

**Developmental environments do not affect thermal  
physiology in reptiles: An experimental test and meta-  
analysis**

Journal:	<i>Biology Letters</i>
Manuscript ID	RSBL-2023-0019.R1
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Zhang, Rose; ACT Wild, Kristoffer H.; ACT Pottier, Patrice; The University of New South Wales, Earth and Environmental Sciences; Iglesias-Carrasco, Maider; Museo Nacional de Ciencias Naturales, Evolutionary Ecology; Sociedad de Ciencias Aranzadi, Herpetology Nakagawa, Shinichi; University of New South Wales, School of Biological, Earth and Environmental Sciences Noble, Daniel; ACT
Subject:	Ecology < BIOLOGY, Evolution < BIOLOGY
Categories:	Global Change Biology
Keywords:	meta-analysis, Developmental, environments, thermal, physiology

**Author-supplied statements**

Relevant information will appear here if provided.

***Ethics***

*Does your article include research that required ethical approval or permits?:*

Yes

*Statement (if applicable):*

Ethics. All experimental procedures followed approved protocols by the ANU Animal Ethics Committee (ARA2019/17). Lizards were caught under NPWS permit LT201917.

***Data***

*It is a condition of publication that data, code and materials supporting your paper are made publicly available. Does your paper present new data?:*

Yes

*Statement (if applicable):*

Data accessibility. Experimental and meta-analytic datasets are available from: [https://github.com/kris-wild/Lampro\\_project.git](https://github.com/kris-wild/Lampro_project.git) and archived in Zenodo (<https://doi.org/10.5281/zenodo.7700383>; Zang, Wild, Pottier et al.55)

***Conflict of interest***

I/We declare we have no competing interests

*Statement (if applicable):*

Competing interests. We declare no competing interests.

# Developmental environments do not affect thermal physiology in reptiles: An experimental test and meta-analysis

Rose Y. Zhang<sup>1\*</sup>, Kristoffer H. Wild<sup>1\*†</sup>, Patrice Pottier<sup>2</sup>, Maider Iglesias Carrasco<sup>1,3</sup>, Shinichi Nakagawa<sup>2</sup> and Daniel W.A. Noble<sup>1,†</sup>

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

2 Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, Australia

3 Doñana Biological Station-Spanish Research Council CSIC, Seville, Spain

**Authors for Correspondence**, †: Kristoffer Wild, [Kristoffer.wild@anu.edu.au](mailto:Kristoffer.wild@anu.edu.au); Daniel Noble, [daniel.noble@anu.edu.au](mailto:daniel.noble@anu.edu.au)

\* Authors Contributed Equally

## Abstract:

On a global scale, organisms face significant challenges due to climate change and anthropogenic disturbance. In many ectotherms, developmental and physiological processes are sensitive to changes in temperature and resources. Developmental plasticity in thermal physiology may provide adaptive advantages to environmental extremes if early environmental conditions are predictive of late-life environments. Here, we conducted a laboratory experiment to test how developmental temperature and maternal resource investment influence thermal physiology (critical thermal maximum:  $CT_{max}$  & thermal preference:  $T_{pref}$ ) in a common skink (*Lampropholis delicata*). We then compared our experimental findings more broadly across reptiles using meta-analysis. In both our experimental study and meta-analysis, we did not find evidence that developmental environments influence thermal physiology. Furthermore, the effects of developmental environments on thermal physiology did not vary by age, taxon, or climate zone (temperate/tropical) in reptiles. Overall, the magnitude of developmental plasticity on thermal physiology appears to be limited across reptile taxa. Our results suggest that behavioural or evolutionary processes, as opposed to developmental plasticity, may be more critical in mitigating the impacts of changing thermal conditions in reptiles in the future.

**Introduction**

Climate warming and anthropogenic stressors pose significant challenges to organisms on a global scale<sup>[1,2]</sup>. Rapidly increasing temperatures are a particularly significant threat for ectothermic species. Indeed, increasing temperatures can drive fitness declines due to physiological intolerance<sup>[3]</sup>, and alter the distribution of species<sup>[4]</sup>. Inevitably, these impacts are primarily mediated by how organisms change their behaviour and physiology through development and evolutionary time in response to shifting environments. Phenotypic changes that occur during an animal's lifetime in response to changing environments (i.e., phenotypic plasticity), are important mechanisms by which ectotherms can cope with climate change over short time scales<sup>[5]</sup>. However, the magnitude of plastic responses is widely trait- and species-specific<sup>[5–7]</sup>

Temperature can also have transgenerational effects by impacting parental generations<sup>[8,9]</sup>. For instance, recent evidence indicates that some ectotherms can tolerate heat events for long periods<sup>[5,10]</sup>. Thermal ecology of ectotherms can also be shaped by other factors, such as diet or maternal investment, which can influence physiological traits that are temperature dependent<sup>[11–13]</sup>. For example, a diet high in nutrients (carbohydrate or protein) leads to higher metabolic rates and  $CT_{max}$ , while a diet low in these nutrients can result in lower physiological estimates<sup>[14–16]</sup>. Additionally, the resources a mother invests in her offspring (i.e., the energetic provisioning of eggs) can influence metabolic processes like growth and development<sup>[17]</sup>. Determining how thermal and resource environments during development affect key thermal physiological traits in various taxa may provide an understanding of how species are likely to cope with changing environments.

