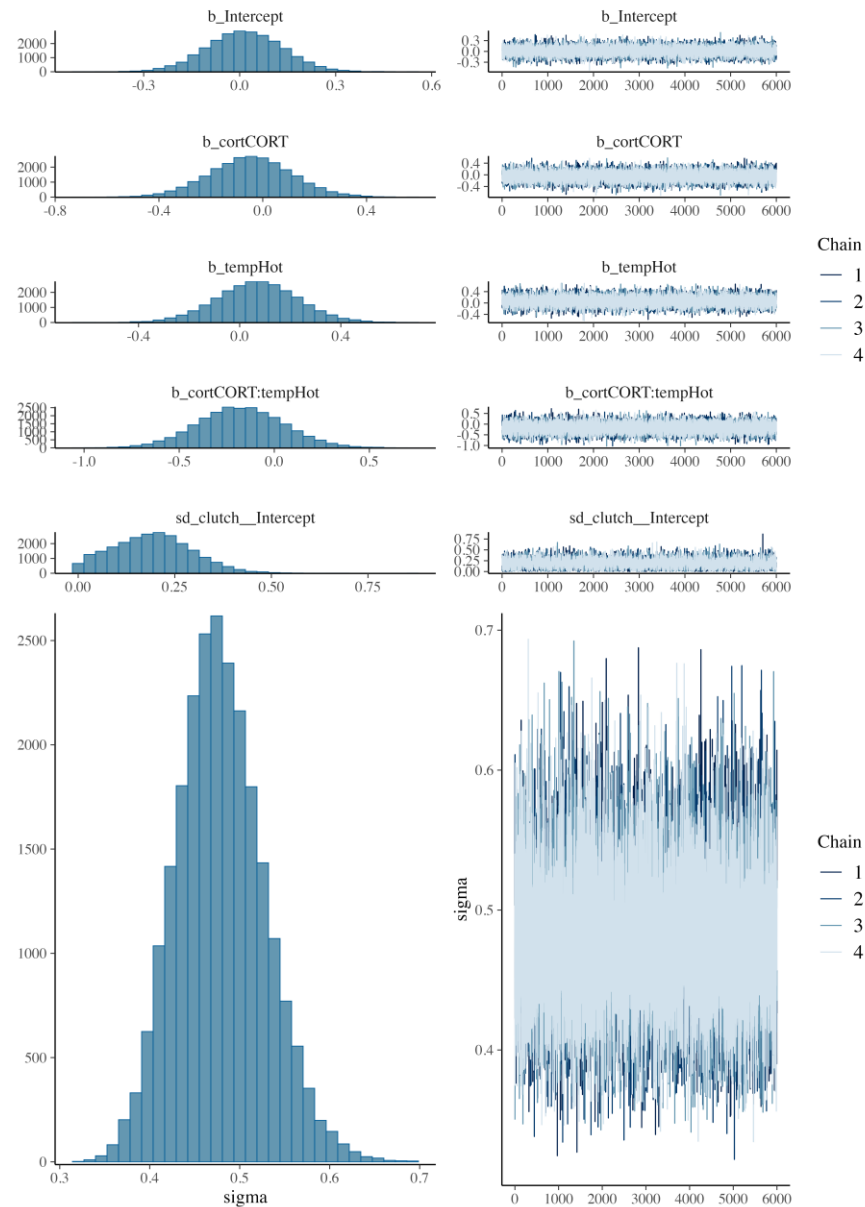
725 Results SEM

726 *Table S3. Estimated direct, indirect, and total coefficients from the multivariate model*

Response	Predictor	Direct effects	Indirect effects	Total effects
Learning	Mitochondrial density	0.068 [-0.270, 0.402]	0.003 [-0.036, 0.049]	0.071 [-0.269, 0.408]

Response	Predictor	Direct effects	Indirect effects	Total effects
	Metabolic capacity	0.025 [-0.311, 0.358]	-0.012 [-0.124, 0.088]	0.013 [-0.329, 0.357]
	ROS	-	-0.011 [-0.109, 0.080]	-0.011 [-0.109, 0.080]
	DNA damage	0.031 [-0.185, 0.241]	-	0.031 [-0.185, 0.241]
	Lipid peroxidation	-0.119 [-0.324, 0.102]	-	-0.119 [-0.324, 0.102]
DNA damage	Mitochondrial density	-	-0.037 [-0.279, 0.145]	-0.037 [-0.279, 0.145]
	Metabolic capacity	-	0.137 [-0.322, 0.654]	0.137 [-0.322, 0.654]
	ROS	0.125 [-0.292, 0.540]	-	0.125 [-0.292, 0.540]
Lipid peroxidation	Mitochondrial density	-	-0.023 [-0.243, 0.159]	-0.023 [-0.243, 0.159]
	Metabolic capacity	-	0.085 [-0.374, 0.583]	0.085 [-0.374, 0.583]
	ROS	0.076 [-0.335, 0.485]	-	0.076 [-0.335, 0.485]
ROS	Mitochondrial density	-0.282 [-0.932, 0.359]	-	-0.282 [-0.932, 0.359]
	Metabolic capacity	1.090 [0.447, 1.738]	-	1.090 [0.447, 1.738]

