Developmental environments do not affect thermal physiology 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 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 [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 exhibiting 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 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]. 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[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

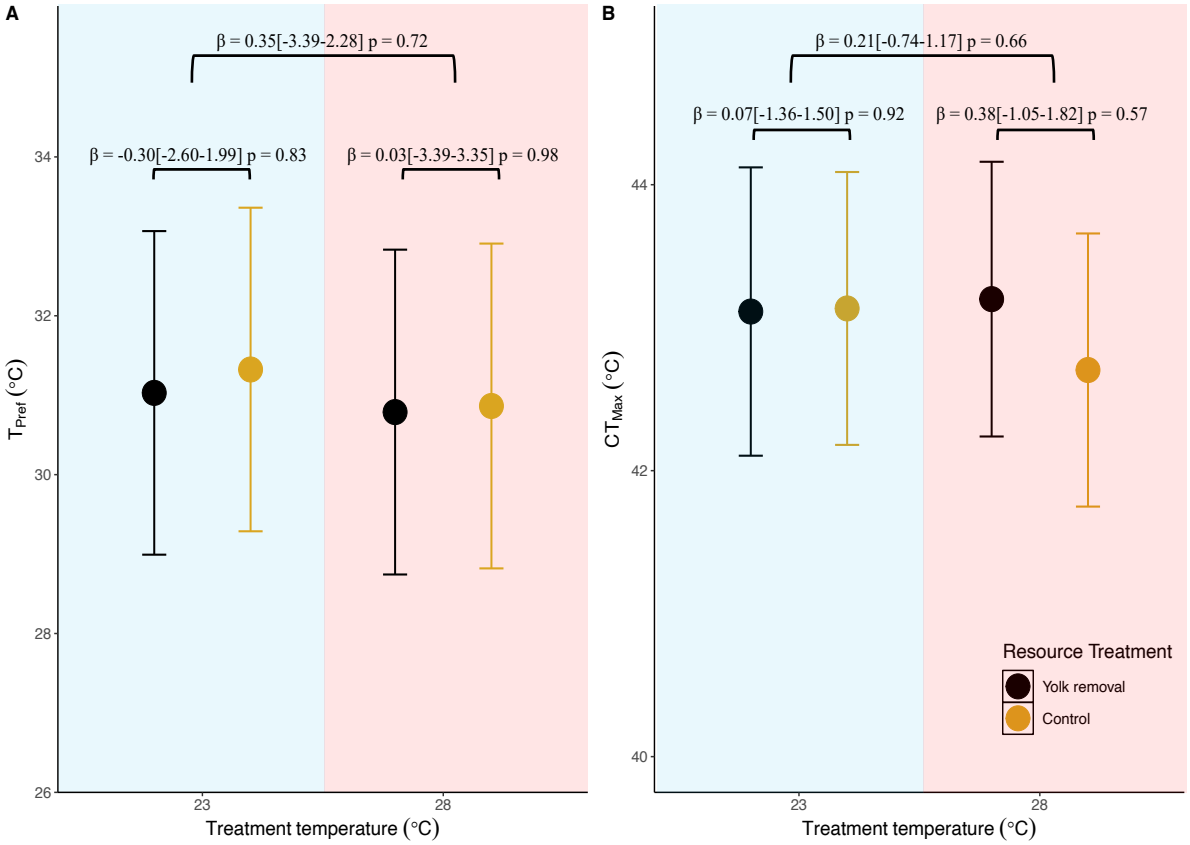

Figure 1. Thermal indices across different incubation temperatures and resource treatments for hatchling *Lampropholis delicata* (n=10 per temperature and treatment). (A) Thermal preference (T_{pref}) in lizards incubated at 23 & 28°C for each resource treatment (yolk ablation & control). (B) Critical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

