EDITOR'S COMMENTS  
  
This study examined the combined effects of prenatal corticosterone (CORT) and incubation temperature on associative learning in two lizard species. Both reviewers agree that the manuscript presents a very interesting and well-designed study, is well written, and sample size is very impressive. One reviewer points out that the timing of the CORT treatments and temperature effects should be addressed as possible confounding factors in the discussion. I am therefore happy to already provide provisional acceptance pending these final revisions. Please carefully revise and comment on all points raised by the reviewers.   
Looking forward to receiving the revised version of the manuscript!

**Response**: Thank you very much for your feedback and for the opportunity to improve our manuscript. We appreciate the time and effort that the reviewers have put into evaluating our work and providing valuable feedback.

In response to their comments, we have made significant revisions throughout the manuscript. We have acknowledged some of the limitations pointed out by Reviewer 2 – alas, that CORT elevations at earlier developmental stages might lead to different outcomes. We have also expanded the introduction to include information on the implications of incubation temperature for CORT levels. While this could be considered a confounding factor, previous research indicates that baseline CORT levels in *L. delicata* increased with identical CORT doses regardless of incubation temperature (Crino et al. 2024, *Journal of Experimental Biology*).

Finally, we have carefully addressed each of the remaining points in detail (see below).

Dr. Eva Ringler  
Editor, Behavioral Ecology  
  
Formatting requirements:  
  
1.  The file of the revised text should contain all parts of the manuscript (including the full cover page, acknowledgements and tables) EXCEPT for the figures.  It should be uploaded either as a Word .doc file or .rtf file and be designated as 'Version for Press'.  
  
2.  Each figure should be submitted as a separate file (i.e. one file per figure) that is a high resolution .tif or .eps file (please see the instructions to authors located at: <http://www.oxfordjournals.org/beheco/for_authors/submission_online.html>, item 3).  If necessary, therefore, figures should be prepared anew using higher resolution graphics than for the reviewed manuscript.  Each should be resubmitted along with the text.  IMPORTANT: no manuscript can receive acceptance unless the figures are provided in the requested format.  
  
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4.'Behavioral Ecology' has a color cover, and so we strongly encourage you to submit high-quality (above 400 dots per inch) photographs for possible use as a cover illustration. Photos must be submitted in color. Please provide a brief caption and include a credit for the author. Please upload both to your manuscript as a supplementary file via the "Manuscripts Accepted for First Look" tab in your Author Centre on ScholarOne.

REVIEWERS' COMMENTS  
  
Reviewer: 1  
  
Comments to the Author  
Overall paper was well written and the experiment was well done, providing good data on the impact of egg CORT and temperature on lizard behavior and cognition. The authors did a nice job presenting the concepts and making sure there was a good logical flow. While the authors do a great in the discussion bringing up important considerations on variables that could be leading to the difference in effecs from what was predicted, there are 2 concepts that would strengthen the paper. Discussion on the timing of doses (ie prelay vs the postlay) and the likelihood that increasing temperature increases CORT would not only help round out the paper, it would also cover 2 large considerations into the measurements and lack of effects found here.    
  
Line 97-104 This (or 116-121) may be a good spot to bring up and discuss that in ectotherms, at least adult lizards, increased temperature also increases GC. You do bring up temperature effecting GC, but only for decreasing, not increasing the effect (Rubalcaba, J. G., & Jimeno, B. (2022). Body temperature and activity patterns modulate glucocorticoid levels across lizard species: A macrophysiological approach. Frontiers in Ecology and Evolution, 10, 1032083.). So is there a difference between temperature induced GC increase or other sources of GCs?

**Response**: We really appreciate the reviewer’s feedback. It is true that temperature elevations can increase GCs production. However, we found the opposite pattern on a previous study (Crino et al. 2024, *Journal of Experimental Biology*). Nevertheless, we have included some information in this regard on lines 143-148: “In contrast, glucocorticoids in endotherms are associated with increased energy demands (e.g., Rubalcaba and Jimeno, 2022), which could lead to higher CORT production in lizards incubated at warmer temperatures. However, previous research on *L. delicata* (Crino et al., 2024) has found that cold-incubated lizards had higher baseline CORT levels, suggesting that cooler incubation temperatures may increase the potential effects of CORT exposure.”

Line 142: Should Latwit be labwit?

**Response**: Yes, we have changed this error.

Line 227: Were you able to ensure that the measurer and trial conductor were blind to the treatment of the lizards?

**Response**: Thanks for the observation. Yes, the person running the trials and making all the analyses was blind to the treatments. We have included that information now: “All the videos were analysed by the same observer (PR) who was blind to the treatment of the lizards.” (Lines 247, 248).  
  
Line 361 Can you exclude the possibility that part of the reason effects were not seen is that temperature increased CORT to the same level as your CORT dose? Do you have measurements on the CORT values from each treatment?

**Response**: Thank you for this comment. While we did not measure CORT levels in this experiment, previous research (Crino et al., 2024, *Journal of Experimental Biology*) indicates that lower temperatures increased baseline CORT levels in *Lampropholis delicata* under identical incubation conditions. However, this study also found significant differences in baseline CORT levels between control and high-dose groups (the dose employed in our experiment), regardless of incubation temperature. Additionally, if temperature had elevated CORT to the same level as our administered dose, we would expect to see differences within treatments incubated at the same temperature – at least for the hot-incubated lizards - which we did not observe.