728 Final univariate models diagnostics (plots)



729 Fig. S2. Posterior predictive checks for the model of Mitochondrial Density. Formula:
 730 $\text{mit_density} \sim \text{cort} * \text{temp} + (1|\text{clutch})$

731

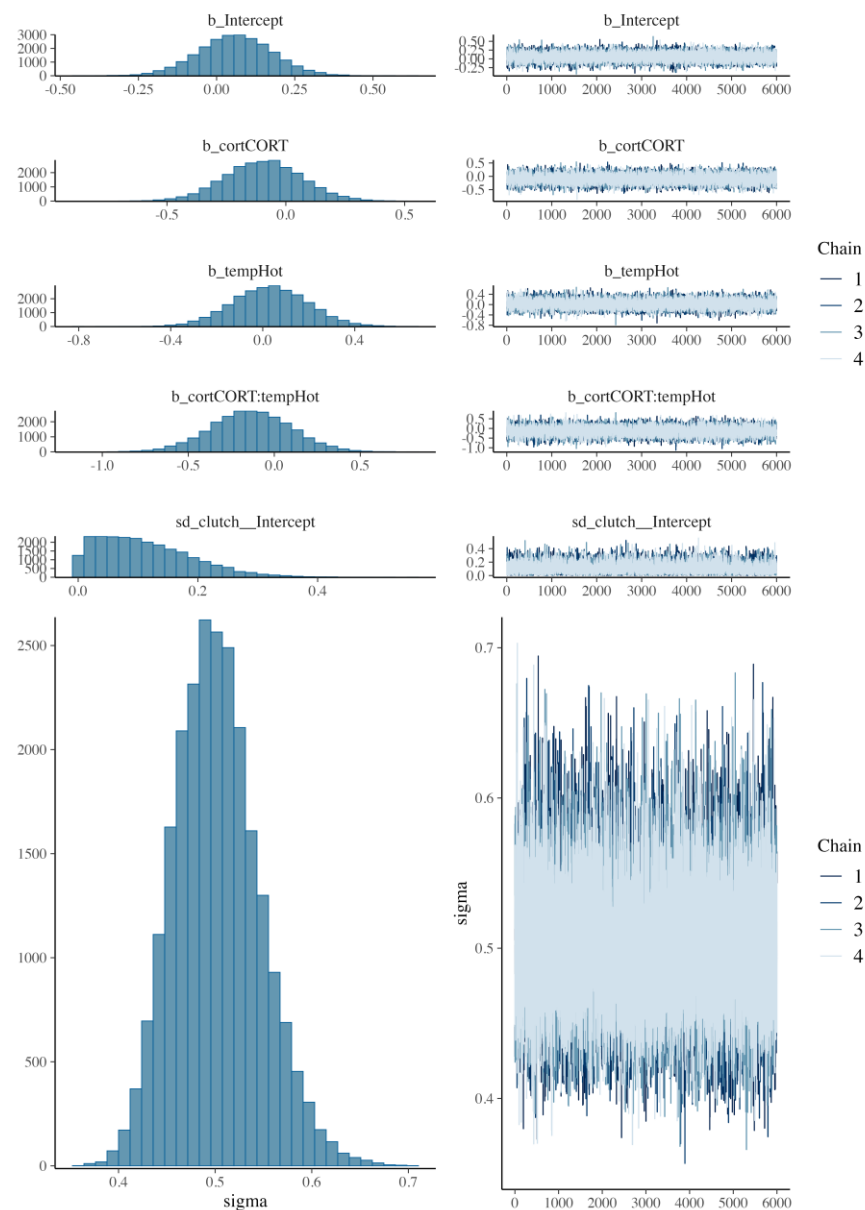
