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

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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 physiological traits (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 (snakes, lizards, turtles) using meta-analysis. In both our experimental study and meta-analysis, we did not find evidence that developmental environments influence CT_{max} or T_{pref} . Furthermore, the effects of developmental environments on thermal physiology did not vary by age, taxon, or climate zone (temperate/tropical). Overall, the magnitude of developmental plasticity on thermal physiology appears to be limited across reptile taxa suggesting that behavioural or evolutionary processes may be more important. However, there is a paucity of information across most reptile taxa, and a broader focus on thermal performance curves themselves will be critical in understanding the impacts of changing thermal conditions on reptiles in the future.

Introduction

Climate warming and anthropogenic stressors pose significant challenges to organisms on a global scale [1,2]. Rapidly increasing temperatures are a particularly significant threat for ectothermic species. Indeed, increasing temperatures can drive fitness declines due to physiological intolerance [3], and alter the distribution of species [4]. 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 [5]. However, the magnitude of plastic responses is widely trait- and species-specific [5–7]

Temperature can also have transgenerational effects by impacting parental generations [8,9]. For instance, recent evidence indicates that some ectotherms can tolerate heat events for long periods [5,10]. 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 [11–13]. 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 [14–16]. Additionally, the resources a mother invests in her offspring (i.e., the energetic provisioning of eggs) can influence metabolic processes like growth and development [17]. 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[6]. 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 [18], most research effort has focused on temperature, which is known to have a profound effect on fitness [19,20]. In reptiles, temperatures during embryonic development are known to affect phenotypes throughout ontogeny [7]. For example, incubation conditions of developing reptile embryos can impact a variety of traits including sex, growth rate, morphology, behaviour, and cognition[7,20,21]. 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[22,23].

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 [3,24,25]. 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°C or 28°C SD ± 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 showing natural thermal fluctuations throughout the day [26]. Yolk removal treatments followed Sinervo[16], 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.*[28].

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 (CT_{max} and T_{pref}) measured. Briefly, after undergoing a 24-hour fasting period, animals were transferred into individual lanes of a thermal gradient (5°C to 55°C) to measure T_{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_{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 [29]. For CT_{max} we followed the same fasting period used for T_{pref} experiments. Here, lizards were placed in falcon tubes in a water bath for 5 min at a temperature of 30°C. The water temperature was increased to 38° C at a rate of 1° C/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 CT_{max} procedure [30]. CT_{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). We used linear mixed-effects models to analyse thermal traits (T_{pref} and CT_{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[31]. 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)[32]. 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 [33]. Sampling variance for the ARR was derived in Pottier et al.,[34]. 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. Studies often had more than two temperature treatments. As such, we derived all pairwise effect size comparisons. 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[35]. 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 [36] and was visually inspected using a funnel plot (*see Supp. for more details*). We present effect size heterogeneity by constructing prediction intervals [37] and presenting I^2 using the *orchaRd* package (version 2.0)[38].

Results

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

Mean T_{pref} was 31°C ±0.47 (mean ±SE) and ranged from 20.99–34.26°C. Mean CT_{max} was 43.04°C ±0.23 and ranged from 38.6–45.2°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 (β=-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 sipdedon*), 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, our results provide valuable insights into possible responses that are plausible under changing thermal conditions.

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Tables & Figures

Table 1. Model outputs coefficients for testing wither sex, body mass, incubation temperature, resource, or the interaction between resource and temperature had an effect on T_{Pref} or CT_{Max} in hatchling *Lampropholis delicata*. Est. value describes the estimated coefficient value and 95% CI describes the lower and upper bound of the 95% credible interval for each coefficient value. 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
Tpref	**(Intercept)**	**30.94**	**28.67**	**33.20**	**0.00**
	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
CTmax	**(Intercept)**	**43.27**	**42.17**	**44.37**	**0.00**
	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

