Dear Dr. Bakker,

Thank you for the opportunity to resubmit our manuscript titled “Do early environmental experiences impact numerical discrimination abilities in a lizard?” Manuscript ID: BEAS-S-24-00429. We appreciate the time and effort that the reviewers have put into evaluating our work and providing valuable feedback.

We have made significant revisions to the manuscript in response to the reviewers’ concerns, and addressed each point raised in detail. We believe these changes have improved the overall quality and clarity of the paper. Below, we provide a summary of the revisions made, and we hope that the updated manuscript meets the standards for acceptance.

We are looking forward to your feedback and hope that our revised submission will be considered for resubmission.

Thank you for your time and consideration.

Pablo Recio

COMMENTS FOR THE AUTHOR:  
  
Associate Editor:  
I have finally, after inviting numerous reviewers, been able to find two expert reviewers to provide comment on this manuscript. One of them states “reject” the other “reject but resubmission possible”. I will therefore provide the authors with the possibility to submit a revised version of the manuscript where all the comments provided by the reviewers are adequately addressed. If I find the revised version of the manuscript has accomplished this, it MIGHT become suitable for publication in BEAS.  
Thomas Madsen  
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**Response**: We would like to thank the Associate Editor for allowing us to re-submit our paper. We have now carefully considered the excellent comments from each of the reviewers and made changes to our manuscript to provide clarity around our findings and questions.

Reviewer #2:   
After carefully reading this manuscript, I regret to say that the experimental results of this study, although quite interesting, are still far from providing publishable data. Although the researchers have carefully designed the experiment and clearly clarify the study's structure, it appears that none of the treatment conditions allowed this lizard species to exhibit numerical discrimination ability under the current experimental design.  
  
I acknowledge the significance of the researchers' experimental design, particularly the investigation of glucocorticoid concentrations and incubation temperature on juvenile lizards, which are academically important and worthy of study. However, the entire paper is structured around comparing their numerical discrimination ability across different experimental treatments. However, since none of the four experimental groups successfully demonstrated the expected behavior, it is difficult to compare an effect that does not exist. Therefore, I sincerely recommend that the authors first refine their experimental design until the animals can reliably exhibit the target behavior before drawing conclusions. Otherwise, despite various controls over experimental conditions, it would be challenging to determine the actual effects of these variables.

**Response**: We appreciate the reviewer’s feedback. In the revised manuscript, we have restructured the introduction to clearly indicate that the primary research question pertains to numerical abilities in *Lampropholis guichenoti*, with the influence of early environmental conditions as a secondary focus. Additionally, we have expanded upon the rationale behind the experimental design choices, illustrating how the current approach still allows for exploration of the potential effects of early-life conditions on cognitive outcomes.

Additionally, I have a few specific concerns:  
  
1. The researchers attempted to alter the placement of cricket food items to standardize object area or length. However, under the current experimental conditions, I believe such control is premature. It remains unknown what cues lizards use to perceive numerical information—whether it is occupied space, area, length, or other visual cues. Before confirming that they indeed possess numerical discrimination ability, introducing a spatial illusion to confuse the animals may not achieve the intended effect.

**Response**: Thank you for this comment. Controlling for non-numerical cues such as length is a standard practice in numerical cognition research to ensure that number is the distinguishing factor (reviewed in Agrillo and Bisazza 2014, *Journal of neuroscience methods*). Our adjustments were not intended to introduce a spatial illusion, but rather to prevent lizards from using total length as the main cue driving their choices. In this regard, other studies use prey of different size (which was not possible in our experiment), or increased the distance between items in the choice with fewer items (see Stancher et al. 2015, *Animal Cognition*). However, the latter option may change the way animals perceive the total area (see Stancher et al. 2015, *Animal Cognition*), which we try to control by rotating the crickets. Results from these analyses show that we could not control for length in the 1 VS 4 and 1 VS 3 trials, but the length difference between the smaller and the larger choices was not significantly different from zero in the other three tests. We have also integrated our approach within the context of the object file system (OFS), as suggested by another reviewer, which could explain better the rationale behind our design.