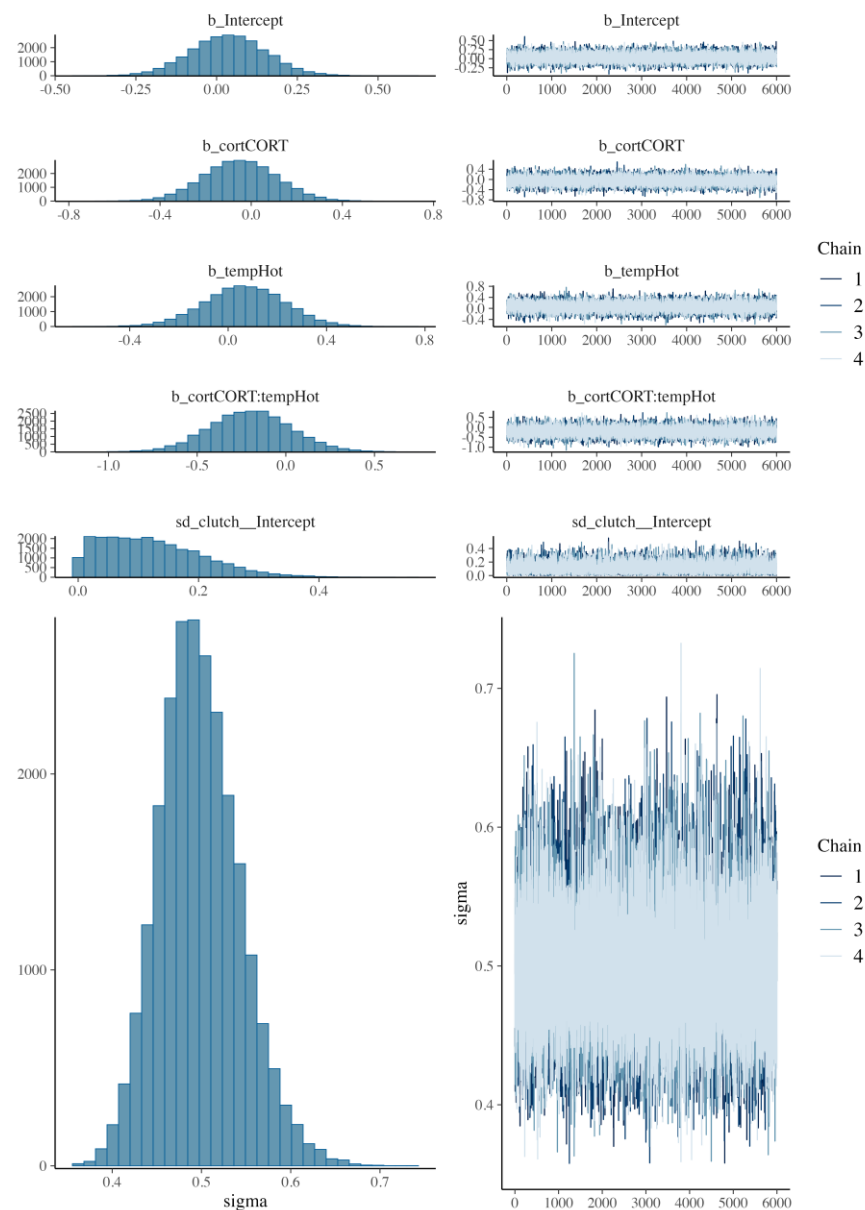
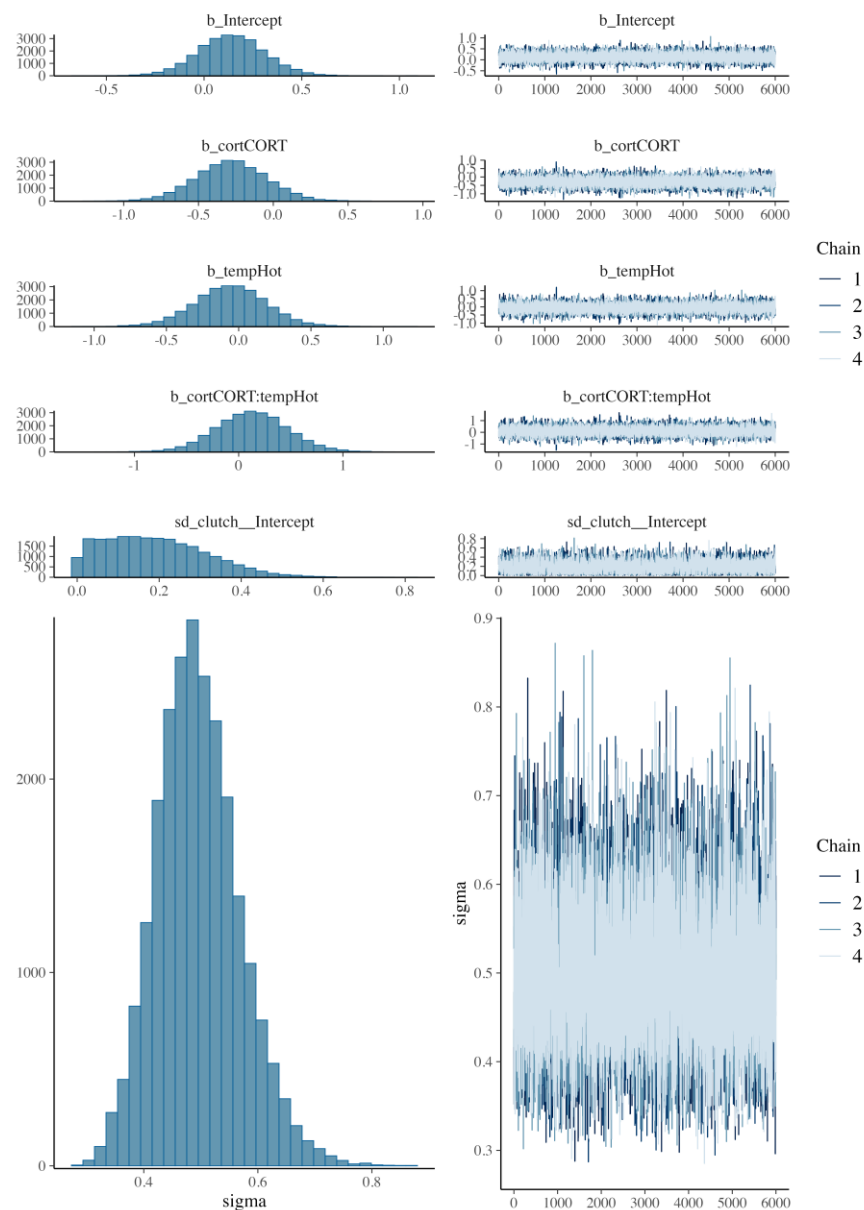


Fig. S3. Posterior predictive checks for the model of metabolic capacity. Formula: $\text{mit_potential} \sim \text{cort} * \text{temp} + (1|\text{clutch})$



