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PROCEEDINGS B

# Rate dynamics of ectotherm responses to thermal stress

Aleksandra Kovacevic, Guillaume Latombe and Steven L. Chown

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**Review timeline**
Original submission: 21 January 2019
Revised submission: 21 March 2019
Final acceptance: 9 April 2019

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

# Review History

## RSPB-2019-0174.R0 (Original submission)

## Review form: Reviewer 1

**Recommendation**
Accept with minor revision (please list in comments)

**Scientific importance: Is the manuscript an original and important contribution to its field?**
Excellent

**General interest: Is the paper of sufficient general interest?**
Excellent

**Quality of the paper: Is the overall quality of the paper suitable?**
Excellent

**Is the length of the paper justified?**
Yes

**Should the paper be seen by a specialist statistical reviewer?**
No

**Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.**
No

**It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.**

**Is it accessible?**
Yes

**Is it clear?**
Yes

**Is it adequate?**
Yes

**Do you have any ethical concerns with this paper?**
No

**Comments to the Author**
This is a timely, well written contribution that contains a comprehensive assessment of a major question in understanding thermal stress of organisms. The authors produce a suite of novel analyses of several theoretical (mathematical/statistical) solutions to understanding thermal ramping rate effects that together produces novel and important insights into this classic problem. I particularly appreciated the distinction between different sources of variation (biological/statistical). The phylogenetic models are cutting edge and further reveal interesting insights that help understand the key sources of variation that may arise. I can find no major faults in the logic employed, methodology or results presented. The manuscript should be of broad interest to the readership of the journal.

I have no major comments but a few small specific points to consider during revision.

The authors may wish to see and cite this paper online early now: Jorgensen et al. How to assess Drosophila heat tolerance: Unifying static and dynamic tolerance assays to predict heat distribution limits. Functional Ecology doi: 10.1111/1365-2435.13279. I think it relevant and would be useful to include a line or two as it supports the author's view of the Rezende tolerance landscapes study too.

Minor comments

L. 79 – 'the expectation is that critical thermal limits should improve' – is it worth adding that this is a 'theoretical' expectation? Or possibly another type of expectation.

L. 273- 'not universally observed pattern among ectotherms' – 'the' is missing?

L. 279 – "Similarly, ontogeny, body size, and sex also have varying effects on heat and cold tolerance [40-42]." Perhaps also worth adding reference to nutritional or desiccation stress which has been the focus of several ramping rate discussions too?

L 324 – 'we obtained different best-fitting model' – missing 'a' before 'different'?

L 349 – 'high stressful temperature' – perhaps rephrase the sentence so it works for both upper and lower thermal limits? E.g. 'high' replaced with 'extremes' of stressful temperature.

Figure 4 – symbols are not explained but should be. Presumably they match taxonomic affiliation of those in Fig. 1 but it is presently unclear.

Figure 5 – I found this figure hard to follow despite the accompanying text in the ms Discussion being quite clear. I think I get what the authors are trying to convey although my confusion probably arises from the way it is illustrated. For example, broken (separated) arrows of the thermal tolerance arrows and lack of an axis legend on the y-axis. Can the authors revisit this and the legend to make the figure standalone more clearly? Is it possible that one of these patterns be considered the null model or null expectation?

## Review form: Reviewer 2

**Recommendation**
Accept with minor revision (please list in comments)

**Scientific importance: Is the manuscript an original and important contribution to its field?**
Good

**General interest: Is the paper of sufficient general interest?**
Acceptable

**Quality of the paper: Is the overall quality of the paper suitable?**
Good

**Is the length of the paper justified?**
Yes

**Should the paper be seen by a specialist statistical reviewer?**
No

**Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.**
No

**It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.**

**Is it accessible?**
N/A

**Is it clear?**
N/A

**Is it adequate?**
N/A

**Do you have any ethical concerns with this paper?**
No

**Comments to the Author**
Review of RSPB-2019-0174, entitled "Rate dynamics of ectotherm responses to thermal stress" submitted to Proceedings B.

Based on existing literature the study analyses and discuss the effect of rate (ramping rate) for estimates of critical thermal limits, and specifically discuss two current frameworks for predicting and explaining these effects.
The paper is generally well written and well argued, and as a thermal biologist I see a lot of merit to the general discussion and premise of the study. However, I am less convinced that the paper in its current form really achieve its full potential for providing novel knowledge or set a clear direction for future research (the statement in the abstract that rate cannot be ignored is probably not surprising to many researchers in the field).

