Author responses to comments made by the reviewers of manuscript "Negative impact of mild arid conditions on a rodent revealed using a physiological approach in natura"

We thank the reviewers for taking the time to assess and help us improve our manuscript. We have addressed all the concerns they raised (text in red), and hope that the revised version of the manuscript is an improvement. The detail of the changes is contained within the "tracked changes" PDF file.

As a side note, we would like to mention that this paper is part of the first author's PhD project; ideally we would prefer to have a response by the end of August (up to the 30th), if possible of course.

Reviewer 1

The title does reflect the main content of the article, but it could nevertheless be improved. First, referring to "a rodent" reduces the significance of the study, since the authors actually used a comparative approach to study several populations of two rodent species. So I would rather suggest referring to "natural rodent populations" or to an equivalent wording. Second, the term "physiological approach" is vague and little informative. Likewise, I would suggest being more precise on what has been done in the study, by referring to "markers of physiological condition", for instance (or something else equivalent but more detailed than the actual formulation).

We agree and propose a new title : *Negative impact of mild arid conditions in natural rodent populations revealed using markers of physiological condition in natura*

Overall, the abstract summarizes properly the objectives and the main findings of the study, except for body condition. However, the authors did not state precisely which physiological metabolites were investigated, and neither did they mention body condition nor habitat characteristics (i.e. bullet points #2 and #3). Thus, I would suggest replacing "blood concentrations of physiologically relevant metabolites" by "blood concentrations of markers of kidney and liver function" (as stated later in the text), and I would at least mention that body condition was also measured. Finally, the idea that "it is crucial to identify the potential for species responses" (i.e. bullet point #1) could be even more explicit, by stating for instance that it is crucial to "investigate/study/define the extent and limits of species responsiveness".

Proposed revision :

1. Understanding how organisms respond to seasonal variations in their environment can be a window to their potential adaptability, a classical problem in evolutionary ecology. In the context of climate change, inducing increased aridity and disruption of seasonality, it is crucial to study the extent and limits of species responsiveness.

2. Here, the physiological response to food and water shortage during seasonally dry conditions were investigated. We studied populations of two rodent species of the genus *Rhabdomys*, one arid and one mesic, in a semi-arid zone where their range overlap in South Africa. We measured blood

concentrations of markers of kidney and liver function, as well as body condition at the onset and the end of the dry season.

3. We found similar shifts in blood metabolite levels in the semi-arid populations of the two species, indicating malnutrition consistent with the observed degradation of habitat quality between the start and the end of the dry season. Furthermore, regardless of the period, differences between the two species in blood metabolite concentrations (ex: amylase, sodium, alkaline phosphatase) were observed, suggesting contrasting diets and water conservation abilities.

4. Overall, we show that, as seasonal dry conditions worsen, organisms are increasingly affected by reduced food availability, and local adaptation to arid conditions may provide the arid species with an advantage to cope with semi-arid conditions. Our study suggests that even mild arid conditions could have a negative impact and questions resilience of animals to harsher arid conditions.

Introduction

The introduction clearly explains the motivation for the study, and both the research question and the associated predictions are well presented. The introduction also builds on relevant research in the field. I only have a few minor comments.

Since "Adaptive evolution" is defined, I would also provide a short definition and an associated reference for "phenotypic plasticity" (eventually within brackets).

At line 72, please provide a definition of homeostasis. This is a central concept in physiology, which will be known by the community of ecophysiologists. Yet, a definition (or a reminder) might be useful for ecologists and biologists who specialized in other fields.

At line 101, it would be more appropriate to refer to "physiological responses" (i.e. plural) as these could involve different processes.

At line 107, reword as "generate additional selective pressures to those already experienced".

At lines 112 and 115 : add "a third species of the same genus" after the first occurrence of "*R. pumilio*", and replace the second occurrence of "*R. pumilio*" by "individuals" since the pronoun "they" is used later in the sentence.

At line 126, please specify there which species you are referring to, since that last one to be mentioned was *R. pumilio*, but that species was not included in the study. Then in the next sentence (line 128), simply mention "parapatric populations of the two species".

At lines 129 and 133, add "that" after "we expected".

We have made all suggested revisions on the structure of this section. Below the sentences added to define plasticity and homeostasis.