While phenotypic plasticity can adjust phenotypes throughout life, developmental plasticity – plasticity occurring during early embryonic development – can have organisational effects on phenotypes that can affect responses later in life<sup>[6]</sup>. For vertebrates in particular, such effects may be adaptive or maladaptive depending on whether early-life environments are predictive of late-life environments. While temperature and early resource provisioning can influence thermal traits in ectotherms<sup>[18]</sup>, most research effort has focused on temperature, which is known to have a profound effect on fitness<sup>[19,20]</sup>. In reptiles, temperatures during embryonic development are known to affect phenotypes throughout ontogeny<sup>[7]</sup>. For example, incubation conditions of developing reptile embryos can impact a variety of traits including sex, growth rate, morphology, behaviour, and cognition<sup>[7,20,21]</sup>. However, there is a dearth of evidence linking developmental factors more generally to thermal traits, and whether these differences persist through various stages of ontogeny in reptiles<sup>[22,23]</sup>.

Here, we aim to determine how early developmental environments affect thermal physiology (critical thermal maximum:  $CT_{max}$  & thermal preference:  $T_{pref}$ ) in reptiles.  $CT_{max}$  &  $T_{pref}$  are two common thermal indices used as proxies for how the environment influences individual fitness and are used to predict how species distributions are predicted to shift with climate change<sup>[3,24,25]</sup>. We first conduct a laboratory experiment to test how maternal investment and developmental temperature both influence  $CT_{max}$  &  $T_{pref}$  in a common skink (*Lampropholis delicata*). We then compare our experimental findings with quantitative results testing this same question more broadly in reptiles using a meta-analysis.

**Method and materials**

*(a) Consequences of incubation temperature and resource allocation on thermal physiology: an experimental manipulation*

We collected gravid *Lampropholis delicata* (common garden skink,  $n = 100$ ) from populations in Sydney (Australia) and transported them back to the Australian National University, where females were housed until eggs ( $n = 40$ ) were laid. We then pseudo-randomly (to ensure equal sample sizes) assigned eggs ( $n = 20$ ) to both a resource allocation treatment ('R' - yolk removal or 'C' – control) and an incubation temperature ( $23^{\circ}\text{C}$  or  $28^{\circ}\text{C}$  SD  $\pm 3.0$ ) treatment (See Supplementary materials for details on husbandry of hatchlings). Egg incubation temperatures were chosen to mimic conditions experienced at extremes of natural nest temperatures in nature while also exhibiting natural thermal fluctuations throughout the day<sup>[26]</sup>. Yolk removal treatments followed Sinervo<sup>[16]</sup>, with 15-20% of the total egg mass being removed via a sterilised syringe. Control treatments were punctured with the syringe without any yolk removal. For further description of husbandry conditions of adults and incubation details, see Kar *et al.*<sup>[28]</sup>.

Hatchlings from their respective treatment were housed in mixed treatment groups of 5-6 within 20 L [40 cm (l) x 29.5 cm (w) x 20.5 cm (h)] plastic enclosures, with UVA/UVB lighting and a 20W heat lamp in each enclosure. Water was provided *ad libitum*, with enclosures misted daily. Lizards were fed calcium and vitamin-dusted crickets (*Acheta domesticus*) every second day. At eight to eleven months post-hatching, lizards were selected at random, and thermal traits ( $\text{CT}_{\text{max}}$  and  $T_{\text{pref}}$ ) measured. Briefly, after undergoing a 24-hour fasting period, animals were transferred into individual lanes of a thermal gradient ( $5^{\circ}\text{C}$  to  $55^{\circ}\text{C}$ ) to measure  $T_{\text{pref}}$ . A FLIR T640 thermal camera was used to take thermal images of all lanes every 15-minutes over an eight-hour observation period.  $T_{\text{pref}}$  was defined as the mean skin surface temperature (on the neck) over the eight-hour observation period. Given the small size of lizards (i.e., 1.3 g) we assumed skin surface temperature reflected body temperature, which has been shown for many small lizards<sup>[29]</sup>. For  $\text{CT}_{\text{max}}$  we followed the same fasting period used for  $T_{\text{pref}}$  experiments. Here, lizards were placed in falcon tubes in a water bath for 5 min at a temperature of  $30^{\circ}\text{C}$ . The water temperature was increased to  $38^{\circ}\text{C}$  at a rate of  $1^{\circ}\text{C}/\text{min}$ . We used a control falcon tub with a thermal couple attached to the bottom of the tub where lizards were positioned to record the temperature of the tube surface, which we took to be the temperature experienced by the lizards. This approach was needed because it was not possible to have a thermal couple in each lizards Falcon tube when measuring righting responses in the  $\text{CT}_{\text{max}}$  procedure<sup>[30]</sup>.  $\text{CT}_{\text{max}}$  was defined as the temperature at which an individual lost their righting reflex (for further details in collection methods, see *Supp.*).

All statistical analyses were conducted using the R environment, ver. 4.1.0 ([www.r-project.org](http://www.r-project.org)). We used linear models to analyse thermal traits ( $T_{\text{pref}}$  and  $\text{CT}_{\text{max}}$ ). We constructed models that contained the main effects of body mass, sex, incubation temperature and resource treatment. We also tested for the interaction between incubation temperature and resource treatment (see *Supp. for more details*). If the interaction was not significant, we removed it and presented the full main effects model.