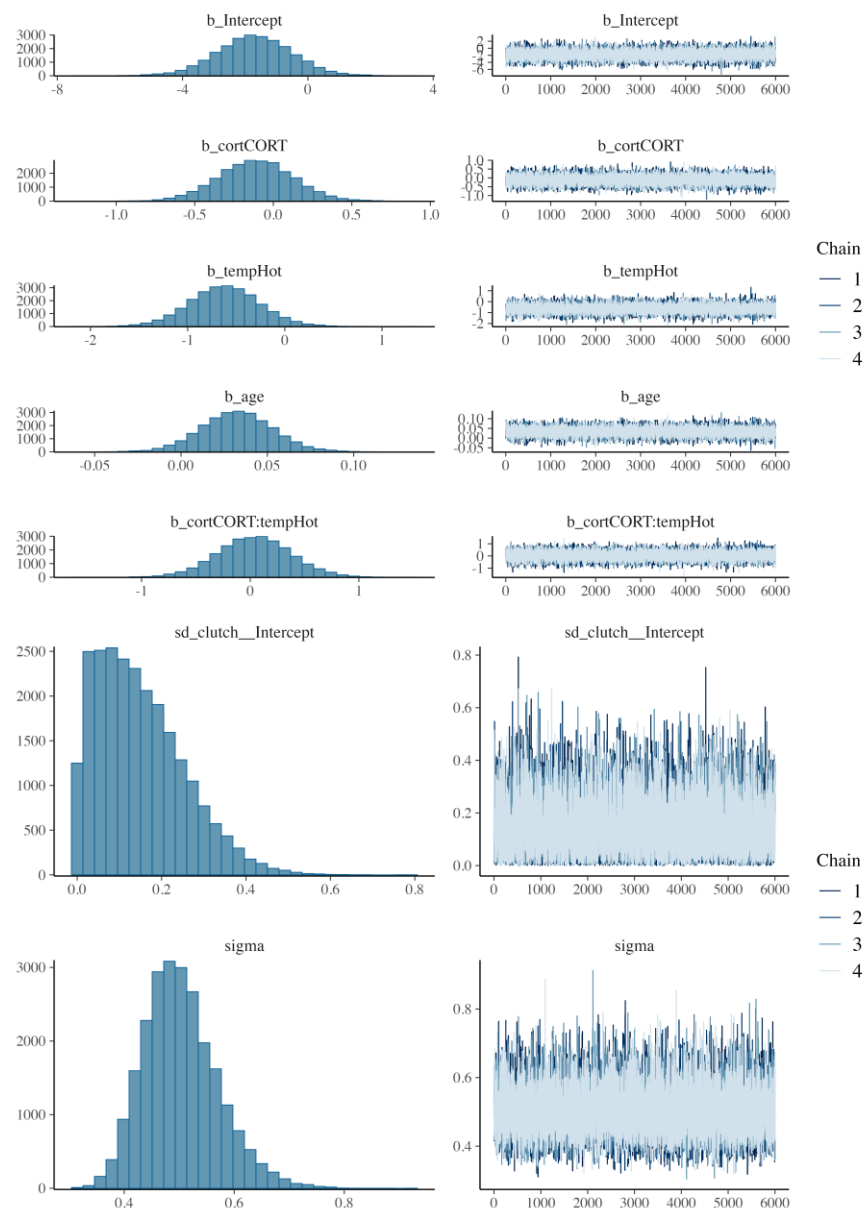
735 Fig. S4. Posterior predictive checks for the model of ROS. Formula: $ROS \sim cort * temp +$
 736 $(1|clutch)$