In contrast, local adaptation *sensu lato*, also considers concepts such as **phenotypic plasticity** (the ability of a genotype to produce distinct phenotypes when exposed to different environments throughout its ontogeny; Pigliucci, 2005).

As a result of climate change, organisms can be faced with warmer and drier environmental conditions (Parmesan et al., 2000), likely to impact their capacity to maintain **homeostasis** (i.e. the state of steady internal conditions allowing optimal body functioning) (Davies, 2016; Fuller et al., 2016).

Materials and methods

This study relies on the combination of several environmental and individual measures to investigate the physiological consequences of seasonal variation in dry conditions, which is a strength of the study. Consequently, the description of the materials and the methods is long, and should be as clear as possible to help the reader follow the experimental plan. In particular, the way this section is organized could be optimized, as I would have expected some information to be given earlier than in the last subsection named “Data preparation and analysis”. Typically, I first thought that information regarding body condition assessment and age class determination were missing because they were not introduced before the data analysis subsection. By contrast, specific paragraphs are dedicated to the description of the measurement of habitat characteristics and physiological markers (i.e. blood metabolite concentrations), before presenting how these data were then analyzed. I thus recommend that this section be rearranged and homogenized to separate variables description from data analysis (i.e. statistics).

Overall, the experimental plan is consistent with the questions. The methods are described in sufficient detail for most parts, but not all. Specifically, I believe that breeding status assessment and age class assignment deserve clarification. Also, I wonder whether the method for body condition calculation is appropriate. Most importantly, data analysis relies on various tests and models, and I found it hard to follow the analytical approach. Actually, some of the chosen methods do not convince me, and I don’t understand the model selection procedure for physiology data (although I briefly checked the statistical scripts, I did not evaluate them in detail). My point-by-point comments are detailed below.

At lines 165-166, it is said that breeding status was assessed (remove the first “s” from “sassessed”) based on external morphological metrics, but these metrics are not specified. Please explain clearly how breeding status was determined.

Breeding status was assessed (i.e. breeder versus non-breeder) based on external morphological features (signs of lactation, perforated vagina, presence of a vaginal plug, abdominal testis, scrotal testis). The text of the manuscript was also modified.

At line 249, the authors stated that trapped mice were assigned to age categories based on their “general appearance, size and/or breeding status”. First, while I agree that both size and breeding status can be objective criteria, I wonder how general appearance could be too without further information. Please provide detail on how mice general appearance, and which particular elements of it, were included in the assignment of age classes. Second, I struggle to understand how many/which categories were considered in the end in the analyses, since it is first said that mice were assigned to either juveniles, subadults or adults (i.e. 3 categories, line 248), but it is then written that 4 length/age classes were considered (lines 255-256). Please clarify.

The study involved only adult animals. We excluded mention to other trapped animals that were not included in the study.

The 4 categories correspond to four adult categories as a proxy of “age” difference. We decided to consider these classes when we realized that we had several “old animals”, which are rarely captured in the traps. Old animals are large animals with dull fur, often bearing several old scars. We suppose that the particularly good rain linked to the “la Niña” period resulted in higher rate of survival of old animals. We clarified the text by removing mention to the juvenile categories and adding the above information.

At line 237, the authors explain that body condition was assessed through the ratio of log(mass) to log(length). Assessing body condition is particularly relevant in the context of this study, and common practice in ecology. Several methods have been used to calculate it, and despite no real consensus, most of them have been criticized (see Peig and Green 2010 *Functional Ecology* for a review). I am under the impression that simply computing the ratio of mass to length is outdated, as this method was not even considered in the above-cited paper. According to the authors of that review, the dominant method consists in calculating the residuals from an OLS regression of mass against length. Yet, these authors also proposed an alternative method, the scaled mass index, and argued that their index is a better indicator of energy reserves than OLS residuals (Peig and Green 2009 *Oikos*). Therefore, I strongly recommend that the authors of the present paper re-evaluate mice body condition, ideally using the scaled mass index, and check whether their conclusions hold using another estimate of body condition.

We thank the reviewer for this suggestion. We have updated the analysis using this more accurate measure of body condition (scaled mass index), as proposed by Peig & Green (2009).