#### (b) Meta-analysis of early thermal effects on thermal physiology in reptiles

To understand more broadly the impact of developmental environments on thermal physiology, we systematically searched for studies manipulating early developmental environments and subsequently measuring thermal physiological traits. Unfortunately, few studies manipulated egg resource investment and measured thermal tolerance. As such, it was only possible to focus on developmental temperature manipulations. Our meta-analysis collected data on offspring's

thermal preference ( $T_{pref}$ ) and critical thermal maximum ( $CT_{max}$ ) in lizards, snakes, tortoises, turtles, and tuatara. Our search string included cold tolerance (i.e., critical thermal minimum,  $CT_{min}$ ), but there were too few studies that manipulated developmental environments and measured this trait to conduct a formal meta-analysis. As such, we focus on  $T_{pref}$  and  $CT_{max}$ .

In brief, we conducted a systematic literature search in Scopus, ISI Web of Science (core collection), and ProQuest (dissertations and thesis) and did not apply a timespan limit. We followed the PRISMA-EcoEvo (Preferred Reporting Items for Systematic Reviews & Meta-Analyses in Ecology and Evolutionary biology) guidelines for reporting<sup>[31]</sup>. Full search strings, search methods, and selection criteria are described in detail in supporting information (Figs. S1&2). We obtained 485 original records, and 15 articles satisfied our selection criteria.

Multilevel meta-analytic (MLMA) models were constructed using the *rma.mv* function in the *metafor* package (version 3.8)<sup>[32]</sup>. To determine the ability of an organism to acclimate to changes in the environment, we used the acclimation response ratio (ARR) as our effect size<sup>[33]</sup>. Sampling variance for the ARR was derived in Pottier et al.,<sup>[34]</sup>. Given that studies often had more than two temperature treatments we included study, phylogeny, and study species were designated as random effects and we included an observation-random effect (effect size ID). A model that included only study, species and effect size ID was best supported over one with phylogeny, so we present meta-analytic results from a model without phylogeny. We derived all pairwise effect size comparisons within a study. This, however, does induce a correlation between effect size sampling errors, which we controlled for through the inclusion of a sampling (co)variance matrix derived by assuming effect sizes are correlated by  $r = 0.5$ <sup>[35]</sup>. Thermal trait ( $T_{pref}$  or  $CT_{max}$ ), life stage at measurement (hatchling, juvenile or adult), climate zone (temperate or tropical), and major taxonomic group (lizard, snake, tuatara or turtle) were included as fixed factors in separate multi-level meta-regression (MLMR) models. We also tested for publication bias using a MLMR model with sampling variance and standard error as predictors<sup>[36]</sup> and was visually inspected using a funnel plot (*see Supp. for more details*). We present effect size heterogeneity by constructing prediction intervals<sup>[37]</sup> and presenting  $I^2$  using the *orchaRd* package (version 2.0)<sup>[38]</sup>.

**Results**

*a) Incubation temperature and resource allocation consequences on thermal preference and critical thermal maximum*

Mean  $T_{pref}$  was  $31^{\circ}\text{C} \pm 0.47$  (mean  $\pm$  SE) and ranged from  $20.99\text{--}34.26^{\circ}\text{C}$ . Mean  $CT_{max}$  was  $43.04^{\circ}\text{C} \pm 0.23$  and ranged from  $38.6\text{--}45.2^{\circ}\text{C}$ . We did not detect any effect of incubation temperature, yolk treatment, sex, or body mass on  $T_{pref}$  or  $CT_{max}$  (Figure 1A|B; Table 1).

*(b) Meta-analysis of early thermal effects on thermal physiology in reptiles*

Across reptiles, developmental temperatures did not influence thermal traits ( $T_{pref}$  or  $CT_{max}$ ), but heterogeneity was high (ARR = 0.05, 95% CI: -0.28-0.37;  $I^2_{Total} = 99.53\%$ , Prediction Interval: -1.23-1.32; Fig. 2A,  $n = 69$  effects from 14 species). Overall, we found no evidence for publication biases ( $\beta = -0.81$ , 95% CI = -1.92-0.3,  $p = 0.15$ ; Fig S3; for further details see electronic supplementary materials). Species effects ( $I^2_{Species} = 70.57\%$ ) drove most of the heterogeneity in ARR, but thermal traits were not influenced by life stage, climate zone, or major taxonomic group (i.e., snakes, turtles, lizards) (Fig. 2B|C). While there was a significant increase in thermal traits in snakes (Fig 2D), this was driven by a single species (*Nerodia sipedon*), and given the small sample sizes, we need to caution whether any true differences between snakes and other groups exists.



## Discussion

Genetic adaptation and phenotypic plasticity are two hypotheses for how ectotherms can cope with warming temperatures associated with anthropogenic climate change [3,39–41]. Plastic responses occurring early in development can have long-lasting effects on organisms, with significant implications for how they cope with environmental stressors.