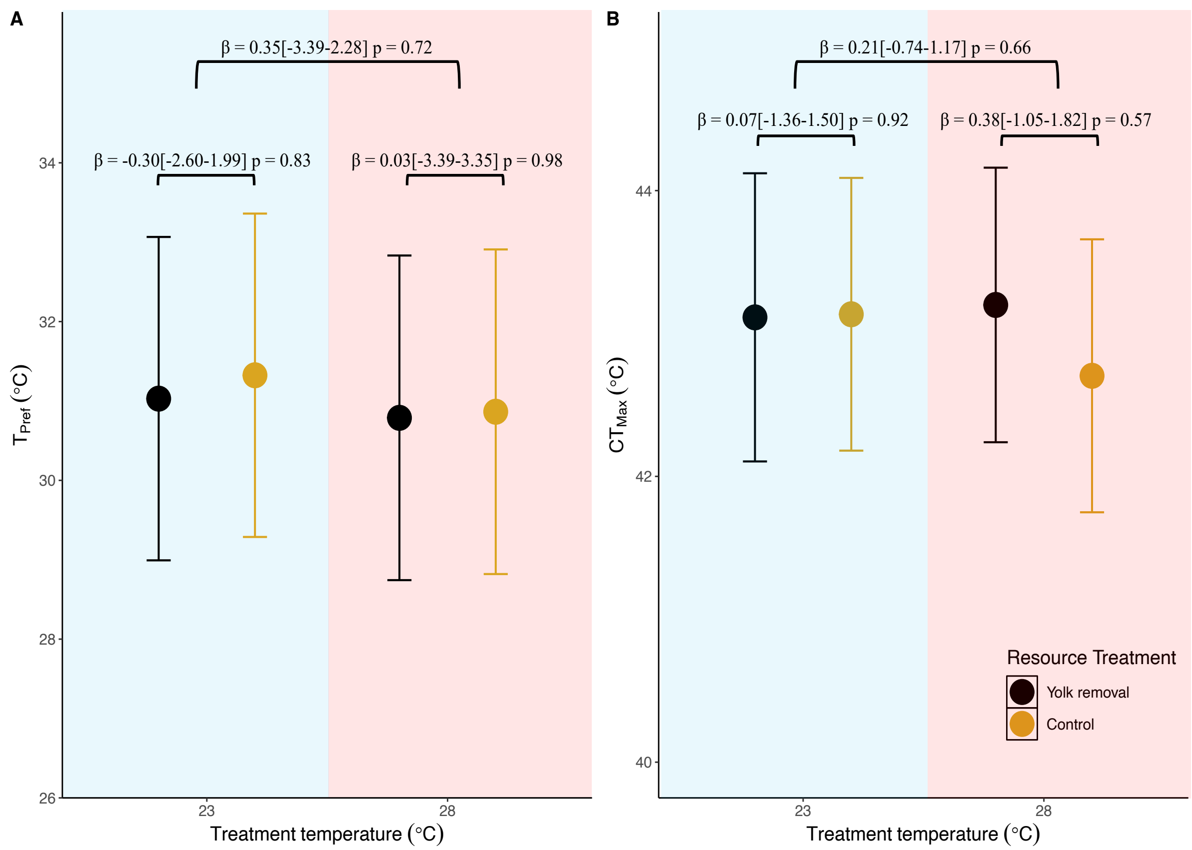

Figure 1. Thermal indices across different incubation temperatures and resource treatments for hatchling *Lampropholis delicata* (n=10 per temperature and treatment). (A) Thermal preference (T_{pref}) in lizards incubated at 23 & 28°C for each resource treatment (yolk ablation & control). (B) Critical 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.