Main topics to consider are:
Analyses are done in the framework of time versus CTmax, and here time is used as a proxy for rate. However, rate and starting temperature are confounded and it is unclear how starting temperature were handled in the analyses. Thus, while the main patterns are likely quite solid, the more specific (e.g. intra-specific patterns) are likely very sensitive to laboratory assay differences or differences in starting temperature. Furthermore, here noise likely drives patterns derived from extrapolation (log time 0 where no data usually exists). The recent paper in Functional Ecology from the Overgaard laboratory might be useful to look up for a take and some data on e.g. the relationship between z and CTmax (or z' and Ctmin) and why such a relationship exists. I also miss
The paper contains some discussion of the mechanistic background for different responses, however, this is not something the data really contribute, so such a discussion really needs to cautiously considered.
A paper like this ought to (in order to be of more general interest for the Proc B readership) make general conclusions, discuss the wider the perspectives and set the direction for future research (new hypothesis, work that needs to be done) – which it does not really achieve.

Small stuff:
How can the 95% CI nor cover the median (e.g. line 229-230 & 240)?
What is an adult species (line 304)

# Decision letter (RSPB-2019-0174.R0)

20-Feb-2019

Dear Ms Kovacevic:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

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If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
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If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,

Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
The manuscript was reviewed by myself and two experts in the field of thermal biology. As you can see from their reviews, the reviewers both liked the manuscript and agree that the manuscript was well written and the ideas and general framework are of broad interest. However, there are some important points and clarifications that were raised and that should be addressed prior to publication including the following: 1) the general framework of the study uses time versus CTmax. How might this impact the analyses? How was starting temperature accounted for in the analyses? 2) There is some important research that should be included/considered, in particular both reviewers point to work from the Overgaard laboratory: Functional Ecology doi: 10.1111/1365-2435.13279. 3) Broaden the perspectives of the discussion for the readership of Proceedings.4) Figures 4 and 5 need further work (Legend/discussion) to allow for easier interpretation. In addition to these issues, there are a few other minor suggested changes the authors should address.

Reviewer(s)' Comments to Author:

Referee: 1

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Referee: 2

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Review of RSPB-2019-0174, entitled "Rate dynamics of ectotherm responses to thermal stress" submitted to Proceedings B.

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The paper is generally well written and well argued, and as a thermal biologist I see a lot of merit to the general discussion and premise of the study. However, I am less convinced that the paper in its current form really achieve its full potential for providing novel knowledge or set a clear direction for future research (the statement in the abstract that rate cannot be ignored is probably not surprising to many researchers in the field).

Main topics to consider are:
Analyses are done in the framework of time versus CTmax, and here time is used as a proxy for rate. However, rate and starting temperature are confounded and it is unclear how starting temperature were handled in the analyses. Thus, while the main patterns are likely quite solid, the more specific (e.g. intra-specific patterns) are likely very sensitive to laboratory assay differences or differences in starting temperature. Furthermore, here noise likely drives patterns derived from extrapolation (log time 0 where no data usually exists). The recent paper in Functional Ecology from the Overgaard laboratory might be useful to look up for a take and some data on e.g. the relationship between z and CTmax (or z' and Ctmin) and why such a relationship exists. I also miss
The paper contains some discussion of the mechanistic background for different responses, however, this is not something the data really contribute, so such a discussion really needs to cautiously considered.
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Small stuff:
How can the 95% CI nor cover the median (e.g. line 229-230 & 240)?
What is an adult species (line 304)

## Author's Response to Decision Letter for (RSPB-2019-0174.R0)

See Appendix A.

# RSPB-2019-0174.R1 (Revision)

## Review form: Reviewer 1

**Recommendation**
Accept as is

**Scientific importance: Is the manuscript an original and important contribution to its field?**
Excellent

**General interest: Is the paper of sufficient general interest?**
Excellent

**Quality of the paper: Is the overall quality of the paper suitable?**
Excellent

**Is the length of the paper justified?**
Yes

**Should the paper be seen by a specialist statistical reviewer?**
No

**Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.**
No

**It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.**

**Is it accessible?**
Yes

**Is it clear?**
Yes

**Is it adequate?**
Yes

**Do you have any ethical concerns with this paper?**
No

**Comments to the Author**
The authors have addressed all my comments thoroughly and I am happy with the revised version. The paper makes a great contribution on a topic of broad importance.