We show that early developmental environments do little to modify thermal physiological traits ( $CT_{max}$  &  $T_{pref}$ ) in most reptile taxa. Both our experimental and meta-analytic approaches suggest that the magnitude of developmental plasticity on thermal indices appears to be canalised across reptile taxa. For example, our meta-analysis indicated that for every 1°C change in developmental temperature, we only expect a 0.05°C change in thermal physiology. Our findings are consistent with those of other ectotherm systems, which show that developmental plasticity has little impact on adult heat tolerance [6,42–44]. Nonetheless, we detected significant species-specific heterogeneity ( $I^2_{species} = 70.57\%$ ), suggesting substantial differences across species that cannot be ignored. Such variability may be driven by species differences in micro-habitat selection of nests or nesting phenology in the wild and whether developmental conditions in the field corroborate with conditions chosen for laboratory experiments. It has been indicated in other studies [45–48] that differences in nest depth, nest location, clutch density or maternal condition may select for developmentally plastic responses in offspring. Together, these data highlight that further ecological data on developmental environments in nature is needed to test if static manipulations in the lab provide a functional link to how species can cope with environmental change.

While there are still limited empirical studies, across reptile taxa, plasticity in thermal physiology did not differ by age, taxon or climate zone. We expected that the earlier age at which thermal traits were measured would be more likely to detect effects of early environments. In addition, tropical species are expected to maintain body temperatures near their thermal limits, and an increase in temperature can push these species to physiological extremes compared to temperate species [3,41,49]. Greater thermal variability in temperate regions should select for greater plasticity. However, our meta-analysis does not support these hypotheses. Instead, the microthermal environments and behavioural flexibility may be a more important driving mechanism as to whether species respond plastically to developmental environments or not [3,50]. Future studies looking at the autocorrelation between early and late developmental environments would be fruitful in helping elucidate species-specific responses to thermal environments. Overall, our results suggest that most reptiles may have limited developmental plasticity in thermal traits, relying instead on energetically expensive behaviours (i.e., thermoregulation) [3,51] or responses that operate on slower time scales (i.e., local adaptation) [40,52]. Given the small effect sizes we observed, statistical power is likely an issue in ours and others' empirical work. However, ethical constraints in measuring thermal limits in large numbers of animals will mean such studies are likely to be common. As such, we will need to rely on meta-analysis to help circumvent power limitations in individual studies (as we have done here) [53]. We have also identified clear gaps in the literature that should help pave the way for future research. First, we encourage measuring thermal physiology under different developmental manipulations across a greater diversity of reptile taxa. Greater taxonomic diversity will clarify when developmental environments matter and allow us to explore reasons for this heterogeneity. Second, we encourage measuring  $CT_{min}$ , in addition to other thermal physiological traits (i.e.,  $CT_{max}$ ,  $T_{pref}$ , etc) as it is often more environmentally flexible than upper thermal limits. Despite these gaps,

213 our results provide valuable insights into possible responses that are plausible under changing  
214 thermal conditions.

For Review Only



**Literature Cited (\*\* indicate studies used in the meta-analysis)**

1. Parmesan C. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*. 2006;37:637–69.
2. Sala OE, Stuart F, Iii C, Armesto JJ, Berlow E, Bloomfield J, et al. Global Biodiversity Scenarios for the Year 2100. *Science*. 2000;287(5459):1770–4.
3. Huey RB, Kearney MR, Krockenberger A, Holtum JAM, Jess M, Williams SE. Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2012;367(1596):1665–79.
4. Peralta-Maraver I, Rezende EL. Heat tolerance in ectotherms scales predictably with body size. *Nat Clim Chang*. 2021;11(1):58–63.
5. Seebacher F, White CR, Franklin CE. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat Clim Chang*. 2015;5(1):61–6.
6. Pottier P, Burke S, Zhang RY, Noble DWA, Schwanz LE, Drobniak SM, et al. Developmental plasticity in thermal tolerance: Ontogenetic variation, persistence, and future directions. *Ecol Lett*. 2022;25:2245–68.
7. Noble DWA, Stenhouse V, Schwanz LE. Developmental temperatures and phenotypic plasticity in reptiles: A systematic review and meta-analysis. *Biological Reviews*. 2018;93(1):72–97.
8. Sales K, Vasudeva R, Dickinson ME, Godwin JL, Lumley AJ, Michalczyk Ł, et al. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nat Commun*. 2018;9(1).
9. Salinas S, Munch SB. Thermal legacies: Transgenerational effects of temperature on growth in a vertebrate. *Ecol Lett*. 2012;15(2):159–63.
10. Kirchhof S, Hetem RS, Lease HM, Miles DB, Mitchel D, McUller J, et al. Thermoregulatory behavior and high thermal preference buffer impact of climate change in a Namib Desert lizard. *Ecosphere*. 2017;8(12).
11. Burton T, Killen SS, Armstrong JD, Metcalfe NB. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences*. Royal Society; 2011. 278:3465–73.
12. Tobler M, Nilsson JÅ, Nilsson JF. Costly steroids: Egg testosterone modulates nestling metabolic rate in the zebra finch. *Biol Lett*. 2007; 3(4):408–10.
13. Zhao CL, Zhao T, Feng JY, Chang LM, Zheng PY, Fu SJ, et al. Temperature and diet acclimation modify the acute thermal performance of the largest extant Amphibian. *Animals*. 2022;12(4).
14. Hardison EA, Kraskura K, van Wert J, Nguyen T, Eliason EJ. Diet mediates thermal performance traits: Implications for marine ectotherms. *Journal of Experimental Biology*. 2021;224(21).
15. Bujan J, Kaspari M. Nutrition modifies critical thermal maximum of a dominant canopy ant. *J Insect Physiol*. 2017;102:1–6.
16. Sinervo B. The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution*. 1990;44(2):279–94.
17. Mousseau TA, Fox CW. The adaptive significance of maternal effects. *Trends Ecol Evol*. 1998;13(10):403–7.