737



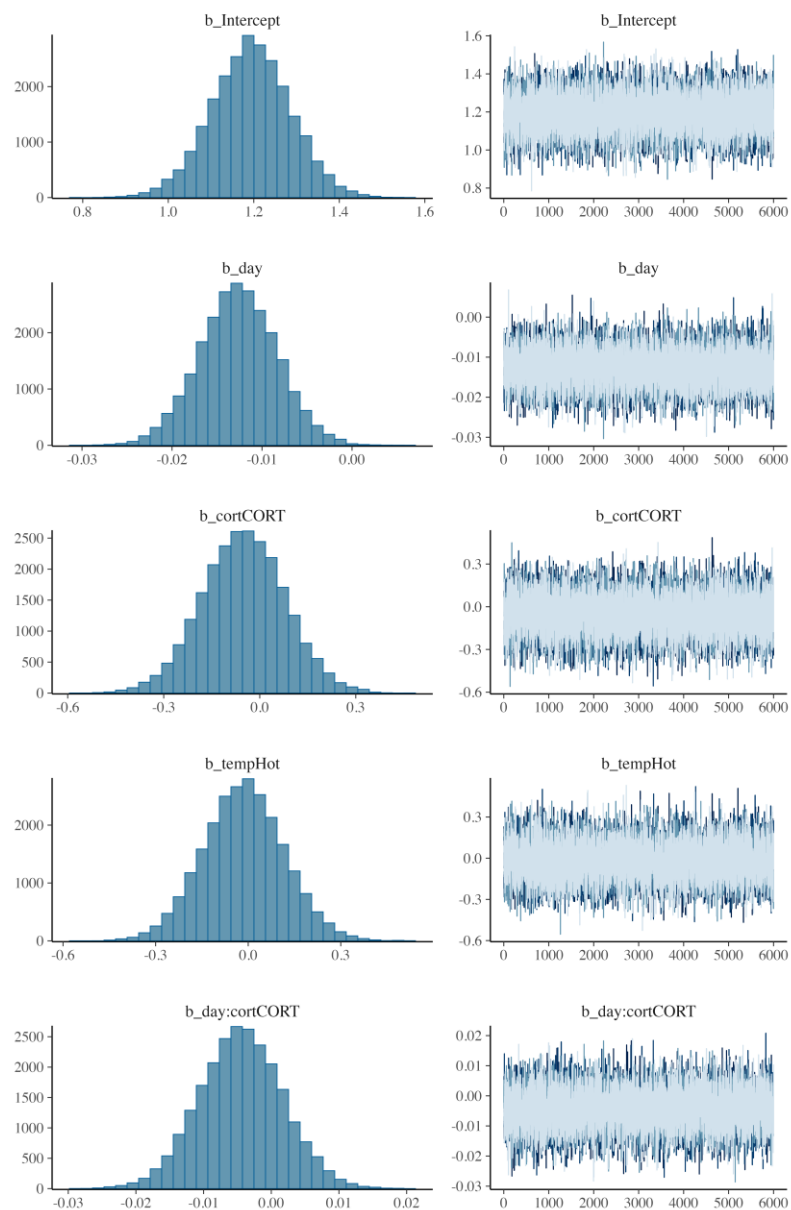
738 Fig. S5. Posterior predictive checks for the model of DNA Damage. Formula: DNAdamage ~
 739 cort * temp + (1|clutch)

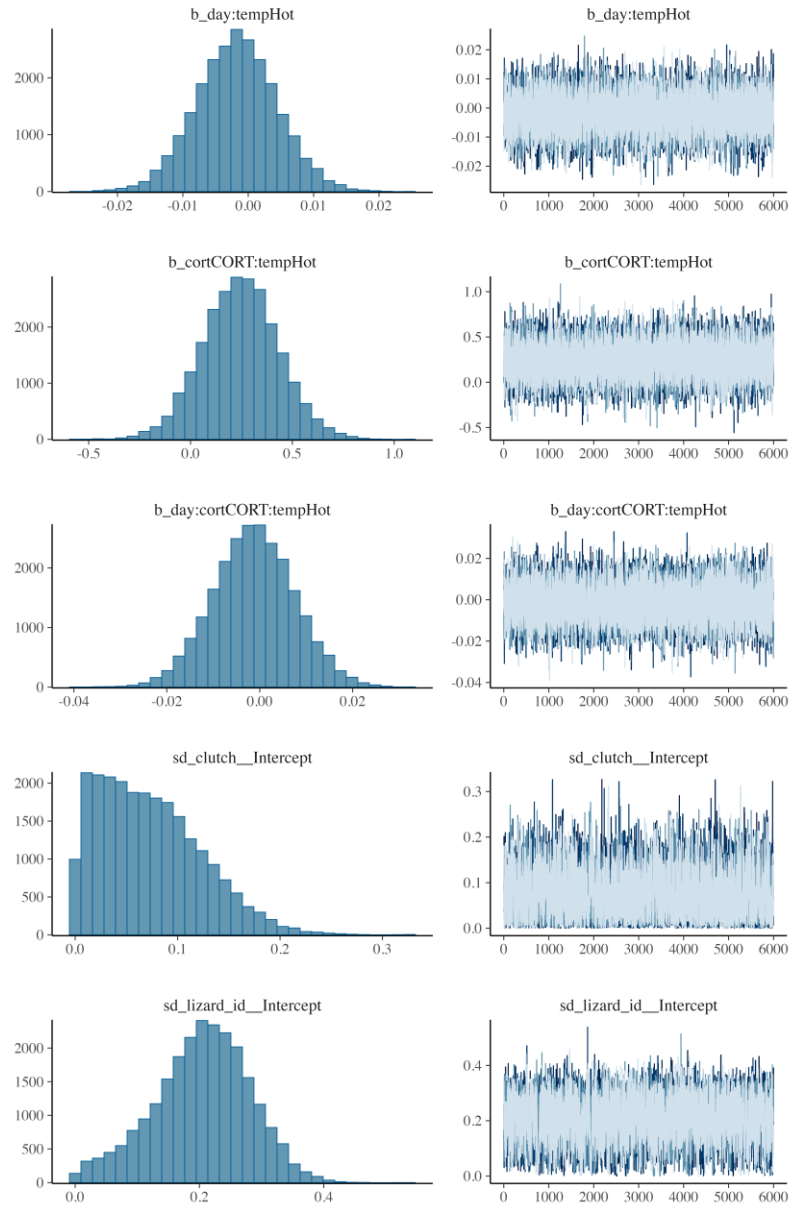
740



741 Fig. S6. Posterior predictive checks for the model of lipid peroxidation. Formula: peroxidation ~
 742 cort * temp + (1|clutch)