The results of the PERMANOVA addressing variation of the physiological response has yielded different results using the new estimation of body condition which is not anymore significant (i.e. physiological response does not vary with body condition). Moreover, the results of the analysis testing the factors explaining variation of the new body condition index also changed. The new analysis revealed a strong sex and “age” effect, while session and NDVI were not significant. We updated the Results and Discussion sections accordingly.

At lines 218-223, the authors stated that they performed an ACP on the 7 variables of vegetation composition. Both the method of measuring these variables and the way to analyze them are puzzling me.

First, it is said that these variables were measured within the $4m^2$ and within the $100m^2$ quadrats for each successful trap, and earlier in the section (i.e. line 184), it was specified that both small and large quadrats were centered on the trap position. So from my understanding, small and large quadrats overlap, in the sense that small quadrats were positioned within the larger ones, meaning that all items that were considered in the small ones were also considered in the larger ones. How is that not redundant? How did the authors take into account the fact that they measured the same grass/bushes/plants twice, but included both measurements in their analyses later on? What was the interest of considering both small and larger quadrats? Actually, the first 2 PCs are displayed in the supplementary figures 4 and 5, and as I would have expected, the position of the different habitat structure variables are very similar in 4m² and 10m² quadrats (only "dry bush" and "no cover" items slightly differ but still remain in the same area on the graph). Then, the authors mentioned that they considered 7 variables in the following PCA (lines 218-219), corresponding to the 7 vegetation variables previously listed (lines 182-183). Thus, it seems that they ran two separate analyses, one for small quadrats and one for large quadrats (it would seem so from the results section, the supplementary information and the R scripts), but again, it appears highly redundant to me.

Second, it is written than 80% of the variance was explained by the five first principal components (PCs), and that these 5 PCs were then used as response variables of a PERMANOVA. While most of the variance was indeed captured by the retained PCs (which is desirable when performing a PCA), retaining 5 PCs when the analysis initially included only 7 variables appears pointless and a failure to summarize the original dataset. Multivariate analyses such as PCA are typically used to deal with datasets containing multiple quantitative variables, aiming at summarizing the information by reducing the number of variables (generally to 2 or 3 PCs are being considered, Greenacre et al. 2022 *Nature Reviews Methods Primers*). In the present case, having 5 composite variables (or PCs) rather than 7 raw variables doesn't seem effective nor relevant to me, even more since I suspect that variables of vegetation composition might be redundant.

Finally, I wonder why the authors did not consider including the NDVI into the PCA, since it is an index of vegetation greenness, which contributed to characterize vegetation and thus habitat quality.

We agree that the text was not clear. We revised the different paragraphs.

We chose to perform independent analysis for each vegetation/habitat quality assessment, because the sampling scales and the methods to obtain the data were different, and indeed they were partially dependent but we believe not redondant. Indeed, the 4m² quadrats were included in the 100m² ones, and in the 60m radius circles. However, the 4m² quadrats were sampled using four 1m² metal squares within each of which a detailed assessment of the ground cover characteristics was done (the data for the four 1m² were then averaged). This allowed us to assess the <u>mice microhabitat around the traps.</u>

Assessment at the scale of the 100 m² quadrats was obviously less precise and allowed to assess the general habitat at the scale of the mouse home-range.

As far as the NDVI was concerned, it was computed using satellite data with a resolution of 10 meters, it covered a larger surface and was therefore considered as a "larger scale" level of information, that allowed to characterize the trapping site. Further the data provided by the NDVI is different to the one collected directly in the field. We believe that analyzing the 3 datasets in a unique model would have been difficult to interpret.

We agree with the reviewer's comment concerning the low variable reduction from 7 raw variables to 5 composite ones in each case. However, our aim was not to reduce the number of variables but to transform their distribution to allow further statistical analysis. It was important for us to keep as much variation as possible not to mask heterogeneity between our studied sites (i.e. to be conservative when concluding that the replicate (sites/populations) for each species were similar). Additionally, in the ACP analyses we saw no obvious cut-off point (i.e., low improvement of inertia, see **Supplementary Tables 4 & 5**) that would have made us include a smaller subset of principal components in downstream analyses.

At lines 240-241, the authors wrote that they performed an ANOVA to test whether several variables (e.g. breeding status, sex, habitat quality) influence body condition, including site as a random factor. It might just be a semantic issue, but what they are describing seems to be a linear mixed effects model to me, leaving me to wonder why they did not say so? Actually, checking the R script, I saw that the "lmer" function of the package "lme4" was used for the analysis, so I really think that it would be more informative to indicate it. I am under the impression that the term "ANOVA" is being used in its general sense throughout the text, while a more precise terminology could be used to describe the models that were computed.