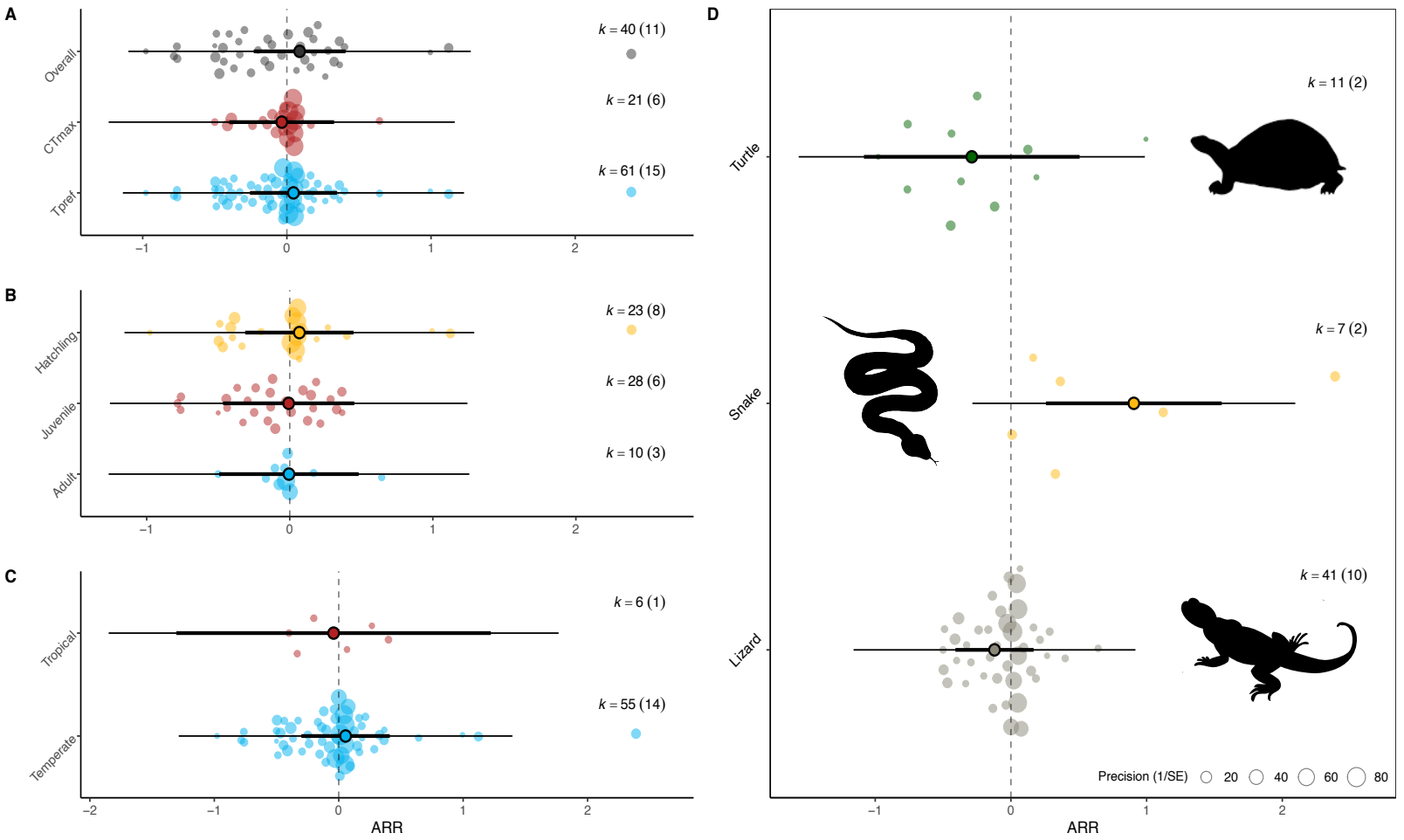

Figure 2. The magnitude of the effect of developmental temperature on thermal indices (T_{pref} & CT_{max}) in reptiles (A) concerning age class of thermal physiological measurement (B), climate zone (C), and taxon (D). Mean meta-analytic ARR estimates (circles) with their 95% confidence intervals (thicker 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)

Electronic supplementary material (ESM) for:

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. Biology Letters

Supplementary Materials and Methods

1. Experimental manipulations of early thermal environment

(a)Thermal Preference – T_{pref}

Animals (n =40) were randomly sampled to form five trial groups (n=8) such that two animals, one male and one female, from each treatment were in each trial group. Before T_{pref} trials, trial animals were moved from mating enclosures to individual enclosures undergoing a 24-hour fasting period. After this period, the mass of all trial animals was measured, after which animals were transferred into individual lanes of a thermal gradient plate spanning temperatures of 5˚C to 55˚C. Animals had a 12- window of acclimation in the thermal gradients prior to data collection. The thermal profiles across the thermal gradient were generated from an immersion cooler, copper tubing, and an electric heating pad. Infrared images were obtained in 15- minute intervals over an eight-hour observation period with a FLIR T640 thermography camera. Animals were returned to individual enclosures after T_{pref} trials. *Lampropholis delicata* body temperatures were extracted from infrared images using Flir Tools, version 5.13.

(b) Critical Thermal Maximum

Critical thermal maximum (CT_{max}) was determined for all individuals at least 24 hours after their T_{pref} trials. For each trial, individual skinks were placed in 50 mL Falcon tubes. Tubes were perforated lengthwise with holes to maintain airflow, while being weighted on the opposite side to maintain stable, horizontal buoyancy. Once lizards were in tubes, they were placed in a water bath for 5 min at a temperature of 30˚C to equilibrate to starting temperatures. To obtain the most accurate T_b for skinks, temperature was monitored with a thermocouple probe secured within a control (empty) Falcon tube and an additional thermal couple that was placed in the water bath. Water bath temperatures and temperatures within the control falcon tube closely matched. While we could not be certain animal body temperature was in fact 30˚C (we needed to avoid disturbance after placing animals within the water bath), it only took the bottom of the control Falcon tube ~1 minute to reach this temperature and remain stable. Given the small size of our lizards (i.e., 1.3 grams) we kept animals ~4 minutes longer before starting as we expected their body temperature to reach equilibrium by this point. Water temperature was then increased to 38˚ C at a rate of 1˚ C/min. If trial temperatures were above 38˚C, the heating rate was reduced to 0.5˚ C/min. Every 1 min tubes were rotated to check righting reflex of skinks. Once CT_{max} was reached, skinks were removed from the tube and placed into room temperature water for cooling. Given the small size of lizards (i.e., 1.3 g) we assumed lizards would reach thermal equilibrium rapidly, and therefore, skin surface temperature reflected body temperature. Skin surface has been shown as an accurate proxy for T_b for many small lizards (Garrick 2008). It is possible T_b lagged behind for our measurements. Any lag would result in an underestimated CT_{max}, which is likely the case for most studies measuring CT_{max} in lizards given the ethical challenges with pushing animals to thermal extremes (e.g[1–3]). Regardless, we do not view this as problematic because body mass did not differ across the treatments, and we do not expect this to affect the relative difference in CT_{max} between treatments.