743





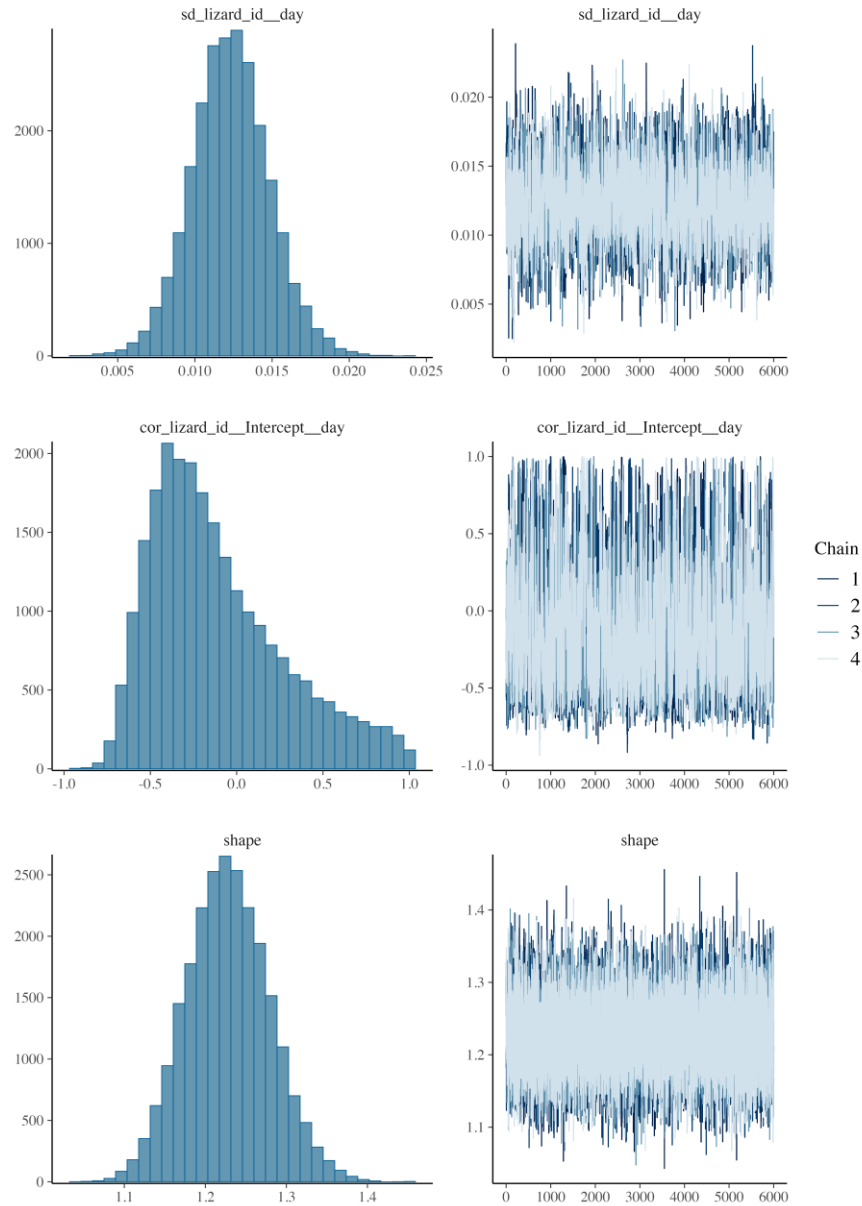


Fig. S7. Posterior predictive checks for the model of spatial learning. Formula: $\text{errors} \sim \text{day} * \text{cort} * \text{temp} + (1 + \text{day} | \text{lizard_id}) + (1 | \text{clutch})$

747 Results of preliminary models

748 *Table S4. Preliminary results of the models testing for Mitochondrial Density.*

variable	mean	median	sd	mad	q5	q95	rhat	ess_bulk	ess_tail
b_Intercept	0.470	0.460	0.866	0.855	-0.947	1.907	1	19,362.26	16,116.88
b_cortCORT	-0.054	-0.055	0.161	0.160	-0.316	0.211	1	23,841.57	18,938.53
b_tempHot	0.177	0.175	0.255	0.254	-0.240	0.598	1	18,264.83	15,601.67
b_age	-0.008	-0.008	0.015	0.015	-0.033	0.016	1	18,809.92	15,827.44
b_sex	-0.012	-0.012	0.122	0.122	-0.212	0.187	1	32,791.21	19,114.69
b_cortCORT:tempHot	-0.163	-0.161	0.234	0.231	-0.547	0.219	1	23,053.19	18,337.06

749 Model formula: mit_density ~ cort * temp + age + sex + (1|clutch). Model convergence was
750 checked through rhat and ess_bulk values. Summary indicates no effect of sex or age, so they
751 were discarded from the final models.

752

753 Table S5. Preliminary results of the models testing for Metabolic capacity.

variable	mean	median	sd	mad	q5	q95	rhat	ess_bulk	ess_tail
b_Intercept	0.302	0.296	0.847	0.839	-1.088	1.697	1	18,682.53	16,852.64
b_cortCO RT	-0.095	-0.094	0.166	0.165	-0.369	0.178	1	22,493.38	18,602.43
b_tempHot	0.092	0.091	0.248	0.247	-0.315	0.501	1	18,415.30	16,061.12
b_age	-0.004	-0.004	0.014	0.014	-0.028	0.019	1	18,669.13	16,525.89
b_sex	0.017	0.016	0.126	0.125	-0.191	0.225	1	31,266.98	17,493.28
b_cortCO RT:tempHot	-0.137	-0.137	0.242	0.240	-0.534	0.258	1	21,436.04	16,972.70

754 Model formula: mit_potential ~ cort * temp + age + sex + (1|clutch). Model convergence was
755 checked through rhat and ess_bulk values. Summary indicates no effect of sex or age, so they
756 were discarded from the final models.