We agree. We have revised the generic mention of "ANOVA" in the text to more accurately reflect the models tested.

Also, the authors included the three-way interaction between session, species and sex (i.e. session*species*sex, line 241 and in R scripts), without explaining the reason for the consideration of such a complex interaction. Three-way interactions are particularly difficult to interpret, and should not be included unless one has solid hypotheses behind them and can express associated predictions. I would recommend removing the triple interaction from the model, eventually including the two-way interactions instead (i.e. session*species + session*sex + species*sex), unless providing a robust justification for it.

The three-way interaction had a biological justification. We could not exclude that sex differences at the two sessions could have been exacerbated in one species compared to the other. For example, reproductive costs could be harder to compensate in one sex at the end of the dry season for the mesic species compared to the arid one. All two-way interactions were also (automatically) included in the full model.

At lines 260-262, the authors explained that they performed another PCA to identify outliers in the data set, what led them to remove 12 data points from further analyses. I never heard of the use of PCA to identify outliers (simply plotting the distribution of a given variable through a boxplot might have worked just fine), and I don't understand how they proceeded exactly without further explanation. Typically, what was the cut-off criterion? In fact, I am concerned by the removal of data points. Again, one must have a solid argument to consider a given data point as an outlier. For instance, it can be the case if its value is far out of the documented physiological range of the variable for the studied species. While I am well aware that reference values are often lacking for wildlife, it remains that data points removal must be properly justified. The authors mentioned that some samples were heavily hemolyzed, what can effectively be an issue, but I don't understand why values from smaller blood volumes would end up as outliers, and I still don't see what the PCA brings to the matter. If the Vetscan was unable to compute reliable values from hemolyzed samples or from low volume samples, simply state it and discard the values for that specific reason.

We acknowledge that the initial wording could wrongly indicate that the position on Principal Components was used as a cut-off value to exclude data points. However, information on each "outlier" sample was gathered first (e.g. hemolysis, insufficient blood volume requiring a dilution) to exclude inaccurate measurements from the analysis. Based on this information, we had reasons to believe the sample measurement as a whole was compromised. Vetscan performs all 14 measurements at the same time. A subsequent Principal Component Analysis was used to represent visually that this divergence from a "typical" range of multivariate values was only found in samples presenting such features (small volumes leading to dilution, hemolysis). However, to avoid confusion we removed reference to the PCA.

At line 272, it is said that a multivariate analysis was performed, please indicate here which analysis (I assume it was a PERMANOVA since this analysis is mentioned later at line 276, but it would be clearer to precise it right there).

We thank the reviewer for pointing this out. We have described the analysis in a more precise manner :

PERMANOVA is a multivariate statistical inference tool using permutational algorithms (Anderson, 2001). This equivalent to *MANOVA* operates in a distribution-free setting and is relatively robust to non-normality of multivariate residuals as well as dispersion heterogeneity, even in slightly unbalanced designs (Anderson, 2013).

I am not familiar with PERMANOVAs, so I cannot really comment on it. However, I am confused with the following procedures of model selection: the authors first state that they performed a backwards stepwise model selection to retain the most parsimonious model (lines 277-278), and then that the best-fitting model was determined by comparing all previous models using the AICc. So it seems that they applied two different selection procedures on the same full/saturated model. While both approaches are valid independently of one another (although their respective use depends on the approach, e.g. exploration *versus* inference), and both aim at defining the final model with the fewest predictors, combining them seems irrelevant. Following the backwards stepwise model selection, the authors were already supposed to get a reduced model that best explains the data.

We actually used the AICc only for the last two models. However, we agree that this approach was not relevant and we modified it in our text.

Then, I do not get either why they performed 12 ANOVA tests using the parameters of the most parsimonious PERMANOVA model (lines 282-284). I would have thought that performing a PERMANOVA including all markers or computing separate models for the 12 markers would be two alternative approaches and that they should chose one or the other. Again, they seemed to have proceeded the same way than before for model selection.