- 261 18. Angilletta Jr MJ, Angilletta MJ. Thermal adaptation: A theoretical and empirical  
262 synthesis. New York, NY, USA: Oxford University Press; 2009.
- 263 19. Huey RB, Berrigan D. Temperature, demography, and ectotherm fitness. *The American*  
264 *Naturalists*. 2001;2:158–210.
- 265 20. Sibly RM, Atkinson D. How rearing temperature affects optimal adult size in ectotherms.  
266 *Ecology*. 1994;8(4):486–93.
- 267 21. Bull JJ. Sex determination in reptiles. *The Quarterly Review of Biology*. 1980;55(1):3–21.
- 268 22. Refsnider JM, Clifton IT, Vazquez TK. Developmental plasticity of thermal ecology traits  
269 in reptiles: Trends, potential benefits, and research needs. *J Therm Biol*. 2019;84:74–82.
- 270 23. Bodensteiner BL, Agudelo-Cantero GA, Arietta AZA, Gunderson AR, Muñoz MM,  
271 Refsnider JM, et al. Thermal adaptation revisited: How conserved are thermal traits of  
272 reptiles and amphibians? *J Exp Zool A Ecol Integr Physiol*. 2021;335(1):173–94.
- 273 24. Hoffmann AA, Chown SL, Clusella-Trullas S. Upper thermal limits in terrestrial  
274 ectotherms: How constrained are they? *Funct Ecol*. 2013;27(4):934–49.
- 275 25. Sinervo B, Mendez-De-La-Cruz F, Miles DB, Heulin B, Bastiaans E, Villagrán-Santa  
276 Cruz M, et al. Erosion of lizard diversity by climate change and altered thermal niches.  
277 *Science*. 2010;328(5980):894–9.
- 278 26. Cheetham E, Doody JS, Stewart B, Harlow P. Embryonic mortality as a cost of communal  
279 nesting in the delicate skink. *J Zool*. 2011;283(4):234–42.
- 280 27. Bilcke J, Downes S, Büscher I. Combined effect of incubation and ambient temperature  
281 on the feeding performance of a small ectotherm. *Austral Ecol*. 2006;31(8):937–47.
- 282 28. Kar F, Nakagawa S, Noble DWA. Impact of developmental temperatures on thermal  
283 plasticity and repeatability of metabolic rate. *Evol Ecol*. 2022;36(2):199–216.
- 284 29. Garrick D. Body surface temperature and length in relation to the thermal biology of  
285 lizards. *Bioscience Horizons*. 2008;1(2):136–42.
- 286 30. Llewelyn J, Macdonald SL, Hatcher A, Moritz C, Phillips BL. Intraspecific variation in  
287 climate-relevant traits in a tropical rainforest lizard. *Divers Distrib*. 2016; 1;22(10):1000–  
288 12.
- 289 31. O’Dea RE, Lagisz M, Jennions MD, Koricheva J, Noble DWA, Parker TH, et al.  
290 Preferred reporting items for systematic reviews and meta-analyses in ecology and  
291 evolutionary biology: A PRISMA extension. *Biological Reviews*. 2021;96(5):1695–722.
- 292 32. Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. *Journal of*  
293 *Statistical Software*. 2010; 36(3)1-48.
- 294 33. Claussen DL. Thermal acclimation in Ambystomatid salamanders. *Comparative*  
295 *Biochemistry and Physiology* . 1977;58(4):333–40.
- 296 34. Pottier P, Burke S, Drobniak SM, Lagisz M, Nakagawa S. Sexual (in)equality? A meta-  
297 analysis of sex differences in thermal acclimation capacity across ectotherms. *Funct Ecol*.  
298 2021;35(12):2663–78.
- 299 35. Noble DWA, Lagisz M, O’dea RE, Nakagawa S. Nonindependence and sensitivity  
300 analyses in ecological and evolutionary meta-analyses. *Mol Ecol*. 2017;26(9):2410–25.
- 301 36. Nakagawa S, Lagisz M, Jennions MD, Koricheva J, Noble DWA, Parker TH, et al.  
302 Methods for testing publication bias in ecological and evolutionary meta-analyses.  
303 *Methods in Ecology and Evolution*. 2022; 13(1), 4-21.
- 304 37. Noble DWA, Pottier P, Lagisz M, Burke S, Drobniak SM, O’Dea RE, et al. Meta-analytic  
305 approaches and effect sizes to account for ‘nuisance heterogeneity’ in comparative  
306 physiology. *Journal of Experimental Biology*. 2022; (Jeb243225).