(c) Statistical analysis

For the experimental analysis (T_{pref} and CT_{max}) on *L. delicata*, we used linear mixed-effects models using the lme4 package (version 1.1)[4]. Each model was constructed with a thermal index (T_{pref} or CT_{max}) as the response variable and body mass, sex, incubation temperature, resource treatment, and the interaction between incubation temperature and resource treatment as predictor variables. Model assumptions were checked using the *performance* package (version 0.10)[5]. Finally, the package *emmeans* (version 1.80)[6] was used to extract marginal means (least-squares means) and standard error for figure purposes.

2. Meta-analysis

(a) Initial literature search and record screening process

We developed search strings to capture experimental studies which measured the thermal traits (in the form of CT_{max} or T_{pref}) of reptiles exposed to different developmental temperatures. We focused only on temperatures given that too few studies manipulated egg resources and measured thermal physiology of offspring. The search strings used in the two databases screened in this study are below:

ProQuest and Scopus:

("temperature*" OR "thermal" OR "cold" OR "cool*" OR "heat*" OR "acclimat*") AND ("incubat*" OR "rear*" OR "development*" OR "ontogen*" OR "nest*" OR "embryo*" OR "early*" OR "juvenile*" OR "hatchling*" OR "young*" OR "egg*" OR "life history stage*" OR "life stage*") AND ("thermal tolerance*" OR "thermotolerance*" OR "cold tolerance*" OR "heat tolerance*" OR "tolerance* to heat*" OR "tolerance* to cold*" OR "tolerance* to temperature*" OR "temperature* tolerance*" OR "physiological tolerance*" OR "critic* thermal" OR "critic* temperature*" OR "thermal limit*" OR "thermal breadth*" OR "tolerance breadth*" OR "thermal range*" OR "performance breadth*" OR "thermal window*" OR "tolerance window*" OR "warming tolerance*" OR "tolerance* to warming" OR "cooling tolerance*" OR "tolerance* to cooling*" OR "CTmax" OR "CTmin" OR "heat coma" OR "chill coma" OR "lethal temperature*" OR "lethal limit*" OR "SCP" OR "supercooling point*" OR "crystal* temperature*" OR "freez* *tolerance*" OR "frost tolerance*" OR "freezing point*" OR "preferred temperature*" OR "preferred body temperature*" OR "selected body temperatures" OR "temperature preference*" OR "thermal preference*" OR "selected temperature*" OR "selected body temperature*" OR "thermal prefer*" OR "temperature* prefer*" OR "temperature* select*" OR "thermal select*" OR "equilibr* temperature*" OR "temperature* at equilibrium") AND ("squamat*" OR "lizard*" OR "reptil*" OR "snake*" OR "serpent*" OR "turtle*" OR "crocodil*", OR "iguan*", OR "anol*" OR "skink*" OR "chameleon*" OR "chamaeleon*" OR "gecko*" OR "tortoise*" OR "adder*" OR "viper*" OR "python*" OR "boa" OR "boas" OR "colubrid*" OR "amphisbaenia*" OR "rhynchocephal*" OR "testudin*" OR "chelon*" OR "alligator*" OR "caiman*" OR "gavial**" OR "garhial" OR "tuatar*" OR "sphenodon*") AND NOT ("endotherm*" OR "bird*" OR "avia*" OR "aves" OR "mammal*" OR "rodent*" OR "mouse" OR "mice" OR "rat" OR "rats" OR "cattle*" OR "livestock*" OR "domesticated" OR "cow" OR "cows" OR "pig" OR "pigs" OR "sheep*" OR "goat*" OR "horse*" OR "rabbit*" OR "chicken*" OR "duck*" OR "turkey*" OR "cat" OR "cats" OR "dog" OR "dogs" OR "plant" OR "plants" OR "plantule*" OR "cultivar*" OR "arabidopsis" OR "leaf" OR "leaves" OR "root*" OR "seed*" OR "tree*" OR "fungi" OR "fungal" OR "alga" OR "algae" OR "algal" OR "seaweed*" OR "seagrass" OR "*phyt*" OR "*bacteri*" OR "unicellular" OR "protist*" OR "archae*" OR "woman" OR "women" OR "man" OR "men" OR "volunteer*" OR "athlete*" OR "military" OR "*fish*" OR "amphib*" OR "frog*" OR

"toad*" OR "salamander*" OR "newt*" OR "invertebrate*" OR "insect*" OR "arthropod*" OR "arachnid*" OR "crustacea*" OR "myriapod*" OR "mollus*" OR "annelid*" OR "*worm*" OR "cnidar*" OR "coral*")

