Dear Professor Johar and Editorial Team,

We would like to thank you and the reviewers for providing extremely constructive feedback on our manuscript entitled: **Developmental environments do not affect thermal physiology in reptiles: An experimental test and meta-analysis”.** We are glad the Editor and Reviewers found the paper to be well written and of general interest.

We have now carefully considered all the comments, and revised our main manuscript and supplementary materials to deal with the comments. Below we provide a line-by-line response (in ‘blue’) to each of the comments raised by the Editor and two reviewers (in ‘black’). Where relevant, we have pasted the section of our manuscript we have edited to provide clarity to what we have done to address comments.

We believe that our revised manuscript is significantly improved. We hope that you now find it suitable for publication in *Biology Letters*.

Sincerely,

Daniel Noble (on behalf of all authors)

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Editor**

The question of how temperature during development affects the thermal physiology of reptiles is an interesting one, especially in times of climate change. Both reviewers agree on this point. However, they differ considerably in their recommendations. Reviewer 1 criticises the sample size in particular, and the authors should now be able to demonstrate that the size is sufficient for their conclusions, or they should include additional data. Since both reviewers acknowledge that the paper is very well written and deals with an interesting topic, I would recommend that the authors be given the opportunity to thoroughly revise their paper.

**Response**: While our sample sizes appear small, they are comparable to samples sizes used in empirical studies in our meta-analysis (mean n; Ctmax = 17.3, SD = 2.9; Tpref = 16.65, SD = 0.92) of the studies included in our meta-analysis, especially given that we had four treatments (n = 40 altogether). Smaller sample sizes in this area of research are not uncommon given that critical thermal limits push animals close to their physiological tolerance limits – pushing them much higher results in death. As such, there are ethical constraints that need to be carefully considered, and it is unlikely we would obtain approval for larger numbers of lizards than the number used in our study. We recognise that this may be a limitation. Our sample size was a major reason why we also included a meta-analysis of existing studies given that this can improve power (Nakagawa et al. 2017). We believe it is re-assuring the meta-analytic results completely support our empirical findings and that of other larger-scale meta-analyses in this area (e.g., Pottier et al. 2022). We have also identified clear gaps in the literature that should help pave the way for future research. We have nonetheless revised our manuscript to make explicit mention of these points (see Discussion). Please also see the responses to Reviewer 1 below.

**Reviewers' Comments to Author**:

**Referee: 1**

Comments to the Author(s)

This is a concisely written, 'hybrid' ms. Part 1 examines effects of developmental temperature on critical thermal maximum and thermal preferences of an Australian lizard. Part II is a meta-analysis of impacts of developmental temperature on reptiles. Both are of general interest, as the vast majority of "acclimation" (plasticity) studies have looked treatments involving adults, not of developmental temperatures. [Note: the main exceptions are studies examining temperature dependent sex determination and the temperature dependence of body size ("temperature size rule".] The authors conclude that developmental temperature effects have minor impacts on their study species in particular, and on reptiles in general, and thus they suggest that behavioral or evolutionary responses will more likely to enable responses to changing climates.

The basic experimental design in Pt. I is to take groups of eggs and to assign them to 1 of 4 treatments (2 temperatures, 2 yolk provisioning), then rear the individual in a common garden, and test CTmax and Tpref months later.

A different design would involve adding a post-hatching treatment. This would enable one to see whether lizards reared at low temperature perform better at low post-hatching temperature than do lizards reared at high temperature (and vice versa). This latter design informs whether the developmental response is adaptive (see Huey et al. '99). However, your treatment has the advantage in that the hatchling were able to thermoregulate as they wished and so is probably more likely reflects what would happen nature, where hatchlings can (often) thermoregulate.

**Response**: We agree that it would be a fantastic future study. However, as noted by Reviewer 1, we had a different question, and our design likely reflects what would happen in nature.

As will be evident below, I have some concerns. I was unclear whether you actually measured body temperature, which is traditional in Tpref and CTmax studies. For Tpref, you measured skin temperature but didn't show whether that matched cloacal temperature. For CTmax, I'm not sure what you measured (see below).