## Review form: Reviewer 2

**Recommendation**
Accept as is

**Scientific importance: Is the manuscript an original and important contribution to its field?**
Acceptable

**General interest: Is the paper of sufficient general interest?**
Acceptable

**Quality of the paper: Is the overall quality of the paper suitable?**
Good

**Is the length of the paper justified?**
Yes

**Should the paper be seen by a specialist statistical reviewer?**
No

**Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.**
No

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**Is it accessible?**
Yes

**Is it clear?**
Yes

**Is it adequate?**
Yes

**Do you have any ethical concerns with this paper?**
No

**Comments to the Author**
In the reivsed version the authors have adequately revised the text in response to the points raised by the referees. Further, the authors have added more anayses to document the robustness of the analyses.

## Decision letter (RSPB-2019-0174.R1)

09-Apr-2019

Dear Ms Kovacevic

I am pleased to inform you that your manuscript entitled "Rate dynamics of ectotherm responses to thermal stress" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

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All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
The authors have addressed all my comments thoroughly and I am happy with the revised version. The paper makes a great contribution on a topic of broad importance.

Referee: 2

Comments to the Author(s)
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# Appendix A

Professor Spencer Barrett
Editor-in-Chief
Proceedings of the Royal Society B

Dear Professor Barrett

RE: RSPB-2019-0174, Kovacevic et al.

Thank you for your e-mail of 20th February 2019. We appreciate the careful reviews and the helpful comments provided by the Associate Editor. We have addressed all of the comments by way of revision of the work and below we set out in detail the changes made, and our rationale for making them in the context of the Associate Editor and Reviewers' comments. We note that we have had to move some display elements to the Supplementary Material to stay within page limits. We would be happy to discuss the decisions we made if these do not sit well with the Editors.

We trust that the ms is suitable for publication, but please do not hesitate to contact us should there be any further queries.