Line 413: While you did a great job showing no effects here, do you think that could be influenced by the timing of your CORT treatment (ie post laying vs pre laying)? Do you think dosing the mothers before the laying and therefore at an earlier developmental stage could have different effects, or is there evidence that this would not be the case?

**Response**: Thank you. We agree that exposure to elevated glucocorticoids at different stages of development can lead to varying effects. We chose to dose eggs instead of mothers to better isolate the effect of CORT, as dosing control mothers could trigger the stress response. Additionally, we consider that dosing the eggs at the beginning of the incubation process mimic maternal transmission during early stages. However, we cannot rule out the possibility that exposing eggs at different stages could change our results. We added the following sentence to address this: “Similarly, exposing embryos at different stages of development could impact the effects of CORT. However, we cannot exclude the possibility that concentrations different from the one used here or exposure at different developmental times does affect learning.” (Lines 389-392)

Reviewer: 2  
  
Comments to the Author  
An increasing number of studies are investigating the causes and consequences of individual variation in cognition, however, relatively few studies have attempted to determine the factors influencing cognitive development. In ectotherms, environmental factors like temperature influence growth, metabolism, and learning. Elevated glucocorticoids (GCs) – stress hormones transmitted from mothers to offspring – may also impact learning. This study examined the combined effects of prenatal corticosterone (CORT) and incubation temperature on associative learning in two lizard species. Despite predictions that high CORT and low temperatures would impair learning, results showed no significant differences. However, both species exhibited a non-learned preference for blue, highlighting the importance of colour selection in cognitive experiments.  
  
This is an elegantly designed study, the sample size is very impressive, and the data are robustly and appropriately analysed. The discussion is comprehensive and does a great job of considering all interpretations. Crucially, I think this study represents an important addition to the field, where studies investigating the factors influencing cognitive development are rare. Accordingly, I only have a handful of very minor comments (please see below). I would like to stress that the brevity of this review is a positive reflection of the manuscript – I found the manuscript to be extremely well written, and it was a pleasure to read.    
  
Minor comments  
L175 – what was the rationale for the temperatures used in the cold and hot temperature regimes? Were these ecologically relevant temperatures where, if there was an effect of temperature on cognitive development, this manipulation would likely capture it?

**Response**: Thank you very much for your comment. The rationale behind our thermal conditions is that they represent the upper and lower limit of the nesting temperatures in the wild. We have included that information in the text: “These temperatures represent the upper and lower limit of the natural incubation temperatures (Qualls and Shine 2000; Cheetam et al. 2011).” (Lines 212, 213)

L379 – can you put these in context – what kind of temperature manipulations did these other studies do? Comparable to yours?

**Response**: Thank you for this comment. We have clarified that these studies were conducted on different species and are therefore not directly comparable. However, in studies where hot-incubated lizards outperformed cold-incubated ones, incubation temperatures remained within the species' natural nesting range—similar to our study. In contrast, when incubation temperatures exceeded natural limits, hot-incubated lizards performed worse than their cold-incubated counterparts.We have included all this information now: “We predicted hot-incubated lizards would perform better in the associative learning task, since most studies in other species demonstrate enhanced learning abilities when eggs are incubated at higher temperatures (Amiel and Shine 2012; Amiel et al. 2014; Clark et al. 2014). These studies employed incubation temperatures within natural nesting thermal limits. In contrast, in those studies where cold-incubated lizards outperformed hot incubated ones, the incubation temperatures employed in the hot condition were far above the natural thermal range of the species (Dayananda and Webb 2017; Abayarathna and Webb 2020).” (Lines 408-414)

L396 – might the bias towards blue be the result of the other ramp being red? Is it possible that red is associated with aposematism, and therefore there was a tendency for individuals to avoid that ramp?

**Response**: We appreciate the reviewer’s feedback. We found this possibility less likely because our analyses about the first choice that lizards chose the red ramps about 30% of the time, which is very close to random choice. If there was a tendency to avoid that ramp, we would expect choice to be lower than 30% of the cases.

L412 – can you provide a bit more information about the two species’ life history and ecology here? For example, social structure has been found to be an important predictor of interspecific differences in cognitive performance and neuroanatomy. In addition, foraging ecology (e.g. folivory vs frugivory in primates), has also been found to be an important predictor in interspecific differences in cognition. How does the ecology and social structure of these two species compare? If similar, it might not be particularly surprising that they perform similarly.

**Response**: Thank you very much for your comment. Both species occupy similar habitats and have similar life history and ecology. However, *L. delicata* is more successful as an invasive species, which could be related to differences in cognitive abilities. We have included some information about their similarities and differences and the implications of our findings in this context: “Since both species occupy similar habitats and have similar ecology (Chapple et al. 2011; 2013; 2014), it may not be surprising that cognitive abilities are similar between both species. Nonetheless, previous studies have shown that *L. guichenoti* is less prone to explore novel environments than *L. delicata*, which may be related to the success of *L. delicata* as an invasive species compared to *L. guichenoti* (Chapple et al., 2011). Our results show that the ability of *L. delicata* to colonize new areas seems not to be related to learning abilities.” (Lines 445-451)