In any case, what matters in terms of the questions posed here is whether developmental temperatures (and egg supplies) impact "indices" of CTmax and Tpref. They do not.

One weakness is power. Each of the 4 treatments had only 10 hatchlings, which came from different dams and unknown sex. Dam and sex effects will inflate the variance, and samples of 10 limited power. The meta-analysis sample sizes are (I suspect) too few given the number of categories. I like the questions being asked, but I'm skeptical that you have sufficient power to address these questions.

**Response**: Actually, we knew the sex of animals, and this was included in our models. See our response to comments below. While our sample sizes appear small, they are comparable to samples sizes used in empirical studies in our meta-analysis (mean n; Ctmax = 17.3, SD = 2.9; Tpref = 16.65, SD = 0.92) of the studies included in our meta-analysis, especially given that we had four treatments (n = 40 altogether). Smaller sample sizes in this area of research are not uncommon given that critical thermal limits push animals close to their physiological tolerance limits – pushing them much higher results in death. As such, there are ethical constraints that need to be carefully considered, and it is unlikely we would obtain approval for larger numbers of lizards than the number used in our study. We recognise that this may be a limitation. Our limited sample size was a major reason why we also included a meta-analysis of existing studies given that this can improve power (Nakagawa et al. 2017). We believe it is re-assuring the meta-analytic results completely support our empirical findings and that of other larger-scale meta-analyses in this area (e.g., Pottier et al. 2022). We have nonetheless revised our discussion noting the sample size limitations of our study. The revised text reads as follows:

*“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 (Nakagawa et al. 2017) in individual studies (as we have done here).”*

that said, asking the question is important, as it might encourage more studies on developmental temperature effects. Towards the end of this review, I make several suggesting for you to consider -- I think highlighting these issues will help others to advance these questions.

**Response**: Thank you! We agree that these are important questions, and we hope that it will provide some guidance for future studies.

Miscellaneous suggestions:

line 49 cite Sinervo's egg manipulations here

**Response**: Thanks. We have now cited Sinervo here.

line 59 probably true for animals, but not for plants (where most research is focused on moisture, nutrients, light)

**Response**: Thanks. We have now reworded to fit reviewer’s point.

*“For vertebrates in particular, such effects may be adaptive or maladaptive depending on whether early-life environments are predictive of late-life environments”*

line 78 how many females?

**Response**: Thanks. We collected about 100 gravid females. We’ve now added this detail.

line 80 Haphazardly not randomly

**Response**: Thanks. This was done randomly, not haphazardly. However, we did ensure we had equal sample sizes across treatments. As such, if more eggs were being randomised to one treatment, we instead randomised them to other treatments. We’ve clarified that we ‘pseudo-randomly’ allocated eggs from treatments.

*“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)”*

So you had 10 eggs per treatment, correct? a better design would have been to take 4 eggs from each female and assign them to the 4 treatments -- even better would be 2 eggs to each treatment. By not including a "dam" effect in your design, you add variance associated with dams, which reduces power to detect treatment effects.

**Response**: This is correct. We had 10 eggs per treatment from different females. It is true a more precise design would be a split clutch design, but *L. delicata* lays around n = 3-4 eggs, so it would only be possible to have a partially crossed design. Given that our meta-analysis and experimental results provide the same result we do not think this design is problematic.

line 81 while it is common for researchers to use fixed temperature treatments (I've done so myself), it is far from ideal. Soil temperatures show daily cycles of temperature and often change during development. More importantly, why did you pic 23 ° and 28 °C temperatures? Has anyone monitored egg temperatures of Lampropholis in the field? As is, a reader has no a priori way of knowing whether these temperatures are at all ecologically relevant for these lizards, or perhaps are stressful.

**Response**: This is an excellent point. Thanks for raising it. We agree that fluctuating treatments are more realistic, but most studies use constant incubation treatments (See Noble et al. 2018). Interestingly, meta-analyses suggest that fluctuating temperature treatments don’t often result in major differences to constant temperature incubation regimes for many traits (See Raynal et al. 2022).