We acknowledge that this section was not sufficiently detailed to provide a satisfactory understanding of the methods. Following a traditional Multivariate Analysis of Variance (MANOVA, assuming multivariate normality and homoscedasticity of residuals), post-hoc tests aiming at checking which individual variables (as opposed to all variables together) differ between groups can usually be carried out.

As no such dedicated function exists for *PERMANOVA*, we employed an approach applying the most parsimonious model obtained with the PERMANOVA to each physiological marker: investigating which of the 12 markers influenced more significantly the outcome of the PERMANOVA. Moreover, to control for multiple testing (same hypothesis tested with several variates) we applied the Bonferroni correction.

<u>Results</u>

Results are properly described and their interpretation makes sense based on the output of the current analyses, although some of these analyses are questionable, as stated previously. I have checked the raw data, but I have not rerun the statistical analyses.

I did not detect obvious manipulation of data apart from the stated removal of 12 outliers (lines 261262), which I already commented on earlier. Overall, the statistical results seem to support the conclusion, although the significance is not always very strong, especially for analyses on body and physiological condition where p-values are often between 0.01 and 0.05. It remains nonetheless that the authors found significant differences between sessions and/or species.

Discussion

The authors discussed all the hypotheses they had initially formulated, based on their own results and published research in the field (on both mice and other species). They thoroughly commented on the observed variations of body and physiological condition, as well as on the differences between species. For each item, they proposed one or several alternative explanations, and their interpretation is in line with their observations and previous findings in the literature. They also commented on the absence of variation for some markers (e.g. sodium, blood urea nitrogen), and considered results that went against their predictions (e.g. body condition, TBIL). Finally, they emphasized a few limitations of the study and suggested a few avenues for future studies, which is nice. I have no major concern on this section, although I think that the structuration could be improved by reorganizing some paragraphs.

Line 362. Please specify here that the sister species were rodent (or at least mammal) species, to clarify the context.

Line 365. The authors state here that the study took place in a relatively wet year, indicating “La Nina” within brackets. Unless I’m mistaken, they did not mention the role of the “El Nino Southern Oscillation phenomenon” in driving South Africa climate in the introduction, and they describe it only after talking about “La Nina” (which should have been the opposite to promote non-familiar readers comprehension). It is an important feature for the study, so I suggest introducing this phenomenon from the introduction.

We have made the suggested revisions.

From lines 375 to 379, the authors sum up their main results. Such a concise summary paragraph is useful at the beginning of the discussion, but comes a bit late here (since it is placed after another longer paragraph on climate and habitat quality). I believe it would be more relevant to move up this paragraph and to combine it with the first one, which sums up the main questions of the study.

We have made the suggested revisions on the structure of this section.

From lines 380 to 394, this paragraph on body condition also deals with seasonal variation of physiological condition to some extent, so I see no reason not to include it the sub-section named “seasonal variation in physiology” (which should then be renamed “seasonal variation of body and physiological condition”, for instance). Otherwise, as it is, it is a bit weird to have most of the discussion shared between 2 sub-sections (i.e. “seasonal variation in physiology” and “interspecific differences”) but a few paragraphs out of any sub-section. An alternative would consist in creating another subsection called “(seasonal) variation in body condition”, which would include the current third and fourth paragraphs.

In line with my previous comment, the last paragraph of the sub-section on seasonal variation in physiology (lines 484-488) deals with body condition, which has already been discussed earlier. For more consistency, and to avoid going back and forth, I suggest grouping all information on body condition within a single sub-section, or at least moving this paragraph closer to the other ones also dealing with body condition.

We agree that including all paragraphs discussing significant seasonal effects (or their absence) on any component of physiological condition in the same sub-section is coherent. Notwithstanding, following the reviewer's advice we used a different index to assess body conditions, resulting in changes in the results and the conclusions.

At lines 522-524, it is written that overall differences between species can be accounted for by lower blood concentrations in *R bechuance*, and on average higher blood concentrations in key metabolites in R. d. *dilecticus*. However, the concentrations involved in the first part of the sentence are not specified, and the key metabolites are not listed either in the second part. Thus, I find it hard to keep track. Please specify which concentrations you are referring to.

In this sentence, we were simply inferring that overall blood osmolality was higher for *R.d.dilectus* compared to *R.bechuanae*, as evidenced by significantly higher concentrations in NA and K (with TBIL and ALP showing the same pattern) . We have provided this clarification in the manuscript.