757

758 Table S6. Preliminary results of the models testing for ROS.

variable	mean	median	sd	mad	q5	q95	rhat	ess_bulk	ess_tail
b_Intercept	0.441	0.439	0.845	0.846	-0.938	1.829	1	19,954.88	17,733.51
b_cortCORT	-0.059	-0.058	0.163	0.162	-0.329	0.206	1	21,973.58	19,467.53
b_tempHot	0.160	0.157	0.245	0.245	-0.241	0.565	1	18,615.90	16,581.43
b_age	-0.007	-0.007	0.014	0.014	-0.031	0.016	1	19,777.02	17,300.71
b_sex	0.021	0.021	0.124	0.123	-0.183	0.224	1	33,495.85	18,274.71
b_cortCORT:tempHot	-0.183	-0.184	0.236	0.235	-0.572	0.203	1	21,404.60	18,058.25

759 Model formula: $ROS \sim cort * temp + age + sex + (1|clutch)$. Model convergence was checked
760 through rhat and ess_bulk values. Summary indicates no effect of sex or age, so they were
761 discarded from the final models.

762

763 Table S7. Preliminary results of the models testing for DNA damage.

variable	mean	median	sd	mad	q5	q95	rhat	ess_bulk	ess_tail
b_Intercept	-0.814	-0.815	1.333	1.286	-2.975	1.384	1	14,193.24	15,015.86
b_cortCORT	-0.286	-0.287	0.249	0.244	-0.695	0.125	1	16,764.42	17,646.72
b_tempHot	-0.278	-0.280	0.402	0.393	-0.936	0.384	1	12,803.98	14,601.59
b_age	0.017	0.017	0.023	0.022	-0.021	0.054	1	13,624.26	14,032.51
b_sex	0.030	0.031	0.185	0.181	-0.274	0.330	1	20,984.11	17,159.64
b_cortCORT:tempHot	0.108	0.107	0.355	0.350	-0.475	0.690	1	14,479.58	15,703.47

764 Model formula: DNAdamage ~ cort * temp + age + sex + (1|clutch). Model convergence was
765 checked through rhat and ess_bulk values. Summary indicates no effect of sex or age, so they
766 were discarded from the final models.

767

768 Table S8. Preliminary results of the models testing for lipid peroxidation.

variable	mean	median	sd	mad	q5	q95	rhat	ess_bulk	ess_tail
b_Intercept	-1.960	-1.944	1.280	1.237	-4.098	0.117	1	17,308.48	15,369.02
b_cortCO RT	-0.139	-0.139	0.242	0.237	-0.537	0.256	1	17,466.19	16,492.63
b_tempHot	-0.691	-0.691	0.386	0.374	-1.328	-0.056	1	14,335.87	14,037.45
b_age	0.036	0.036	0.022	0.021	0.000	0.073	1	16,551.88	15,440.72
b_sex	0.119	0.119	0.179	0.175	-0.171	0.414	1	23,967.02	17,240.50
b_cortCO RT:tempHot	0.051	0.049	0.337	0.330	-0.508	0.603	1	18,089.57	17,414.62

769 Model formula: peroxidation ~ cort * temp + age + sex + (1|clutch). Model convergence was
770 checked through rhat and ess_bulk values. Summary indicates no effect of sex, so it was
771 discarded from the final models. However, we saw an effect of age and we included it in our
772 final models.

773

774 Table S9. Preliminary results of the models testing for learning.

variable	mean	median	sd	mad	q5	q95	rhat	ess_bulk	ess_tail
b_Intercept	1.350	1.348	0.546	0.537	0.455	2.256	1	14,157.44 ₃	16,878.70
b_day	-0.013	-0.013	0.004	0.004	-0.020	-0.005	1	9,398.846	13,567.40
b_cortCORT	-0.054	-0.052	0.134	0.136	-0.275	0.164	1	10,693.40 ₉	14,518.00
b_tempHot	0.023	0.022	0.180	0.178	-0.269	0.322	1	10,924.74 ₂	15,119.67
b_sex	-0.005	-0.005	0.080	0.079	-0.136	0.128	1	16,727.89 ₁	17,160.45
b_age	-0.003	-0.003	0.009	0.009	-0.018	0.012	1	13,505.07 ₃	16,391.78
b_day:cortCORT	-0.004	-0.005	0.006	0.006	-0.015	0.006	1	9,216.274	12,908.06
b_day:tempHot	-0.002	-0.002	0.006	0.006	-0.012	0.008	1	9,945.670	13,744.26
b_cortCORT:tempHot	0.242	0.241	0.194	0.194	-0.076	0.563	1	10,111.70 ₀	14,279.42
b_day:cortCORT:tempHot	0.000	0.000	0.009	0.009	-0.015	0.014	1	9,169.257	12,700.42

775 Model formula: errors ~ day * cort * temp + sex + age+ (1 + day | lizard_id) + (1|clutch). Model
776 convergence was checked through rhat and ess_bulk values. Summary indicates no effect of sex
777 or age, so they were discarded from the final models.