Regardless, our temperature treatments were not constant, and we have fixed this in our revision. Temperatures fluctuated +/- 3 degrees Celsius around 23 and 28 C. We have now added this in our revised manuscript (revised text: “…*an incubation temperature (23°C or 28°C SD 3.0) treatment”*).

With respect to our choice of temperatures. Egg temperatures have been monitored in natural nests of *L delicata* (see Cheetham et al. 2011). Our choice of incubation temperatures are at the extremes of the natural temperatures. We have now cited this paper and made this point clearer in our revised manuscript.

*“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]”*

If you used a large number of developmental temperatures, the extreme would be pathological, and some temperature range would be optimal for physiology. But we don't know whether 23 and 28 are within that range, or whether 28 °C is in fact stressful.

**Response**: Please see our response above. These temperatures are at the extremes of naturally occurring nest temperatures. We don’t believe our treatments were pathological as we did have high hatching success (~90%) across our treatments.

line 85 Were groups from mixed treatments, or single treatments? If the latter, then you have a "group" effect (i.e., the treatment and post treatment environments are confounded.

Also, group housing can lead to social dominance effects.

Regal, P. J. (1971). Long term studies with operant condition techniques of temperature regulation studies in reptiles. Journal de Physiologie, 63, 403-406.

**Response**: Thanks. No, animals from different treatment groups were mixed which does not confound treatment and post treatment environment. We have now clarified this point in our revision as follows:

*“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.”*

line 89 Was the order of measurement independent of treatment group? I hope so.

**Response**: Yes. We ensured the order was random and not confounded with treatment. We have revised as follows:

*“At eight to eleven months post-hatching, lizards were selected at random, and thermal traits (CTmax and Tpref) measured.”*

line 104 I suspect some reviewers will object to this wide 'phylogenetic' grouping. Also, were only 15 species represented? If so, you have very few species per taxon, suggesting limited phylogenetic power.

**Response**: We believe it depends on the question. The wide phylogenetic grouping is not a problem if one wishes to make inferences to non-avian reptiles, which was our goal. It is true that we may have lacked power to detect a phylogenetic signal, but equally, it may not really exist as there is no *a priori* reason to believe that the pattern of developmental plasticity in thermal physiology should be evolutionarily constrained (it’s likely more relevant at the population-level). We believe that it is still important to at least test, and attempt to control for, phylogenetic non-independence (See Cinar et al. 2022; Sakamoto & Vendittie 2018).

line 114 define ARR

**Response**: The definition has now been provided.

*“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”*

line 115 what is "study"? does that mean some papers reported on more than 1 species?

**Response**: This is correct, some studies had multiple species or studies often had more than two temperature treatments.

*“*Studies often had more than two temperature treatments. As such, we derived all pairwise effect size comparisons”

line 126 Good to have checked for publication bias. (syntax bad here -- publication bias wasn't using a funnel plot.)

Note: I don't do meta-analyses so I can't comment on the methods, but the text conveys the impression that the authors were careful here.

**Response**: Thank you! Yes, we have tried to conduct the analysis as carefully as possible.

line 134 So none of your hatchlings died? That's a good sign that rearing conditions were tolerable.

**Response**: Hatching mortality was very low. See comments above (<10%).

line 136 Personally, I would like some mention of methods of CTmax and Tpref in the text. There's big debate on CTmax methods, and a reader should not have to find the Supplement to learn the basics of your methods. With Tpref, you probably had multiple measurements/individual, but did you analyze means/medians for each individual or did you pool all measurements?