38. Nakagawa S, Lagisz M, O’dea RE, Pottier P, Rutkowska J, Senior AM, et al. orchaRd 2.0: An R package for visualizing meta-analyses with 2 orchard plots. *EcoEvoRxiv*. 2023.
39. Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, et al. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences*. 2014;111(15):5610–5.
40. Kellermann V, van Heerwaarden B, Sgrò CM, Hoffmann AA. Fundamental evolutionary limits in ecological traits drive drosophila species distributions. *Science*; 2009; 325(5945):1244–6.
41. Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, et al. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*. 2008;105(18):6686–6672.
42. MacLean HJ, Sørensen JG, Kristensen TN, Loeschcke V, Beedholm K, Kellermann V, et al. Evolution and plasticity of thermal performance: An analysis of variation in thermal tolerance and fitness in 22 *Drosophila* species. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2019;374(1778).
43. Enriquez-Urzelai U, Sacco M, Palacio AS, Pintanel P, Tejedo M, Nicieza AG. Ontogenetic reduction in thermal tolerance is not alleviated by earlier developmental acclimation in *Rana temporaria*. *Oecologia*. 2019;189(2):385–94.
44. Gunderson AR, Fargevieille A, Warner DA. Egg incubation temperature does not influence adult heat tolerance in the lizard *Anolis sagrei*. *Biol Lett*. 2020;16(1).
45. Shine R, Harlow PS. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology*. 1996;77(6):1808–17.
46. Mitchell TS, Warner DA, Janzen FJ. Phenotypic and fitness consequences of maternal nest-site choice across multiple early life stages. *Ecology*. 2013;94(2):336–45.
47. Bonduriansky R, Head M. Maternal and paternal condition effects on offspring phenotype in *Telostylinus angusticollis* (Diptera: Neriidae). *J Evol Biol*. 2007;20(6):2379–88.
48. Parker GA, Begon M. Optimal egg size and clutch size: Effects of environment and maternal phenotype. *The American Naturalist*. 1986;128(4):573–92.
49. Janzen DH. Why Mountain passes are higher in the tropics. *The American Naturalist*. 1967;101(919):233–49.
50. Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, et al. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences*. 2014;111(15):5610–5.
51. Kearney M, Shine R, Porter WP. The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. *Proceedings of the National Academy of Sciences*. 2009;10(10):3835–40.
52. Gunderson AR, Stillman JH. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*. 2015; 20;282(1808).
53. Nakagawa S, Noble DWA, Senior AM, Lagisz M. Meta-evaluation of meta-analysis: Ten appraisal questions for biologists. *BMC Biology*. 2017; 15(1) 1-14.
54. Nakagawa S, Lagisz M, O’Dea RE, Rutkowska J, Yang Y, Noble DWA, et al. The orchard plot: Cultivating a forest plot for use in ecology, evolution, and beyond. *Res Synth Methods*. 2021;12(1):4–12.
55. Rose Y, Zhang, Kristoffer H. Wild, Patrice Pottier, Maider Iglesias Carrasco, Shinichi Nakagawa and Daniel W.A. Noble. (2023) Developmental environments do not affect

thermal physiology in reptiles: An experimental test and meta-analysis. *Zenodo*.  
<https://doi.org/10.5281/zenodo.7700383>

- \*\* Abayarathna MG. *The effect of incubation temperature on offspring phenotypes and survival of velvet gecko, Amalosia lesueurii* (Doctoral dissertation). \*\*
- \*\* Alberts AC, Perry AM, Lemm JM, Phillips JA. Effects of incubation temperature and water potential on growth and thermoregulatory behavior of hatchling Cuban rock iguanas (*Cyclura nubila*). *Copeia*. 1997; 766-76. \*\*
- \*\* Arnold SJ, Peterson CR, Gladstone J. Behavioural variation in natural populations. VII. Maternal body temperature does not affect juvenile thermoregulation in a garter snake. *Animal Behaviour*. 1995; 1;50(3):623-33. \*\*
- \*\* Beltrán I, Durand V, Loiseleur R, Whiting MJ. Effect of early thermal environment on the morphology and performance of a lizard species with bimodal reproduction. *Journal of Comparative Physiology B*. 2020;190(6):795-809. \*\*
- \*\* Blouin-Demers G, Kissner KJ, Weatherhead PJ. Plasticity in preferred body temperature of young snakes in response to temperature during development. *Copeia*. 2000; (3):841-5 \*\*
- \*\* Dayananda B, Murray BR, Webb JK. Hotter nests produce hatchling lizards with lower thermal tolerance. *Journal of Experimental Biology*. 2017;15;220(12):2159-65. \*\*
- \*\* Goodman RM, Walguarnery JW. Incubation temperature modifies neonatal thermoregulation in the lizard *Anolis carolinensis*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*. 2007;307(8):439-48. \*\*
- \*\* Llewelyn J, Macdonald SL, Moritz C, Martins F, Hatcher A, Phillips BL. Adjusting to climate: acclimation, adaptation and developmental plasticity in physiological traits of a tropical rainforest lizard. *Integrative Zoology*. 2018;13(4):411-27. \*\*
- \*\* Nelson NJ, Keall SN, Hare KM. Temperature selection by juvenile tuatara (*Sphenodon punctatus*) is not influenced by temperatures experienced as embryos. *Journal of Thermal Biology*. 2017;69:261-6. \*
- \*\* O'Steen SH. Embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles *Chelydra serpentina*. *The Journal of Experimental Biology*. 1998;201(3):439-49. \*\*
- \*\* Qualls CP, Andrews RM. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard, *Sceloporus virgatus*. *Biological Journal of the Linnean Society*. 1999;67(3):353-76. \*\*
- \*\* Spotila JR, Zimmerman LC, Binckley CA, Grumbles JS, Rostal DC, List Jr A, Beyer EC, Phillips KM, Kemp SJ. Effects of incubation conditions on sex determination, hatching success, and growth of hatchling desert tortoises, *Gopherus agassizii*. *Herpetological Monographs*. 1994; 1:103-16. \*\*