778

Appendix IV.3: Brain validation

To ensure that neurons were not unintentionally lost during homogenisation, we performed a pilot study where we euthanized four lizards and prepared medial cortex homogenates using the same procedures as before. However, to ensure we could identify neurons, homogenates here were dyed with marker that specifically targeted neuron nuclei (Farrow et al. 2021; Storks et al. 2023). We employed fluorescence microscopy and flow cytometry to check for the presence of neurons in the homogenates.

Euthanasia and homogenisation for each of brain region followed the procedures outlined above (see *Methods 2: Flow cytometry*). Before staining the samples, cells were also fixed and permeabilised as described previously (see *Methods 2: Flow cytometry*). Following permeabilisation, we centrifuged the samples at 1000 RCF for 10 minutes and resuspended the pellet in 100 μ L of a 1:100 dilution of NeuN + Alexa488 fluorescent conjugate to dye the neuronal nuclei (Farrow et al. 2021; Storks et al. 2023). The samples were incubated at +4 °C overnight.

The following day we centrifuged the samples at 1000 RCF for 10 minutes and resuspended the pellet in 100 μ L of PBS. Samples were split in two: 50 μ L from each was reserved for examination under a Zeiss AxioObserver Z1 microscope, while the remaining 50 μ L from each sample was pooled into a single tube for flow cytometry analysis. . Flow cytometry of stained cells allowed us to validate that our gating strategy was correct.

Samples examined under the microscope showed a clear presence of neuronal nuclei in the homogenates (Figure S13). Flow cytometry analysis also confirmed the presence of neuronal nuclei in the homogenates, that were similar to the size of the cells employed in the experiment (mean size cells experiment = 2.765; 95% CI = [2.093, 3.139], n = 80; size neurons = 3.977495).

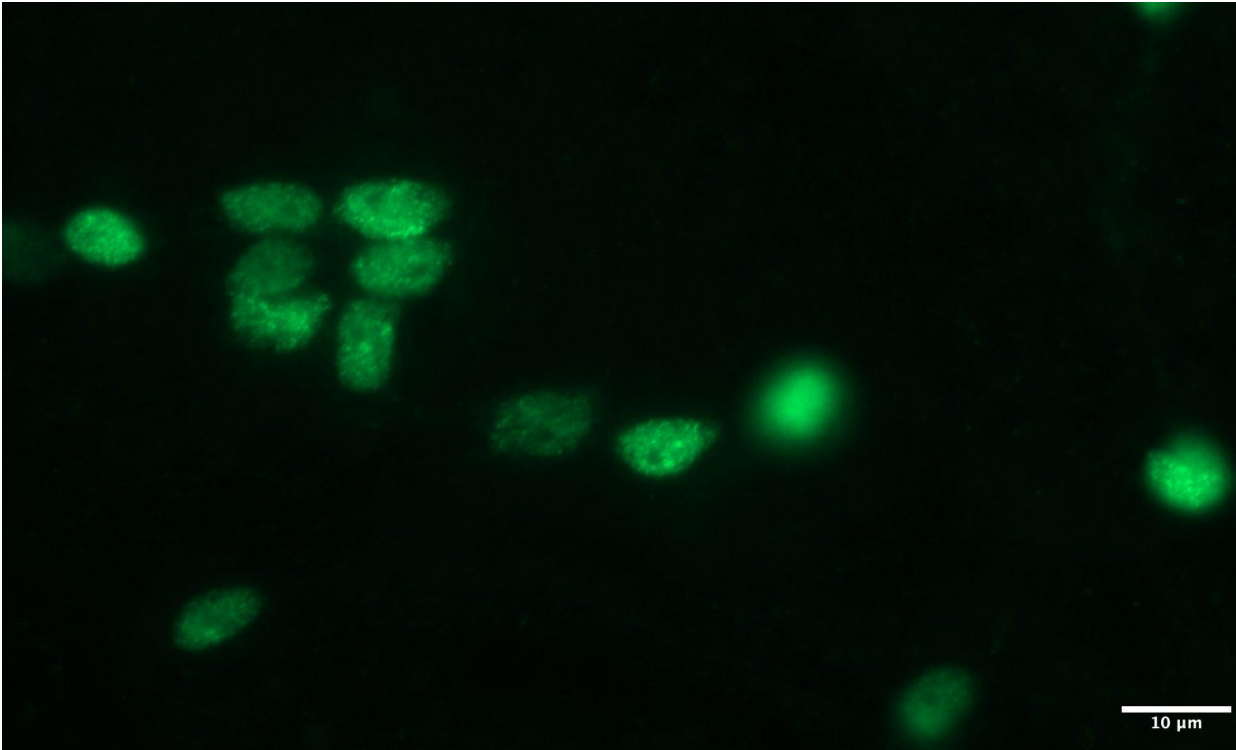


Figure S8. Fluorescence microscopy image of neuron nuclei stained with NeuN-Alexa 488. Images were taken on a Zeiss AxioObserver Z1, equipped with Zeiss AxioCam 506 monochrome camera. A Zeiss 38HE fluorescent filter set (450-490nm Ex, 500-550nm Em.) was used in conjunction with a 63x 1.4 NA.

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