**Response**: We agree. We have now provided more details in the methods and our definition for each thermal trait in the methods section of the actual manuscript. The section now reads as follows:

*“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 Tpref. A FLIR T640 thermal camera was used to take thermal images of all lanes every 15-minutes over an eight-hour observation period. Tpref 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 CTmax we followed the same fasting period used for Tpref 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 CTmax procedure [30]. CTmax was defined as the temperature at which an individual lost their righting reflex (for further details in collection methods, see Supp.).”*

Fig. 1 suggests no obvious treatment effect, and the statistics support that. But you have very limited power (only 10/treatment). Incidentally, why didn't you include sex in your statistical model? Can you sex hatchlings?

**Response**: We did include sex in our model. Please see Table 1 and line 107 and supplement. Sex was not significant.

line 147

Fig. 2 what does "overall" mean"? Fig. 2D shows that sample sizes are small-- 2 turtles, 2 snakes, 10 lizards. Fig. 2C shows that you have 1 species of tropical something. I realize that that is what is available, but my feeling is that any conclusions from this meta-analysis are premature. You just have no good sampling (not your fault) and no power. The utility of a meta-analysis here is to highlight the paucity of information

**Response**: Thanks for catching that. We have revised the figure 2 caption. Reviewer 2 is correct that these are all the studies out there currently that met our inclusion criteria. However, the meta-analysis is based on >600 individuals across the treatments, so we don’t agree that we have ‘no power’. This approach is still more superior than a single empirical study on its own.

line 153 What about behavior? In any case genetic adaptation and plasticity need not be competing.

**Response**: We agree. We have made it clear that behaviour is important in the discussion:

*“Overall, our results suggest that most reptiles may have limited developmental plasticity in thermal traits, relying instead on energetically expensive behaviours (i.e. thermoregulation) [3,48] or responses that operate on slower time scales (i.e. local adaptation)”*

We have also removed the word ‘competing’.

line 155 On "anticipatory" see Padilla and Adolph

**Response**: We have removed “anticipatory”.

line 162 I think you are overstating the pattern here, as studies with Drosophila (papers by Gilchrist, Crill, Huey, and others) have shown effects of developmental temperature on heat tolerance and other traits. The MacLean study used flies from stock centers, which have been maintained (often for decades) at fixed temperatures. I'm skeptical of their results, though obviously they are note.

**Response**: Thanks for pointing this out. We were unaware MacLean et al., flies were from stock centres. The *Drosophila* reference, MacLean et al., has now been removed from our statement. The other studies mentioned (i.e., Gunderson, Deutsch, and Pottier et al. ), one being a meta-analysis, explicitly show adults had limited plasticity.

Seems to me that you need to add a paragraph discussing the limitations of your approach. First, you apparently know nothing about developmental environments of these eggs in nature, so your treatments may not be ecologically relevant. Basic natural history information is always needed (though almost always absent!). But you can encourage it. You hint at this in lines 169-170, but you don't concede ignorance of nest conditions for your species. Second, fixed temperatures in particular may yield misleading results -- there is a growing literature on the impacts of fluctuating temperatures. Third, power -- you have limited power in your experiments and in your meta-analyses. Call attention to this as it might encourage more studies of developmental effects.

**Response**: As indicated above, we do know natural nest conditions of eggs in nature. We know our manipulations are ecologically relevant. Natural history information of selected treatments for *L. delicata* has now been provided.

Reviewer 1 is correct that there is a growing literature on fluctuating developmental environments versus constant. A meta-analysis on this very topic in reptiles shows it can impact incubation duration, and hatching success but there is little evidence that they result in greatly different effects for many traits (See Raynal et al. 2022).

We have now called greater attention to the power issue raised. Please see our responses to comments above.

line 176 Janzen's classic paper ('67 Am Nat) should b cited here

**Response**: Great suggestion. This reference has been added here.

line 173 Interesting suggestion. I don't recall any studies that have examined this.

**Response**: Thanks! We’re glad Reviewer 1 found this interesting.

\*\*\*\*\*\*\*\*\*

Supplement

line 8 Tpref is NOT the optimal body temperature. See Martin, T. L., & Huey, R. B. (2008). Why suboptimal is optimal: Jensen's inequality and ectotherm thermal preferences. American Naturalist, 171, E102-E118.