We argue that the concern around not yet knowing which cues lizards rely on does not invalidate our approach; rather, it underscores the importance of controlling for potential confounds. If lizards primarily rely on non-numerical traits, this would be reflected in their choice patterns. Previous research has shown that other skinks rely exclusively on the OFS (Szabo et al 2021, *Behavioral Ecology*), which is why we chose to use this design over testing the Approximate Number System (ANS).

2. Furthermore, how exactly was this placement manipulation expected to achieve the described effect? The authors did not clearly explain this in the manuscript (Lines 210-218).

**Response**: Thank you. We agree that we were not clear in explaining and justifying our design in our previous manuscript. The intention of our manipulation was to control for length while minimising the effects of increasing the distance between items (in options with more than one) as that could affect the perceived total area (Stancher et al. 2015, *Animal Cognition*). The intention, therefore, was to base our experiment exclusively in the OFS (see also above). We have included new data and more information in the Introduction, Methods and the Supplementary Material to provide clarity and support to our reasoning and the choice of our assay.  
  
3. Line 318: "the relevant stimuli involved either vegetable or large quantities." This sentence seems not precise. Based on my understanding of these species, being herbivorous or preferring larger food items is unlikely to be the key determinant of numerical discrimination ability. Carnivorous animals are also known to possess numerical abilities. Improving the experimental design may be more critical than focusing on whether the animals are herbivorous or carnivorous.  
  
**Response**: We appreciate the reviewer’s concern but would like to clarify that we are not suggesting diet determines numerical discrimination ability. Rather, we are considering whether foraging strategies might shape spontaneous numerical preferences. In fact, insectivorous lizards have demonstrated strong numerical discrimination skills in learning paradigms (e.g., Miletto Petrazzini et al. 2018, *Frontiers in Psychology)*. However, the same species has also been shown to perform poorly in spontaneous choice tasks (Miletto Petrazzini et al. 2017, *Biology Letters*). Our argument, which aligns with the conclusions of Miletto Petrazzini et al. (2017, *Biology Letters*), is that diet may not influence numerical ability but could influence decision-making strategies.

For an insectivorous lizard, foraging success may not be significantly different when choosing between a single prey item versus multiple ones, at least when numbers below than 4 are compared. This differs from species that rely on larger or aggregated food resources, for which numerical evaluation may play a more crucial role in optimizing foraging decisions. Thus, our argument is not that insectivores lack numerical ability, but rather that their foraging ecology may not drive strong spontaneous numerical preferences in the context of small prey numbers.

We have clarified this in the resubmitted manuscript: “As a result, even if diet might not affect quantity discrimination abilities, insectivorous lizards might not have evolved a preference for larger patches of prey, at least with small numbers” (Lines 347-350).

Reviewer #3:   
This paper is an important contribution because it is the first study that I am aware of that tackles the combined effect of both cort and temperature on cognitive ability, in this case quantity discrimination. the authors use the garden skink (Lampropholis guichenoti) as a model system, which is entirely appropriate. Overall, the paper is well-written and certainly well analysed. Having said that, the introduction fell a little short when it came to describing the two core systems (see below) used for quantity discrimination. This is an issue that is easily addressed. However, it is perhaps a prelude to an unorthodox method for measuring quantity discrimination. I going to this in more detail below (see comments about Fig. 1). The authors themselves raise this as a potential issue in the discussion. I guess the key question here is to what degree their design addresses the role of court and temp on a measure of cognitive ability - quantity discrimination. Added to this is the issue that the authors don't find any quantity discrimination ability, which makes testing for the role of cort and temperature slightly problematic. I can't help thinking they likely have some QD ability that just wasn't uncovered using their design but I could certainly be wrong.