ISI Web of Science:
("temperature*" OR "thermal" OR "cold" OR "cool*" OR "heat*" OR "acclimat*") AND ("incubat*" OR "rear*" OR "development*" OR "ontogen*" OR "nest*" OR "embryo*" OR "early*" OR "juvenile*" OR "hatchling*" OR "young*" OR "egg*" OR "life history stage*" OR "life stage*")) AND ("thermal tolerance*" OR "thermotolerance*" OR "cold tolerance*" OR "heat tolerance*" OR "tolerance* to heat*" OR "tolerance* to cold*" OR "tolerance* to temperature*" OR "temperature* tolerance*" OR "physiological tolerance*" OR "critic* thermal" OR "critic* temperature*" OR "thermal limit*" OR "thermal breadth*" OR "tolerance breadth*" OR "thermal range*" OR "performance breadth*" OR "thermal window*" OR "tolerance window*" OR "warming tolerance*" OR "tolerance* to warming" OR "cooling tolerance*" OR "tolerance* to cooling*" OR "CTmax" OR "CTmin" OR "heat coma" OR "chill coma" OR "lethal temperature*" OR "lethal limit*" OR "SCP" OR "supercooling point*" OR "crystal* temperature*" OR "freez* *tolerance*" OR "frost tolerance*" OR "freezing point*" OR "preferred temperature*" OR "preferred body temperature*" OR "selected body temperatures" OR "temperature preference*" OR "thermal preference*" OR "selected temperature*" OR "selected body temperature*" OR "thermal prefer*" OR "temperature* prefer*" OR "temperature* select*" OR "thermal select*" OR "equilibr* temperature*" OR "temperature* at equilibrium")) AND ("squamat*" OR "lizard*" OR "reptil*" OR "snake*" OR "serpent*" OR "turtle*" OR "crocodil*", OR "iguan*", OR "anol*" OR "skink*" OR "chameleon*" OR "chamaeleon*" OR "gecko*" OR "tortoise*" OR "adder*" OR "viper*" OR "python*" OR "boa" OR "boas" OR "colubrid*" OR "amphisbaenia*" OR "rhynchocephal*" OR "testudin*" OR "chelon*" OR "alligator*" OR "caiman*" OR "gavial**" OR "garhial" OR "tuatar*" OR "sphenodon*")) NOT ("endotherm*" OR "bird*" OR "avia*" OR "aves" OR "mammal*" OR "rodent*" OR "mouse" OR "mice" OR "rat" OR "rats" OR "cattle*" OR "livestock*" OR "domesticated" OR "cow" OR "cows" OR "pig" OR "pigs" OR "sheep*" OR "goat*" OR "horse*" OR "rabbit*" OR "chicken*" OR "duck*" OR "turkey*" OR "cat" OR "cats" OR "dog" OR "dogs" OR "plant" OR "plants" OR "plantule*" OR "cultivar*" OR "arabidopsis" OR "leaf" OR "leaves" OR "root*" OR "seed*" OR "tree*" OR "fungi" OR "fungal" OR "alga" OR "algae" OR "algal" OR "seaweed*" OR "seagrass" OR "*phyt*" OR "*bacteri*" OR "unicellular" OR "protist*" OR "archae*" OR "woman" OR "women" OR "man" OR "men" OR "volunteer*" OR "athlete*" OR "military" OR "*fish*" OR "amphib*" OR "frog*" OR "toad*" OR "salamander*" OR "newt*" OR "invertebrate*" OR "insect*" OR "arthropod*" OR "arachnid*" OR "crustacea*" OR "myriapod*" OR "mollus*" OR "annelid*" OR "*worm*" OR "cnidar*" OR "coral*")

On 2021/01/28 a literature search using Scopus returned 289 records. On 2021/02/01 an additional search was performed using ISI Web of Science (core collection) and ProQuest (dissertation and theses) returning 346 records. During this search, four additional records were obtained from a review paper[7] and two unpublished records from additional studies were also included. These were combined to generate 639 records. Within these records, we removed 154 duplicates and obtained 485 unique documents. RZ screened titles, abstracts, and key words in Rayyan QCRI[8]. We selected studies based on eight eligibility criteria: (i) the study was done on a non-avian reptile (lizard, snake, turtle/tortoise, crocodile/alligator, or tuatara), (ii) the study was experimental, (iii) CT_{max} or T_{pref} (also referred to as T_{sel}) was measured, (iv) studies experimentally manipulated two or more incubation temperatures, (v)

measurements of T_{pref} and CT_{max} were performed on individuals acclimated to the same temperatures, (vi) means, sample sizes and variances were reported. Full details of our selection criteria at the abstract and full-text screening stages are provided in Table S1 and Figure S1.

The PRISMA flowchart illustrating the systematic literature search and workflow is also shown in Figure S2. Following preliminary selection, full-text eligibility criteria were used to screen 52 full-text documents (Figure S2). Of the full-text documents, 19 documents fulfilled all eligibility criteria. We contacted the primary authors of five different studies to request unprocessed data that was not included in the publication but received no responses.