**Response**: Corrected in the supplement.

*“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 Tpref 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 Tpref trials. Lampropholis delicata body temperatures were extracted from infrared images using Flir Tools, version 5.13.”*

line 19 Check for consistency over the 8-hours. Brattstrom (decades ago) found (if I remember correctly) that lizards often take some time in a gradient before they "settle in".

You measured dorsal skin temperature, not body temperature. Did you ever check whether skin temperature = cloacal temperature? These are moderately large lizards (8 to 10 cm), so skin temperature may be different from cloacal.

**Response**: Actually, these are small lizards. Mean mass at measurement was only 1.3g (~4cm snout to vent length). While it is possible that skin temperature differs from body temperature lizards of this size are expected to equilibrate with environmental temperatures quickly (i.e. heat/cool rapidly given the environmental temperature; *See* Garrick 2008 on comparison Tsurf and Tb estimations for multiple species of small lizards). We have revised our manuscript to be more explicit about this and provide better justification where we discuss the methods in more details. The revised section reads as follows:

“*A FLIR T640 thermal camera was used to take thermal images of all lanes every 15-minutes over an eight-hour observation period. Tpref 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].”*

We have also added these details to the supplement.

line 27 are you sure they reached 30 °C? what is the time constant for a lizard of this size?

**Response**: No, we are not 100% certain they reached this temperature because we avoided opening Falcon tubes when animals were placed in the water bath. We have revised to be more forthcoming on this point:

*“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 Tb 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.”*

line 34 Suggests that you used water temperature as index of body temperature. But with a fast heating rate, Tb might lag, especially in relatively large individuals. You can easily test this -- assuming you have access to some lizards.

**Response**: We used Tair as an index of body temperature (well, more precisely skin temperature, as indicated in the comments above and below). It is possible Tb lags. This would suggest our CTmax measurements are underestimated. Importantly, however, we do not expect these effects to vary systematically with developmental treatment because of their small size and lack of size differences in animals across treatments (see below). We have now revised our text here to make this explicit.

*“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 Tb for many small lizards (Garrick 2008). It is possible Tb lagged behind for our measurements. Any lag would result in an underestimated CTmax, which is likely the case for most studies measuring CTmax in lizards given the ethical challenges with pushing animals to thermal extremes (e.g., Phillips et al., 2016; Llewelyn et al., 2016; Claunch et al., 2021). 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 CTmax between treatments.”*

From Fig. S1, I'll guess the "operative" temperature inside the tube reflects water temperature and also air temperature (tube + air circulating from above the water).

Sorry I'm confused as the legend is ambiguous. Did you put a thermocouple probe inside the Falcon tube or inside a lizard in the Falcon tube? I've been assuming you were using Twater as your estimate of CTmax, but not I'm not sure what you measured. Sorry if I missed an explicit statement.

**Response**: Thank you. This diagram was indeed unclear. We have now revised our wording and removed the figure to avoid further confusion. The thermocouple probe was inside a control falcon tube so that we were measuring Tair in the tube. However, we also had a thermal couple in the water bath itself. These temperatures were nearly identical, but we used Tair as we expected it to more accurately measure lizard temperature.

“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”

Even if you used Twater (or Tair inside the tube) as your metric, that should be ok for detecting developmental temperature effects, unless the size range of lizards was substantial. The temperature lag of a large lizard might be substantial.

but if you did not actually measure Tb, then I strongly encourage you not to call this CTmax, as you did not measure body temperature.

**Response**: We used Tair inside the tube as indicated above. The size range of the animals was minimal because they were all the same age (average mass = 1.3 g, SD = 0.27 g). Importantly, the mass of animals across the treatments did not differ (see Table below), so there should not be a body size effect on heating rate across the treatments. Our methods for measuring “CTmax” follow much of the literature on lizards (See Llewelyn et al., 2016; Phillips et al., 2016). As such, we have opted to keep it as CTmax but we have been clear that not measuring body temperature

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Mean** | **SD** |
| A-23 | 1.24 | 0.289 |
| A-28 | 1.34 | 0.363 |
| C-23 | 1.31 | 0.286 |
| C-28 | 1.27 | 0.193 |

Has anyone measured CTmax of Lampropholis the traditional way? Check Spellerberg's old papers, Greers, and John-Alder and Bennett. And check GlobTherm.