**Response**: We thank the reviewer for their valuable comments and insights. Their suggestions have been extremely helpful in refining our manuscript. We acknowledge that our design needed more clarification and justification, which we have now provided in the Introduction, Methods, and Supplementary Material (see replies below). We also agree with both reviewers that exploring the effects of the early environment on numerical discrimination in this species may be somewhat premature, given the lack of prior knowledge on this topic. However, we still believe it is an important and unexplored question. Nonetheless, we believe that our study provides useful information on how early environmental factors influence decision-making in *L. guichenoti*. As such, we have restructured our paper to position this question as a secondary focus and have clearly acknowledged that our design does not allow us to answer whether the early environment affects cognitive abilities in the numerical domain but still provides important information on how it impacts decision making more generally.  
  
Title: I would probably have gone with "Does early environmental experience impact quantity discrimination ability in a lizard?"

**Response**: Thanks. Given this comment and others we have changed out title to: “Quantity discrimination, decision making, and the role of early-life conditions in a lizard”.   
  
22.     Again, I think "quantity discrimination" is more appropriate than quantitative abilities. For example, on a very elementary level an animal might simply be able to tell whether one quantity is larger than another. In this case, they are not really doing any form of mathematics or quantification. Bear this in mind for the rest of the manuscript, I will not highlight any other instances.

**Response**: Thanks. We agree. We have now modified the concept of quantitative abilities in the text.  
  
Abstract. 33. Yes, and were you examining numerical vs size discrimination or both?

**Response**: We examined numbers when controlling by continuous cues like length, but we did not evaluate size, just different numbers of items. We have clarified this in the abstract: “Using a repeated measures design, we subjected lizards to five numerical tests (1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4 prey items), while controlling for total length and area” (Lines 33-35).

83-85 I wasn't aware of any studies? You should cite them.

**Response**: We apologize for the incorrect statement about there being 'few studies' on this topic. Our search, documented in the Supplementary Material, revealed that there are no studies addressing this matter as correctly pointed out by the Reviewer. This sentence has been removed from the text following the restructuring of the Introduction.  
  
The intro is very well written and nicely tackles the role of the early thermal developmental environment and glucocorticoids, in affecting cognition. It's missing some useful background information on quantity discrimination (QD). For example, the brain uses two core systems, the object file system (OFS) and the approximate number system (ANS). The OFS considers items as discrete units. So, this system employs absolute numbers albeit typically only up to 4. The ANS is ratio dependent and deals more with magnitude. The ANS does seem to follow Weber's law, which the authors mention, and allows an animal to discriminate when one quantity is larger than the other. The authors appear to be focusing on the ANS and not the OFS. This is fine given the context, but they just need to make this clear.

**Response**: Thank you very much for this excellent comment. It is extremely helpful in making clear the distinctions in this area of research. We have now incorporated this information into the Introduction and framed our paper within the context of the use of both systems. Further clarification in the Methods section should make it clear that our focus was on the OFS rather than the ANS. The rationale for this choice is that other species within the Scincidae family have been shown to rely exclusively on the OFS (Szabo et al 2021, *Behavioral Ecology*). The intention of the rotation of the crickets was to control for continuous cues (length and area) to test numerical discrimination abilities in the context of the OFS (see below).

159.     snout-vent length, tail length (lower case).

**Response**: Changed as suggested. Thanks.

170.     I'm quite surprised that the control was 100% ethanol. I see that the court was dissolved in 100% ethanol, but wouldn't ethanol itself potentially have an adverse effect on development? Or is it too small a volume to worry about and perhaps just short-term?

**Response**: We appreciate the reviewer’s concern regarding the use of 100% ethanol as a control. Ethanol was used as a solvent to dissolve corticosterone in our experimental design, and only a very small volume was administered (5 uL). While ethanol can indeed have developmental effects at higher concentrations or with prolonged exposure, we believe that the minimal volume and short-term exposure in our study were unlikely to impact the animals in a meaningful way. Moreover, in previous studies using the same prenatal manipulations, animals performed normally in other cognitive tasks without any observable complications (Recio et al 2025, *Animal Behaviour*).  
  