Line 526. I am under the impression that "rather than" would better fits than "although" in this sentence, unless I misunderstood it.

Line 547. Replace "is" by "being" in "starch is the best substrate for […]".

We have made the required changes.

From line 552 to 566. This last paragraph is a bit out of topic within the sub-section on interspecific differences, as it has a broader scope. Its ideas are more in line with those presented in the conclusion section, part of which does not fall within the scope of a conclusion *per se*. Thus I wonder whether it would be preferable to merge the text of the last 2 paragraphs (or at least most of it) into a single subsection named "perspectives" (or "perspectives and conclusions" if the entirety of the text was to be merged).

We have merged the above mentioned paragraphs in a "Perspectives and Conclusions" section to improve coherence.

Tables and figures

The manuscript includes a lot of tables and figures, of which some are presented as supplementary material. I believe a few could be merged to reduce their total number.

Table 2. It is said in the legend of this table that values in the column "Aridity Index" correspond to the average index ± standard errors. Yet, in the table, the symbol "±" does not appear between the 2 values, only a "-". Please correct it.

We thank the reviewer for pointing this mistake out and have corrected it.

Tables 3, 4 and 5. Titles and captions are not detailed or specific enough to understand these tables without reading the manuscript. More precision is required about the analysis/model that was performed in each case. In particular, tables 3 and 4 have the same title except for the name of the response variable within brackets, and the term "physiological response" in table 5's title remains vague and uninformative.

We have provided further details in the table titles.

Figure 1. I personally do not see the difference between translucent and full color dots on the map.

We have heightened the contrast between translucent and full-color dots to improve the readability of the map.

Figures 3, 4, 5 and 6. Overall, labels on y and x axes are too small, especially compared to the heading of each panel.

We have updated the figures to improve readability.

Supplementary table 4. Please provide the significance of the different acronyms in the caption (e.g. ALB, ALP, ALT, etc.).

The meaning of each acronym is now explained in the Table caption.

Supplementary methods. In the section about the Aridity Index formula, the reference for “PET by Thornthwaite method” is missing (i.e. [REF]).

We thank the reviewer for pointing out this mistake and have corrected it.

Supplementary table 2. Is this table really useful? Mean value for trapped surface ± standard deviation (and min-max values) could have simply been indicated in the main text, in the sub-section on sampling periods and sites. This would allow the reader to get the main information without having to consult the supplementary material.

We believed that this table, though not crucial for the understanding of the study, could be useful for later meta-analyses, which is why we initially chose to include it in the supplementary material rather than in the main text.

Supplementary figures 2, 3, 4 and 5. It would be nice to plot on the same graph both the habitat structure variables and the traps position (i.e. biplots, see Fig 3 in Greenacre et al. 2022 *Nature Reviews Methods Primers* for an example), so that the reader could easily see how sites and traps differ in vegetation structure. This would consist here in combining figures 2 and 4 on one hand, and figures 3 and 5 in the other hand. Not only this would contribute reducing the number of figures, it would also improves the visualization of inter-site differences.

We understand the need to reduce the total number of Supplementary Figures, as well as improving the visualization of inter-site differences. On this basis, we have updated the above-mentioned figures.

Reviewer 2

Major comments:

1. They are a lot of tables and figures in the scientific article proposed, either in the results or in the supplementary documents. I think the choice of which figures to include in the article itself or in the supplementary documents needs to be reviewed. It is also important to legend the figures and tables properly to make them easier to understand. Beware also of the organization of figure titles (below) and tables (above).

2. I suggest that the authors rearrange a little the introduction section. I detail my request below.

a. Lines 40 - 47: the authors talk about short- and long-term changes and their possible effects on organisms, which it may be interesting to assess in order to understand the impact of climate change on organisms.

Lines 48 - 70: the various local adaptations are presented, and in particular the importance of physiology to study the impact of environmental variations.

Lines 71 - 83: the authors discuss climate change and its possible impact on semi-arid organisms, linked to food and water scarcity, with a possible distinction in responses between arid and mesic species.

Lines 84 - 92: the importance of physiology to understand the ability of species to adapt to environmental change is discussed.

All the ideas are there, but I suggest that the different paragraphs be reorganized to make the article easier to read and understand. For example, start by talking about short- and long-term changes in organisms, and in particular drought conditions and their importance in understanding the possible impacts of climate change on these organisms. Then, for example, talk about the adaptive capacities of organisms and finish with physiological adaptations, which may be of interest.