## 394 Tables & Figures

395 Table 1. Model outputs coefficients for testing whether sex, body mass, incubation temperature,  
 396 resource, or the interaction between resource and temperature had an effect on  $T_{pref}$  or  $CT_{max}$  in  
 397 hatchling *Lampropholis delicata*. Est. value describes the estimated coefficient value and 95% CI  
 398 describes the lower and upper bound of the 95% credible interval for each coefficient value.  
 399 Intercept is the estimated mean of each thermal trait from the null model.

Thermal Index	Covariate	Estimate	l-95% CI	u-95% CI	p value
$T_{pref}$	<b>(Intercept)</b>	<b>30.94</b>	<b>28.67</b>	<b>33.20</b>	<b>0.00</b>
	Body Mass	0.44	-0.97	1.86	0.53
	Sex	0.30	-2.50	3.09	0.83
	Incubation Temperature	-0.35	-2.36	1.66	0.72
	Resource	0.19	-1.83	2.20	0.85
	Incubation Temperature*Resource	-0.22	-4.31	3.87	0.91
$CT_{max}$	<b>(Intercept)</b>	<b>43.27</b>	<b>42.17</b>	<b>44.37</b>	<b>0.00</b>
	Body Mass	-0.41	-1.08	0.25	0.21
	Sex	-0.03	-1.35	1.28	0.96
	Incubation Temperature	-0.18	-1.14	0.78	0.70
	Resource	-0.24	-1.20	0.71	0.61
	Incubation Temperature*Resource	-0.52	-2.47	1.44	0.59

400

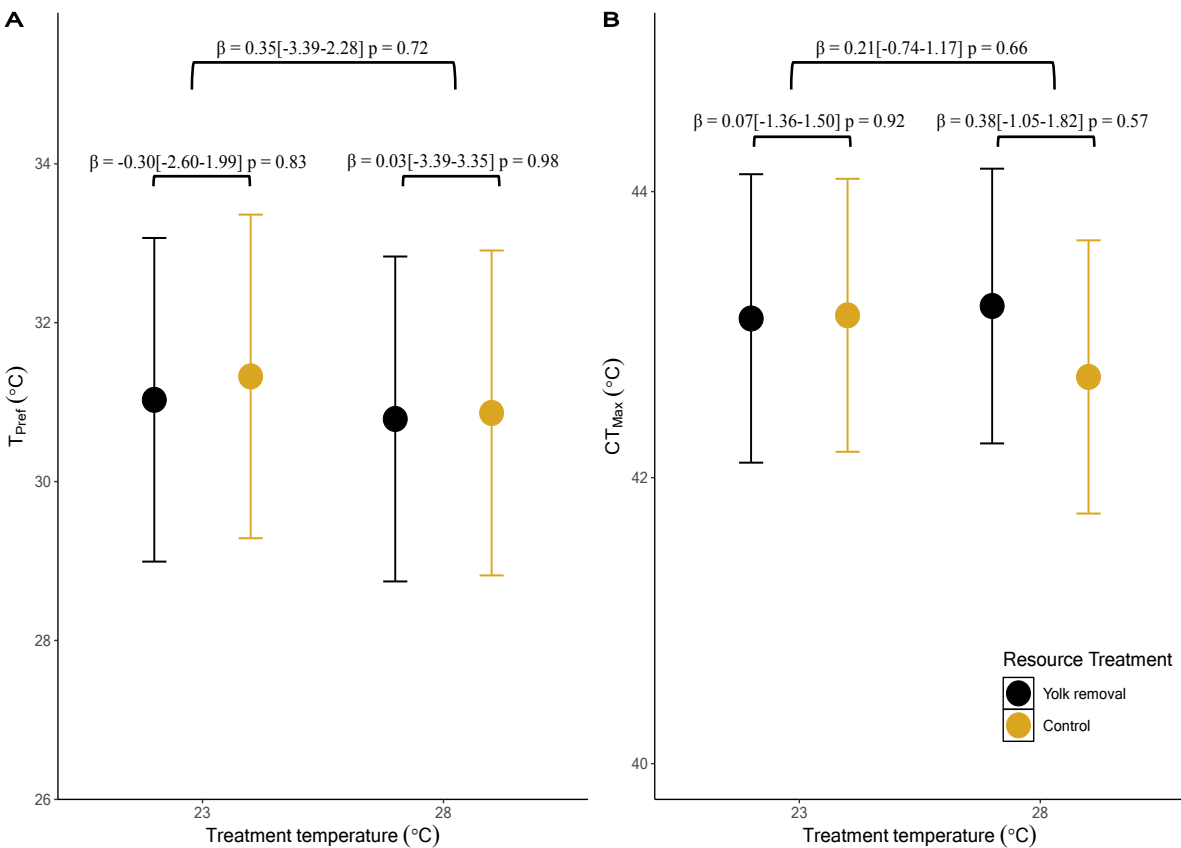


Figure 1. Thermal indices across different incubation temperatures and resource treatments for hatchling *Lampropholis delicata* (n=10 per temperature and treatment). (A) Thermal preference ( $T_{pref}$ ) in lizards incubated at 23 & 28°C for each resource treatment (yolk ablation & control). (B) Critical thermal maximum ( $CT_{max}$ ) in lizards incubated at 23 & 28°C for each resource treatment. Bars above plots indicate pairwise comparisons of thermal indices between treatment temperature and the interaction between treatment temperature and resource treatment. Means and 95% confidence intervals are provided along with the  $p$ -value for each contrast.