(b) Data extraction

Overall, we obtained a total of 69 unique effect sizes from 14 studies spanning 13 different species. All data were extracted by RZ. Data presented in the text or tables were directly extracted from the study. Data shown in the figures were digitised using the *metaDigitise* package[9] in R (version 1.0.1). Alongside effect size data, we also extracted any available information regarding experimental species, life stage at the time of measurement (hatchling, juvenile, or adult), life history (latitude of origin, terrestrial, or aquatic), and reptilian class (lizard, snake, turtle, or tuatara).

(c) Statistical analysis

We analysed our data using multi-level meta-analysis (MLMA) (i.e., intercept only models with random effects) and multi-level meta-regression (MLMR) models (i.e., models with 'fixed' and random effects). The acclimation response ratio (ARR) was used as our effect size and was defined as the variation in heat tolerance associated with a one-degree change in developmental temperature. Acclimation response ratio was defined as:

$$ARR = \frac{\mu HT_{T2} - \mu HT_{T1}}{T_2 - T_1}$$

Where HT is the mean heat tolerance estimates (CTmax or Tpref), and T is the incubation temperature in Celsius. T_1 is defined as the control developmental temperature and T_2 is defined as the warm or treatment developmental temperature. When ARR = 0 the heat tolerance measurement remains static, and no acclimation occurs as developmental temperature increases. In contrast, perfect compensation would be considered when ARR =1, where heat tolerance changes in concordance with developmental temperature. The sampling variance for AAR was derived as:

$$s^2ARR = \left(\frac{1}{T_2 - T_1}\right)^2 \left(\frac{sd^2_{[T1]}}{n_{[T1]}} + \frac{sd^2_{[T2]}}{n_{[T2]}}\right)$$

Where s^2(ARR) is the sampling variance of AAR, *sd* is the standard deviation and n is the sample size (number of individuals). In studies with more than two temperatures we calculated a pairwise effect between each developmental temperature comparison. Given the same data are used to derive different effect sizes this induces non-independence between effect size sampling errors and the effects themselves (*See* Noble et al.[10]). We accounted for this through the inclusion of a sampling (co)variance matrix derived assuming effect sizes are correlated by r = 0.5[10]. We also re-fit models using robust variance estimation methods as these do not make assumptions about the nature of correlation within studies and have been shown to perform extremely well with complex sources of non-independence [11,12]. In all cases, RVE did not make any difference to conclusions. As such, we only included the sampling covariance matrices in our models.

All meta-analytic models were constructed using the '*rma.mv'* function in the package *metafor* (version 3.8-1)[13]. In all models we included phylogeny, species, study, and observation as random effects. We created a phylogenetic correlation matrix of species in the data set using the Open Tree of Life [14]. We used the *rotl* package (version 3.0.12) [15] to access the Open Tree of Life in R. Branch lengths were calculated for trees using the 'compute.brlen' function in the *ape* package (version 5.6.2)[16]. Using the *ape* '*vcv'* function, we built a correlation matrix of phylogenetic relatedness among species which was included in our models. We compared three intercept models where we accounted for 1) species, 2) phylogeny, and 3) species and phylogeny (Table S2). [7]we used the function AIC scores from *metafor*[4] *to* evaluate which model was the best fit for the data.

We estimated the overall meta-analytic mean and calculated measures of heterogeneity by constructing prediction intervals and calculating I^2 from our MLMA models (Nakagawa & Santos 2012; Noble et al. 2022). I^2 allowed us to estimate the proportion of variation explained by species differences, phylogeny, and study-specific effects while accounting for known sampling variance[17,18]. Prediction intervals were calculated using *metafor* whereas I^2 was calculated using the *orchaRd* package (version 2.0).

We then fit MLMR models by including the same random effects, but adding in a single moderator (i.e., predictor) at a time. The models included those with the following moderator variables: thermal trait measurement type (T_{pref} or CT_{max}), climate zone (temperate or tropical), and life stage when thermal physiological trait measurements took place (hatchling, juvenile or adult). We explored publication bias using visual interpretation using a funnel plot and a modified version of Eggers regression [19] that included a multi-level meta-regression model with sampling variance or sampling standard error as a moderator[17].

Supplementary Results

1. Meta-analysis

We found minimal difference in AIC support for our intercept-only MLMA models when accounting for phylogeny, species, or phylogeny and species (Table S2). Therefore, we selected species in our final intercept model. We did not find evidence for developmental temperatures to influence CT_{max} (ARR = -0.08, 95%CI: -0.75–0.58; $p = 0.79$) or T_{pref} (ARR = 0.08, 95%CI: -0.36–0.53; $p = 0.68$; Table S3). We also did not find evidence for developmental temperatures affecting ARR across age classes in reptiles, where the confidence intervals overlapped with zero for hatchlings, juveniles, and adults (Table S4). We did not find differences in plasticity between animals found in the tropics (ARR = -0.08, 95%CI: -1.39–1.24; $p = 0.90$), and temperate animals (ARR = 0.04, 95%CI: -0.35–0.43; $p = 0.81$; Table S5). We acknowledge, however, that the sample size for tropical species was low and these results must be considered preliminary. We also did not find evidence for differences in plasticity between turtles, lizards, and tuataras (Table S6). In snakes, however, developmental temperatures did have a significant increase effect on thermal traits, but this effect is primarily driven by one species, *Nerodia sipedon*. Visual inspection of funnel plots did not show data distribution of publication bias (Figure S3), and statistically, we found no evidence for publication biases (β=-0.81, 95%CI=-1.92-0.3, $p=0.15$).