We have made the suggested revisions on the structure of this section.

b. Lines 50 – 61: "For example, adaptive variation in lethal temperature […] different latitudinal niches (Somero, 2010)". I suggest that the authors develop this idea in order to make the link with the previous sentences: why is it interesting to present adaptations to lethal temperatures in marine invertebrates?

We have reworded the given example to make the link with the previous sentence clearer.

"Specifically, physiological limits can drive and be driven by evolution, shape species distributions and niches, and define species response capacities to future climate change, directly impacting risks of extinction (Somero, 2012). For example, latitudinal niches are associated with adaptive variation in thermal limits in marine invertebrates (Somero, 2010)."

c. Lines 92 – 94: the problematic of the study is given.

Lines 94 – 108: the ecology of the two rodents used for the study is given. At lines 100 – 102: questions addressed by the study are presented.

Lines 110 – 118: Physiological responses of *R. pumilio* face to dry season are introduce.

Lines 119 – 138: the authors discuss the lack of study in natural condition and their advantages and give details on measurements that will be conducted in the study, as well as the hypotheses.

In these last paragraphs, the problematic of the study seems to me to be hidden among the abundance of information. However, it is important that it should appear clearly and prior to the hypotheses. Certain sections concerning the ecology of the species studied or the physiological responses of *R. Pumilio* to dry conditions could, for example, appear earlier in the introduction.

We have made the suggested revisions on the structure of this section.

3. Table 1: I do not understand the last part of the table entitled “aseasonal differences: predictions” (Lines 906 – 912).

We acknowledge that this wording was not clear enough. This part of the table reflects predictions of differences that are independent of seasonal effects. As a consequence, the wording in this section of the table has been revised.

4. Supplementary material section: “PET by Thornthwaite method [REF] was calculated”, what is the reference in question? I suggest to reorganize the part from “with exponent c” until the formula for PETi(0) to make it more clearer. The last sentence “This was the final value […] which was divided over annual precipitation to obtained aridity index” is not correct and causes confusion. If I understood correctly, it is annual precipitation that is divided by PETi(L), not the contrary.

We thank the reviewer for pointing out this mistake we have corrected it in the new version of the manuscript.

5. The other sections of supplementary material is not used and referred in the main text of the article. I suggest that authors either remove these sections if they are not necessary, or make good use of them by referencing them in the main text.

We have updated the main text to contain references to the Supplementary Material in the relevant sections.

6. Lines 162 – 163: “They were placed approximatively every 15m along roughly 150 to 300m transects”. There were how many transects per site?

Depending on habitat features, the number of transects was highly variable. We instead reported the number of traps per day for a more accurate measure of the trapping effort (Supplementary Table 1).

7. Line 176: “until we reached our target of 20 adult individuals”. Is it the number per species or per site? Only for physiological analyses? Because it is mentioned later that “273 adult mice were euthanized” (Line 197).

20 individuals was the target number per site, per species, with 273 being the total adults trapped that complied to the conditions (“low relatedness”) to participate to the physiological study. We have clarified this in the manuscript.

8. Lines 187 – 188: “We characterized 236 quadrats of each type (100m² and 4m²)”. Is this for each site or in total? How many quadrats of each type were there for each site and each species?

236 quadrats of each type were characterized in total. for *R.bechuan*ae 39 traps in Gariep Dam, 50 in Kalkfontein Dam, 24 in Benfontein; for *R.d.dilectus*, 49 traps in Wolwespruit, 48 in Bloemhof, 24 in Barberspan. This info is available in the tables in the raw data.

9. I have some questions about statistical analysis. Could the authors explain their choice of doing a permanova on the 5PCs rather than on the 7 variables directly for vegetation composition? Why not on 2PCs if the aim is to reduce the number of explanatory variables? Regarding the physiology section, I do not really understand the statistical analyses that have been carried out. Perhaps there are some elements missing to better understand the work done.

We chose to perform independent analysis for each vegetation/habitat quality assessment, because the sampling scales and the methods to obtain the data were different, and indeed they were partially dependent but not completely redundant. Indeed, the 4m² quadrats were included in the

100m^2 ones, and in the 60m radius circles. However, the 4m^2 quadrats were sampled using four 1m^2 metal squares within each of which a detailed assessment of the ground cover characteristics was done (the data for the four 1m^2 were then averaged). This allowed us to assess the mice microhabitat around the traps.

