Editor’s letter BEAS

Dear Mr Recio,  
  
Based on the evaluations of two referees and an Associate Editor's recommendation, I have to reject the present version of your manuscript for publication in Behavioral Ecology and Sociobiology. However, although reviewers identified significant deficiencies in your study, they also recognized that your work has merit and the potential to develop into a substantial contribution.  If after careful consideration of reviewer comments you are able to fully address all concerns, you may resubmit a thoroughly revised manuscript for reconsideration.   
  
When submitting a revision, please reply point-by-point to the referees' comments.  Provide a clear rationale if you disagree with any comment. Refer to the new line numbers to identify your revisions. Submit your work as a new submission while referring to the original manuscript number together with a marked manuscript in which the changes have been highlighted.  
  
For resubmissions, the marked manuscript should be uploaded as "supplementary material" and response to reviewers as "authors' response to reviewers' comments".  
  
The reviewers' comments can be found at the end of this email.  
  
Thank you for giving us the opportunity to consider your work.  
  
Sincerely,  
Theo C. M. Bakker  
Editor-in-Chief for Behavioral Ecology and Sociobiology, vertebrates  
  
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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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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.

We sincerely appreciate the reviewer’s constructive feedback. We recognize that without the animals exhibiting the target behaviour, comparisons between experimental conditions could be misleading. Nevertheless, we believe it is important to: a) transparently acknowledge that one of our research questions focused on the effects of prenatal treatments, even though our experimental design did not allow us to fully investigate this question as originally intended; and b) emphasize that, despite our inability to conclusively assess the impact of early life conditions on numerical discrimination, our data still provide valuable insights into how early environmental factors may influence cognitive processes and decision-making.

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.

We acknowledge the reviewer’s concern regarding the cues lizards might use to perceive numerical information. However, controlling for non-numerical cues such as length is a standard practice in numerical cognition research to ensure that number is the distinguishing factor. Our adjustments were not intended to introduce a spatial illusion, but rather to prevent lizards from using alternative quantitative traits to drive their choices. In this regard, we considered that rotating the crickets would control for total length while minimizing the effect of convex hull. Although the latter was not explicitly controlled for, we have now included additional analyses in the Supplementary Material, examining the length difference between choices in each test. 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.

In addition, the concern that we do not yet know 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 rather than not controlling for continuous variables.  
  
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).

We understand that the design requires to be more detailed. The intention of our manipulation was to control for length while minimizing the effects of convex hull, and 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.  
  
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.  
  
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 (Miletto Petrazzini et al. 2018, *Frontiers in Psychology*. However, the same species performed poorly in spontaneous choice tasks. 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).

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.

We sincerely thank the reviewer for their valuable comments and insights. Their suggestions have been extremely helpful in refining our manuscript. We acknowledge that our design required further clarification, 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 is somewhat premature, given the lack of prior knowledge on this topic. However, we still believe it is an important and unexplored question. Even though we were unable to address it properly, our study provides useful information on how early environmental factors influence decision-making in *L. guichenoti*. However, 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.  
  
Title: I would probably have gone with "Does early environmental experience impact quantity discrimination ability in a lizard?"

We understand and appreciate the reviewer’s suggestion. We have changed our title according to this suggestion plus the new structure: “Quantity discrimination in the common garden skink and the role of early-life conditions”.   
  
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.

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

We examined numbers when controlling by continuous cues like length, but we did not employ size, just different number 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 continuous variables.” (Lines).

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

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. However, 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.

We sincerely appreciate your comment. We have 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 convex hull) to test numerical discrimination abilities in the context of the OFS (see below).

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

Thank you, modified.  
  
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?

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. 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.

We have included that information in the same paragraph now (Lines).

Regarding the use of calcium and multivitamins, lizards in captivity need for this kind of supplements, 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.

We appreciate the reviewer’s suggestion. We have included the information in the Introduction (lines). 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.

We sincerely appreciate the reviewer’s thoughtful 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. 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. Specifically, we rotated the crickets to control for total length while minimizing the effect of convex hull.

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. 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?

PR was not bind to the ‘amount’ because it was visible in the videos. We have indicated it now: “All the analyses were performed by PR, who was blind to the treatment of the lizards, but not the type of test or the cricket's number as the videos were analysed manually.” (Lines)  
  
The data were rigorously and appropriately analysed, and well presented.

We do really appreciate the reviewer’s comment  
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.

We greatly appreciate the thorough review. We have carefully checked the entire document.  
  
The discussion is thorough and I appreciate the authors detailing their literature search queries in the ESM.

Thank you for your kind words regarding the Discussion and literature search sections. We truly value the reviewer’s encouraging remarks.