**Response**: No, not that we have found.

Inconsistent capitalization of titles in Literature cited.

**Response**: Thanks. We should have fixed all these inconsistencies now.

Some relevant references:

Cheetham, E., J. Sean Doody, B. Stewart, and P. Harlow. "Embryonic mortality as a cost of communal nesting in the delicate skink." *Journal of Zoology* 283, no. 4 (2011): 234-242.

Garrick, D. (2008). Body surface temperature and length in relation to the thermal biology of lizards. *Bioscience Horizons*, *1*(2), 136-142.

Huey, R. B., D. Berrigan, G. W. Gilchrist, and J. C. Herron. (1999). Testing the adaptive significance of acclimation: a strong inference approach. American Zoologist 39:323-336.

Nakagawa, S., Noble, D. W., Senior, A. M., & Lagisz, M. (2017). Meta-evaluation of meta-analysis: ten appraisal questions for biologists. *BMC biology*, *15*(1), 1-14.

Noble, D. W., Stenhouse, V., & Schwanz, L. E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: A systematic review and meta‐analysis. *Biological Reviews*, *93*(1), 72-97.

Padilla, D. K., & Adolph, S. C. (1996). Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. Evol. Ecol., 10, 105-117.

Pottier, P., Burke, S., Zhang, R. Y., Noble, D. W., Schwanz, L. E., Drobniak, S. M., & Nakagawa, S. (2022). Developmental plasticity in thermal tolerance: Ontogenetic variation, persistence, and future directions. *Ecology Letters*, *25*(10), 2245-2268

Raynal, R. S., Noble, D. W., Riley, J. L., Senior, A. M., Warner, D. A., While, G. M., & Schwanz, L. E. (2022). Impact of fluctuating developmental temperatures on phenotypic traits in reptiles: a meta-analysis. *Journal of Experimental Biology*, *225*

Paranjpe, D. A., Bastiaans, E., Patten, A., Cooper, R. D., & Sinervo, B. (2013). Evidence of maternal effects on temperature preference in side-blotched lizards: implications for evolutionary response to climate change. Ecology and Evolution, 3, 1977-1991.

Sakamoto, M., & Venditti, C. (2018). Phylogenetic non-independence in rates of trait evolution. *Biology letters*, *14*(10), 20180502

**Referee: 2**

Comments to the Author(s)

The authors used a two-pronged approach to investigate whether developmental conditions influence the thermal physiology of reptiles. First, using the delicate skink (Lampropholis delicata), they conducted a lab experiment to determine whether developmental temperature and maternal resource investment (via egg yolk reduction) influenced two thermal traits, CTmax and thermal preference. They found that it didn’t. They then did a meta-analysis more broadly for reptiles, to see whether developmental temperature influences thermal physiology. Their results suggest that there is limited developmental plasticity in these thermal traits in reptiles.

Overall, the study is of great interest to the readership of Biology Letters. It is extremely well-written, and analyses were outlined in detail and were appropriate for the questions being addressed. However, I do have one key question regarding the study….why focus on CT max, rather than CT min? CTmax has been show to be evolutionarily conservative in ectotherms, without much response to selection or plasticity. Conversely, CTmin is more evolutionarily labile, and the authors may have been more likely to observe a response in this trait. Perhaps it would be beneficial to explain the choice of CTmax for this study.

**Response**: Great question. Actually, we did set out to find studies measuring CTmin as well. Please see our search string in the supplement. However, very few studies do developmental manipulations and subsequently measure cold tolerance. Hence, this is the reason why we focused on CTmax/Tpref. We have now clarified this in our revised MS:

*“Our search string included cold tolerance (i.e., critical thermal minimum, CTmin), 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 Tpref and CTmax”*

In addition, I have outlined a series of more minor comments that the authors should address in their revision.