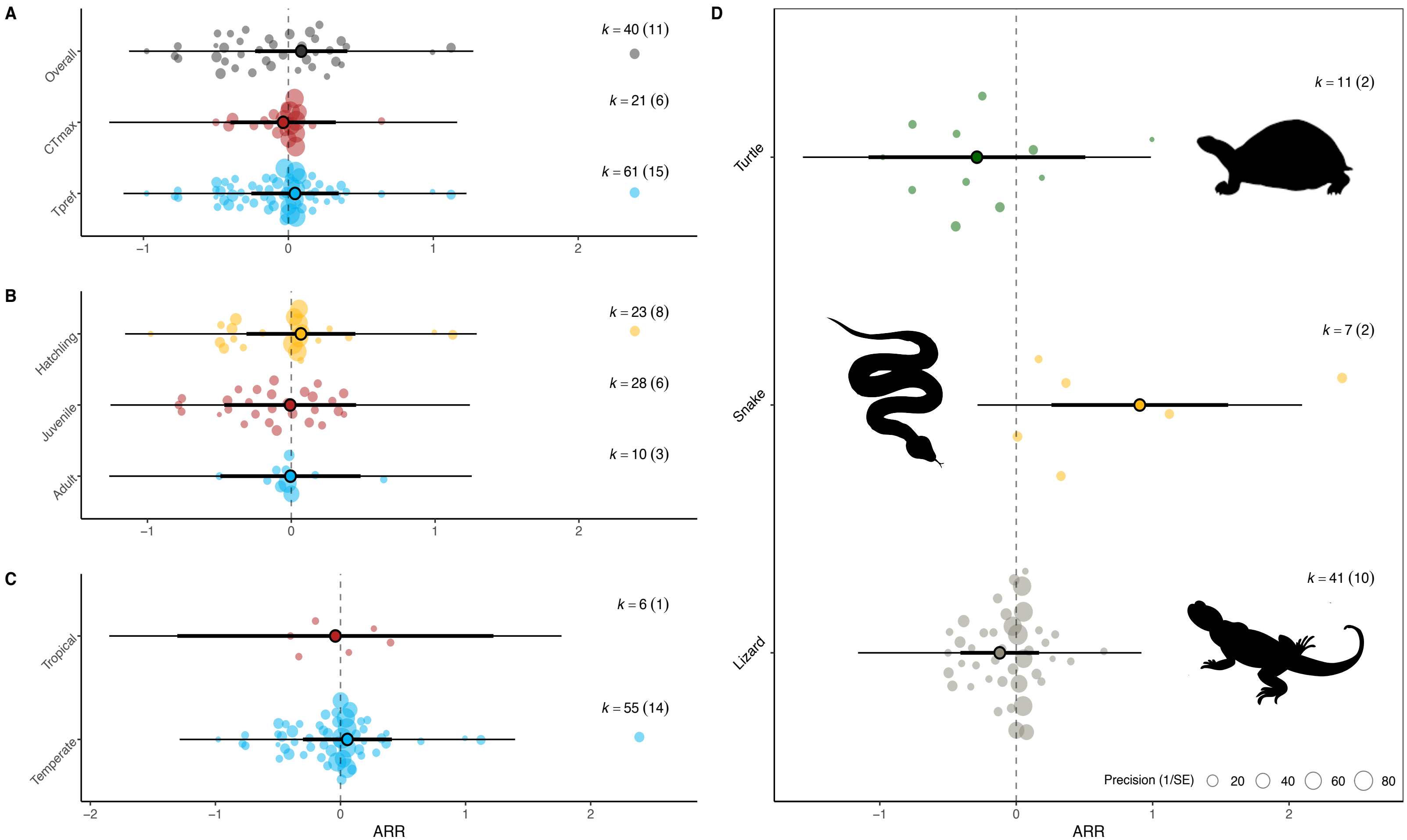

Figure 2. The magnitude of the effect of developmental temperature on thermal indices (T_{pref} & CT_{max}) in reptiles (A) concerning age class of thermal physiological measurement (B), climate zone (C), and taxon (D). Mean meta-analytic ARR estimates (circles) with their 95% confidence intervals (thicker 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[54]. Tuatara was removed for visual purposes due to the small number of effect sizes (n=3)