Table S1. Description of the inclusion criteria used to screen full texts of studies used in Figure S1 (decision tree).

Term	Definition
1. Reptile	Only included studies where the study species belonged to the class *Reptilia*. Studies examining bacteria, fungi, plants, invertebrates, non-reptilian vertebrates, or cells isolated from reptilian animals were excluded.
2. Experimental study	Only studies were included where researchers performed manipulative laboratory experiments. As a result, data obtained from field experiments, theoretical studies, observational laboratory experiments and qualitative reviews or models were excluded.
3. Measurement of T_{pref} or CT_{max}	Thermal preference (T_{pref}) and critical thermal maximum (CT_{max}) were selected as the two desired measures of thermal traits. Accordingly, we excluded experimental studies measuring other thermal traits like the lethal temperature for 50% of animals (LT_{50}), critical thermal minima (CT_{min}), heat knockdown time (HKT), or thermal optima (T_{opt}) of reptiles. Studies that measured preferred body temperature (PBT) or preferred temperature (T_p) were included, as these are analogous measures to T_{pref}.
4. Manipulation of developmental temperature	Only studies were included where independent groups of animals were exposed to two or more controlled (laboratory setting) temperatures during their embryonic development and subsequently assessed for thermal tolerance. A brief (e.g. less than 24hrs) exposure to a particular temperature condition was not considered to be sufficient manipulation of developmental temperature. Studies containing fluctuating developmental temperature treatments were permitted so long as the mean temperature between treatments differed. In circumstances where embryos were collected from the wild, we only included studies that performed a subsequent developmental temperature manipulation. Any studies which manipulated juvenile or adult developmental temperature were excluded. We also excluded any studies where juveniles or adults were collected from the wild and subsequently measured for T_{pref} or $CT_{max,}$ but included studies where embryos were collected for controlled developmental temperature manipulation.
5. Developmental temperature not confounded with adult acclimation	Studies were excluded where other known factors like chemical exposure, hormone addition and humidity were confounded with developmental temperature treatments. We included studies that manipulated developmental temperature alongside one or more factors in a fully factorial design, as it is possible to have independent manipulations of developmental temperature.

6. Is developmental temperature not confounded with additional factors?	Studies were excluded where other known factors like chemical exposure, hormone addition and humidity were confounded with developmental temperature treatments. We included studies that manipulated developmental temperature alongside one or more factors in a fully factorial design, as it is possible to have independent manipulations of developmental temperature.
7. Sample sizes and variances reported	Only included studies where measures of dispersion in the form of standard deviation or standard error were reported for each group of animals. If such data were not reported, the study's primary author was contacted for further information.

Table S2. Multi-level meta-analysis (MLMA) (i.e., intercept only models with random effects) of phylogeny, species, or phylogeny and species. Akaike information criterion was used to compare model fits.

Model name	AIC	est	ci.lb	ci.ub	pi.lb	pi.ub	pvalue	I^2_{Total}	$I^2_{study\ ID}$	I^2_{phylo}	I^2_{spp}	I^2_{obs}
Phylogeny	77.38	0.03	-0.28	0.34	-1.23	1.28	0.86	99.52	0.52	0.00	-	20.99
Species	78.11	0.05	-0.28	0.37	-1.23	1.32	0.76	99.53	7.87	-	70.57	21.10
Species and Phylogeny	80.11	0.05	-0.28	0.37	-1.23	1.32	0.76	99.53	7.87	0.00	70.57	21.10

Table S3. The magnitude of the effect of developmental temperature on ARR on CTmax and Tpref of reptiles. The number of effect sizes is denoted by k and n indicates the number of species. Estimates are species mean meta-analytic estimates with their 95% confidence intervals (lowerCL = lower bound & upperCL = upper bound) and prediction intervals (lowerPR = lower bound & upperPR = upper bound). P values indicate if values are significantly different from zero. The conditional r^2 (0.79) and the marginal r^2 (0.01).

Thermal metric	k	n	Estimate	upperCL	lowerPR	upperPR	p value
Ctmax	21	6	-0.04	0.33	-1.2	1.2	0.84
Tpref	61	15	0.09	0.41	-1.1	1.3	0.58

Table S4. The magnitude of the effect of developmental temperature on ARR when accounting for age class. The number of effect sizes is denoted by k and n indicates the number of species. Estimates are species mean meta-analytic estimates with their 95% confidence intervals (lowerCL = lower bound & upperCL = upper bound) and prediction intervals (lowerPR = lower bound & upperPR = upper bound). P values indicate if values are significantly different from zero. The conditional r^2 (0.80) and the marginal r^2 (0.01).