Assessment at the scale of the 100 m^2 quadrats was obviously less precise and allowed to assess the general habitat at the scale of the mouse home-range.

As far as the NDVI was concerned, it was computed using satellite data with a resolution of 10 meters, it covered a larger surface and was therefore considered as a "larger scale" level of information, that allowed to characterize the trapping site. Further the data provided by the NDVI is different to the one collected directly in the field. We believe that analyzing the 3 datasets in a unique model would have been difficult to interpret.

We agree with the reviewer's comment concerning the low variable reduction from 7 raw variables to 5 composite ones in each case. However, our aim was not to reduce the number of variables but to transform their distribution to allow further statistical analysis. It was important for us to keep as much variation as possible not to mask heterogeneity between our studied sites (i.e. to be conservative when concluding that the replicate (sites/populations) for each species were similar). Additionally, in the ACP analyses we saw no obvious cut-off point (i.e., low improvement of inertia, see **Supplementary Tables 4 & 5**) that would have made us include a smaller subset of principal components in downstream analyses.

We acknowledge that this section was not sufficiently detailed to provide a satisfactory understanding of the methods. Following a traditional Multivariate Analysis of Variance (MANOVA, assuming multivariate normality and homoscedasticity of residuals), post-hoc tests aiming at checking which individual variables (as opposed to all variables together) differ between groups can usually be carried out.

As no such dedicated function exists for *PERMANOVA*, we employed an approach applying the most parsimonious model obtained with the PERMANOVA to each physiological marker: investigating which of the 12 markers influenced more significantly the outcome of the PERMANOVA. Moreover, to control for multiple testing (same hypothesis tested with several variates) we applied the Bonferroni correction.

10. I would like to point out that the authors have done a remarkable effort in the discussion section to interpret their results. Nevertheless, I suggest that the authors reorganize a little this section to make it more comprehensible. For example, the authors divided their discussion into two parts: "seasonal variation in physiology" and "interspecific differences". However, some physiological results are presented before the relevant section.

The physiological results presented before the two main parts of the discussion were irrelevant to both seasonal, or species effects, which is why they were not included in these two main parts. However, following re-analysis of the data, following the advice of referee 1, we have made structural changes to the discussion.

Minor comments:

1. Line 166: please, replace "sassessed" by "were assessed"

We thank the reviewer for pointing this mistake out and have corrected it.

2. Line 170: "trapping effort was 9688 trap nights". Does this mean that 9688 traps were set overnight? Then, were they checked twice a day, as indicated in line 164? In addition, I suggest to the authors to put this sentence before "Since Rhabdomys is mostly diurnal […]".

This means that a total of 9688 traps were set and checked twice a day IN TOTAL over the course of the study (details in Supplementary Table 1).

3. Line 194: I did not find the supplementary table 3 (only the title). In addition, supplementary table 2 is not mentioned in the text of the article. So, is it necessary?

We excluded "suppl table 3" from the new version of the manuscript as it contained raw data that will be available on DRYAD upon the paper acceptance.

We have updated the main text to contain references to the Supplementary Material in the relevant sections.

4. Supplementary Table 1: title is not placed correctly.

corrected

5. Supplementary table 4: the title should appear before the table. I suggest to add a legend to explain the different abbreviations (ALB, ALP, ALT, AMY, etc).

The meaning of each acronym is now explained in the Table caption.

6. Line 247: the paragraph about "age classes" should be appear before the section about "body condition" because it is considered as explanatory variable for body condition of individuals by the authors.

We have made the suggested revisions on the structure of this section.

7. Figure 2 is not used in the article.

We have updated the main text to contain references to Figure 2 in the relevant section.

8. Lines 316: "A post-hoc Tukey […] showed that all sites had a significantly lower NDVI in September compared to May (Figure 4)". In Figure 4, statistical results are not visually indicated.

We have updated the figure to visually indicate significant differences in NDVI.

9. Line 352: please, indicate the figure.

10. A lot of tables and figures are not mentioned and used (in supplementary section).

We have updated the main text to contain references to the Supplementary Material in the relevant sections.