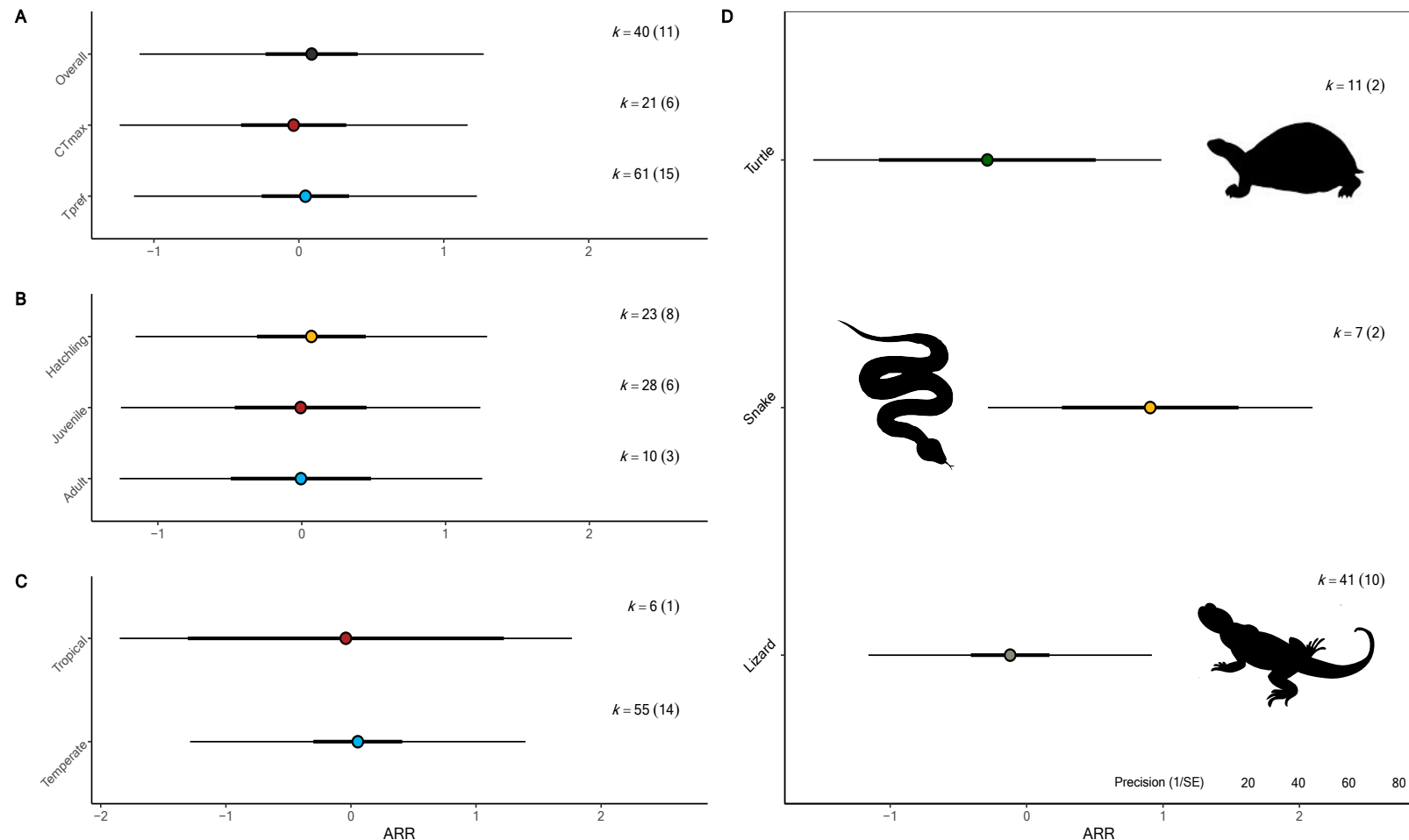


Figure 2. The magnitude of the effect of developmental temperature on thermal indices ( $T_{pref}$  &  $CT_{max}$ ) in reptiles (A) concerning age class of thermal physiological measurement (B), climate zone (C), and taxon (D). Mean meta-analytic ARR estimates (circles) with their 95% confidence intervals (thicker error bars) and prediction intervals (thinner error bars). Data points from each study from the meta-analysis are scaled by precision (inverse of standard error), and  $k$  is the number of effect sizes with the number of species in brackets. ARR is the acclimation response ratio. 95% confidence intervals not overlapping 0 are statistically significant. Graphs were constructed using the *orchaRd* package<sup>54</sup>. Tuatara was removed for visual purposes due to the small number of effect sizes ( $n=3$ )



418

For Review Only