Age class	k	n	Estimate	upperCL	lowerPR	upperPR	p value
Adult	10	3	-0.01	0.48	-1.3	1.3	0.98
Juvenile	28	6	-0.01	0.45	-1.3	1.2	0.97
Hatchling	23	8	0.07	0.45	-1.2	1.3	0.72

Table S5. The magnitude of the effect of developmental temperature on ARR when accounting for the species origin. The number of effect sizes is denoted by k and n indicates the number of species. Estimates are species mean meta-analytic estimates with their 95% confidence intervals (lowerCL = lower bound & upperCL = upper bound) and prediction intervals (lowerPR = lower bound & upperPR = upper bound). P values indicate if values are significantly different from zero. The conditional r^2 (0.80) and the marginal r^2 (0.01).

Geographic zone	k	n	Estimate	upperCL	lowerPR	upperPR	p value
Temperate	55	14	0.06	0.41	-1.3	1.4	0.74
Tropical	6	1	-0.04	1.22	-1.9	1.8	0.94

Table S6. The magnitude of the effect of developmental temperature on ARR when accounting for reptile taxa. The number of effect sizes is denoted by k and n indicates the number of species. Estimates are species mean meta-analytic estimates with their 95% confidence intervals (lowerCL = lower bound & upperCL = upper bound) and prediction intervals (lowerPR = lower bound & upperPR = upper bound). P values indicate if values are significantly different from zero. The conditional r^2 (0.80) and the marginal r^2 (0.38).

Taxa	k	n	Estimate	upperCL	lowerPR	upperPR	p value
Lizard	41	10	-0.12	0.17	-1.16	0.92	0.37
Snake	7	2	0.91	1.55	-0.28	2.10	0.01
Tuatara	2	1	0.37	2.08	-1.60	2.35	0.63
Turtle	11	2	-0.29	0.51	-1.56	0.99	0.44

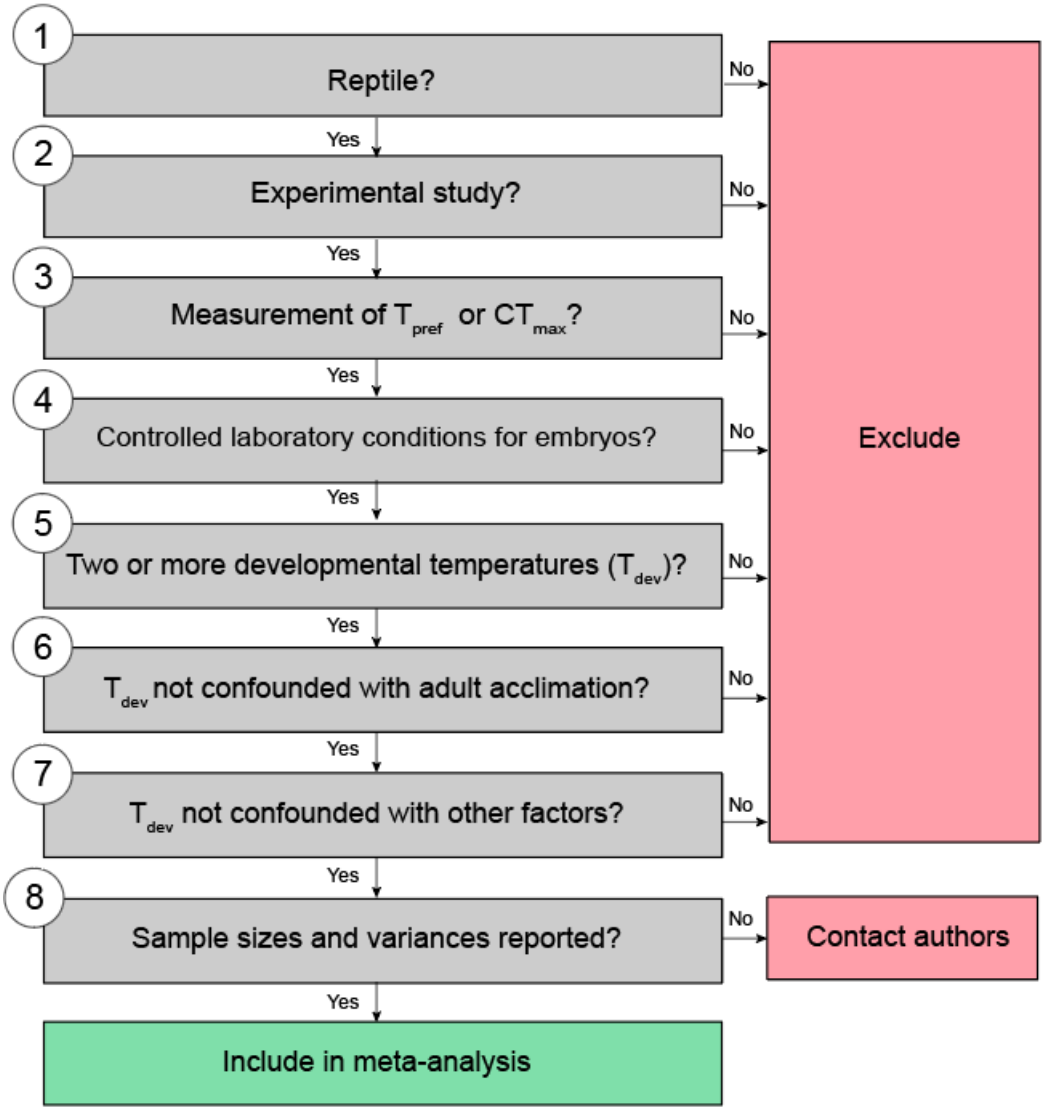

Figure S1. Decision tree showing the eligibility criteria used to assess full-text articles.