Minor comments

1. Lines 71-72. Please provide the common name for Lampropholis delicata as well.

**Response**: This detail has been provided in the methods section.

*“We collected gravid Lampropholis delicata (common garden skink, n = 100)*”

2. Lines 78-80. It would be better to refer the reader to the supplementary methods here, rather than in Line 84.

**Response**: This has been done to fit the reviewer's suggestion.

3. Lines 78-80. Is there a reference that you can provide that outlines the housing conditions that the females were kept in prior to oviposition? If not, it would be great to add this information to the supplementary methods. Likewise for the egg incubation details.

**Response**: Yes, a citation for husbandry and egg incubation conditions has been added.

*“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]). “*

4. Lines 134-136. This is repetition from the methods.

**Response**: Thanks. This redundancy has been removed.

5. Line 137. A Tpref of 20.99 C seems unusually low for the species. Was this a valid measurement, or was there some issue with this individual(s) during the experiment?

**Response**: We agree with Reviewer 2 that this outlier seems biologically low for the species and was in fact a point we checked prior to submission. We have thoroughly interrogated the raw data (i.e., pictures from thermal imaging camera), but we are convinced that this is correct. We also checked our data to see if this individual had any issues prior to measurement, but there was nothing noted. We also checked if individuals collected that measurement day demonstrated low Tpref’s. They did not. We also checked whether it was a common issue with that specific lane, but all other individuals performed fine. We are not sure why this specific individual has such a low Tpref, but this is the datum!

6. Lines 153-156. This paragraph is repetition. It would be better to focus on your key results, and how they address these competing hypotheses.

**Response**: We have summarised the two hypotheses of how ectotherms are expected to be impacted by warming temperatures. We then address how each of these hypotheses are addressed with our data in subsequent paragraphs in the discussion.

*“*We show that early developmental environments do little to modify thermal physiological traits (CTmax & Tpref) 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”

Editorial office comments to authors:

Please ensure that you include;

\*A description of your dataset in the Data Accessibility section as described on our website: https://royalsocietypublishing.org/rsbl/for-authors#question4

**Response**: We have uploaded all raw data, descriptions of metadata, model outputs, scripts and code to Zenodo (<https://doi.org/10.5281/zenodo.7700383>). There you will find a README file that describes a dataset along with the raw files in the same location as described on your website.

\*Due to its lack of permanence, we do ask that datasets of soon to be accepted articles are uploaded to other repositories other than GitHub, such as Dryad or figshare. Please upload your data into another repository and amend your data section accordingly to reflect this.

**Response**: Thanks. We have loaded the data up to Zenodo (<https://doi.org/10.5281/zenodo.7700383>).

\*Please also ensure that your data is cited in your reference list as described here https://royalsociety.org/journals/ethics-policies/data-sharing-mining/

**Response**: Thanks. This has now been cited as reference 55.

\*As long as they are accurate, your Ethics, Competing Interests, Data, and Author Contributions sections should be removed from your main manuscript and added to ScholarOne in the relevant sections instead (Step 6 in ScholarOne)

**Response**: Thanks. Removed.

\*Please also confirm in your cover letter whether all the figures are your own or whether permission has been obtained for their use

\*If you have any images that can be used to promote your article on social media (should this be accepted) please upload them as a supplementary file

\*Please do ensure that your supplementary files themselves include the title and authors of your main manuscript, and that you also provide an appropriate title and description for each of these on ScholarOne

\*Please upload a version with the changes clearly marked as a supplementary file

\*Please confirm whether your manuscript should be available via open access, as we note that this may be a requirement of your funder. Please also check if your institution has a Read & Publish deal by visiting our website here: https://royalsociety.org/journals/authors/read-and-publish/read-publish-agreements/

\*Further tips on how to approach revisions that you may find useful can be found on our blog post here: https://royalsociety.org/blog/2021/07/responding-to-decision-letters-tips/