202.    Maybe add that lizards were happy to eat dead crickets. Okay, I see you do this in 203. That being said, why the new pg? I'm surprised these were dusted with calcium and multivitamins in an experiment with a food reward, but totally fine.

**Response**: We have included that information in the same paragraph now (Lines 220, 221).

Regarding the use of calcium and multivitamins, lizards in captivity need for this kind of supplements when young, especially if the experiment includes a habituation process of one month plus the trials.   
  
213-215. This is the first mention of discriminating between using the ANS vs OFS although these terms are not used. This needs to be addressed in the introduction and mentioned early in the methods.

**Response**: We appreciate the reviewer’s suggestion. We have included the information in the Introduction (lines 83-98). Furthermore, we have now framed great part of the paper in this context to add clarity to our design and the rationale behind it (see below).   
  
Fig. 1. I'm slightly confused by this experimental design. Typically, you would separately test discrete (OFS) versus overall quantity/size (ANS). When testing OFS the different quantities would still have the same surface area. When testing ANS it would be the same number of objects but they would differ in overall size. I get this is a little difficult with crickets. But the current design seems to make a few assumptions. I think the authors need to explain this a little bit more in light of what people typically do in these studies and do a better job explaining what we can infer from the results using this design. I think the key result is that cort and temperature have no significant effect on QD. I'm just not too sure about the specific metric of QD that is being measured. (After writing this, I saw in the discussion [line 340-352] they mention potential issues with their design.) But perhaps more importantly, the authors don't actually find any QD ability and as such a reader may wonder why you are measuring an effect of cort and temp on a phenomenon that has not been demonstrated. This perhaps needs to be fleshed out a bit more.

**Response**: We appreciate the reviewer’s concerns and would like to clarify our experimental design decisions. We acknowledge that some aspects of the design were not explained as clearly as they should have been. When referring to the potential conflict between number and other quantitative traits, we were addressing the conflict between the expected relationship of number with other continuous variables like total length or area (see below). For example, length increases with the number of items, but we aimed to keep the size consistent despite the number of items, which conflicts with the typical expectation. In other words, we aimed to maintain continuous variables constant while modifying number, as in conventional designs (see reviewed in Agrillo and Bisazza 2014, *Journal of neuroscience methods*). Specifically, we rotated the crickets to control for total length and, at the same time, reduce the space between items to reduce potential effects of area perception (see Stancher et al. 2015, *Animal Cognition*).

We agree that our approach could have been further clarified, particularly within the context of the object file system (OFS) and approximate number system (ANS) strategies. To address this, we have revised the Methods section to better explain our reasoning. Additionally, we have included new data indicating that the length of both choices did not differ from zero in at least three of the tasks We believe this data demonstrates that we effectively controlled for total length, in line with the OSF framework. However, we acknowledge that we were unable to control for length in the 1 VS 4 and 1 VS 3 trials, despite rotating the crickets. We have provided these details in the Introduction, Methods, and Supplementary Material sections and have acknowledged the potential implications of this limitation in the Discussion.  
  
236-237. Was PR also blind to the actual 'amount' in addition to the treatment? Maybe that was not possible?

**Response**: PR was not bind to the ‘amount’ because it was visible in the videos. We have indicated it now: “All the analyses were by one observer (PR), who was blind to the treatment of the lizards, but not the type of test or the number of crickets because the videos were analysed manually. (Lines 256, 257)  
  
The data were rigorously and appropriately analysed, and well presented.

**Response**: We are grateful for the reviewer’s recognition of the thorough analysis and clear presentation of the data.

325.    Spelling. Maybe do a spell check on entire document.

**Response**: Thanks. We have carefully checked the entire document now for any other spelling errors.  
  
The discussion is thorough and I appreciate the authors detailing their literature search queries in the ESM.

**Response**: Thank you for your kind words regarding the Discussion and literature search sections. We truly value the reviewer’s constructive feedback.