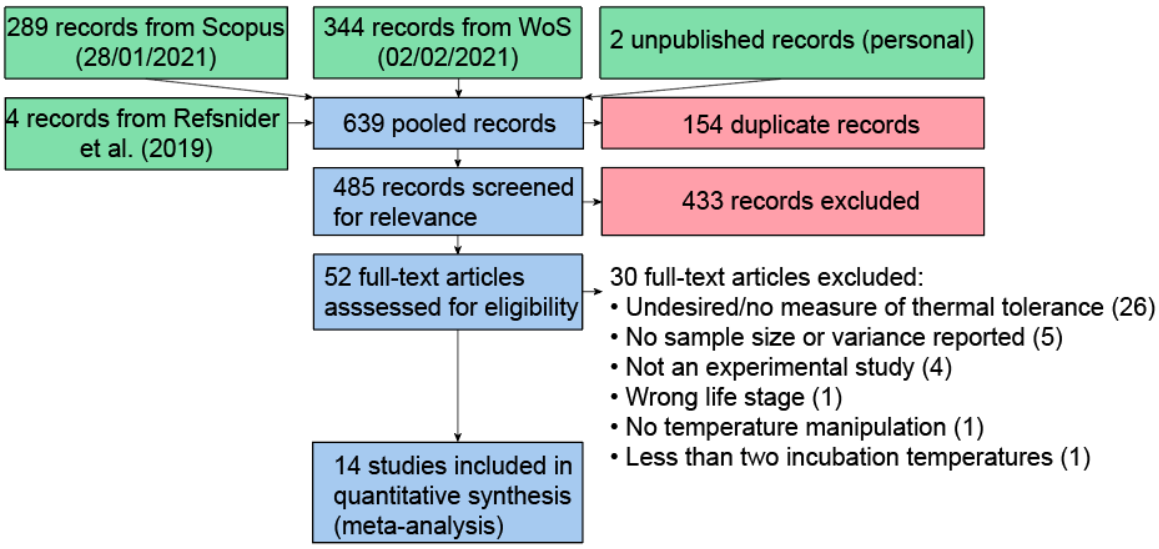

Figure S2. PRISMA flowchart illustrating the systematic literature search and record screening process.

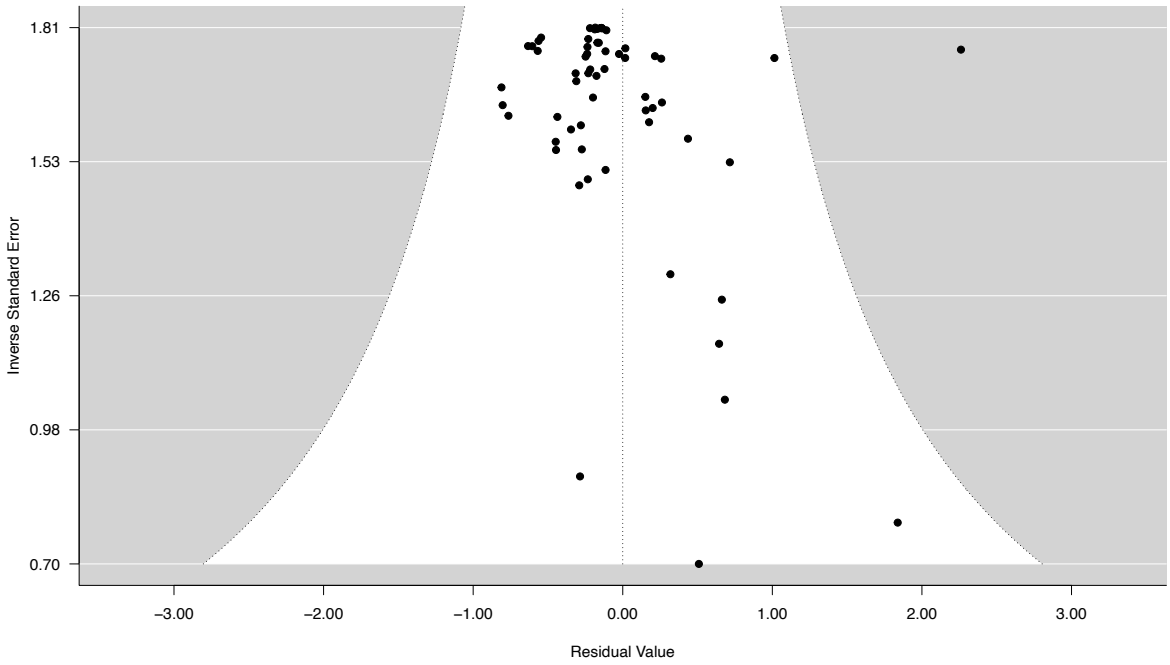

Figure S3. Funnel plot of the meta-analytic residuals against precision (1/SE) to test for publication bias. Each point represents a pair-wise temperature comparison. There is no detectable asymmetry across our samples.

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