Sincerely,

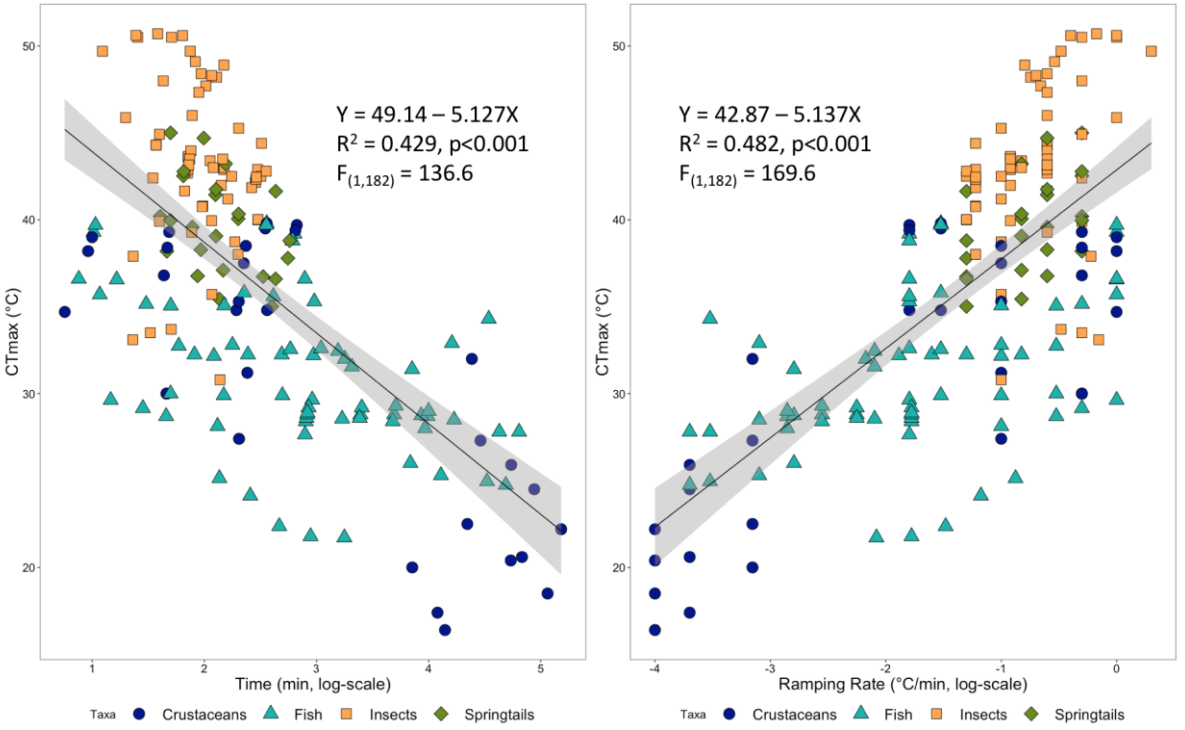
Aleksandra Kovacevic

On behalf of the authors

Associate Editor
Board Member: 1
Comments to Author:
The manuscript was reviewed by myself and two experts in the field of thermal biology. As you can see from their reviews, the reviewers both liked the manuscript and agree that the manuscript was well written and the ideas and general framework are of broad interest. However, there are some important points and clarifications that were raised and that should be addressed prior to publication including the following: 1) the general framework of the study uses time versus CTmax. How might this impact the analyses? How was starting temperature accounted for in the analyses? 2) There is some important research that should be included/considered, in particular both reviewers point to work from the Overgaard laboratory: Functional Ecology doi: 10.1111/1365-2435.13279. 3) Broaden the perspectives of the discussion for the readership of Proceedings.4) Figures 4 and 5 need further work (Legend/discussion) to allow for easier interpretation. In addition to these issues, there are a few other minor suggested changes the authors should address.

*Thank you for the opportunity to revise our manuscript. In response to the specific matters raised:*
*1) We note that in the interspecific analyses, starting temperature is explicitly included in the calculation of time (equations 1 and 2 of the original ms and also the revision). This explains*

*our use of time rather than rate. That is, we include both variables independently. Of course, this raises the question of what the effect might have been had we included rate only. We redid the analyses using this approach, which we believe would be the more problematic approach because we would then be ignoring starting temperature (contrary to the advice of Terblanche et al. 2007, Proc B). Using either time or rate does not significantly change the final outcome of the analysis or form of the response patterns exhibited by the species as illustrated below (time left, rate right for CTmax). As might be expected, there is a slightly better fit using rate because the variance introduced by different starting temperatures is ignored. Likewise, some difference in intercept is present, again, as might be expected. The slopes of the relationships do not differ.*




*For these reasons we do not think that our interspecific analyses are impacted in any way except to the extent that they are in keeping with the need to include both starting temperature and rate in analyses. We have included this figure in the Supplementary Material (SI Appendix, Fig. S4) and refer to it in the Results section.*

*In the case of the intraspecific investigations, we note that it is only Terblanche et al. (2007, Proc B) whom undertook an extensive analysis of rate and starting temperature interactions. No other studies we included took this approach. To determine what, if any, effect might be had on the outcomes of the modelling (using Kingsolver & Ubanhowar's method) we completed an analysis of the mean data as we had in the original ms., but now using all starting temperatures from the Terblanche et al. (2007) investigation. The table below shows no effect of starting temperature.*

| Species | RampRate (°C/min) | StartTemp (°C) | CTmax (°C) | FailTime (min) | M (1/min) | Best-fitting model per Start Temp |
|---|---|---|---|---|---|---|
| GP35 | 0.06 | 35 | 40.8 | 96.66666667 | 0.010344828 | Exponential Threshold |
| GP35 | 0.12 | 35 | 43.6 | 71.66666667 | 0.013953488 | |
| GP35 | 0.25 | 35 | 44.3 | 37.2 | 0.02688172 | |
| GP38 | 0.06 | 38 | 41.9 | 65.17 | 0.015344484 | Exponential Threshold |
| GP38 | 0.12 | 38 | 44.1 | 50.5 | 0.01980198 | |
| GP38 | 0.25 | 38 | 45.7 | 30.88 | 0.03238342 | |
| GP41 | 0.06 | 41 | 42.9 | 31.67 | 0.031575624 | Exponential Threshold |
| GP41 | 0.12 | 41 | 43.7 | 22.42 | 0.044603033 | |
| GP41 | 0.25 | 41 | 45 | 15.88 | 0.062972292 | |

*Because only a single study takes such a comprehensive approach, and because no effect of starting temperature is detected, we have not included this analysis in the ms. Should the Editors be of the view that we should do so, we would be pleased to oblige.*

*2) We appreciate the importance of the new research from the Overgaard laboratory, which we did not initially have a chance to include because we saw it only after submission, but have now done so.*

*3) We now broaden the perspective in the discussion and suggest directions and new avenues for future research. We have set out, in the response to Reviewer 2, the exact nature of the changes and have not repeated them here. We appreciate the guidance from the Reviewer and from the Associate Editor.*

*4) We have modified Figures 4 and 5 for easier interpretation.*

*5) All minor matters for clarification have been attended to.*

Reviewer(s)' Comments to Author:

**Referee: 1**

Comments to the Author(s)
This is a timely, well written contribution that contains a comprehensive assessment of a major question in understanding thermal stress of organisms. The authors produce a suite of novel analyses of several theoretical (mathematical/statistical) solutions to understanding thermal ramping rate effects that together produces novel and important insights into this classic problem. I particularly appreciated the distinction between different sources of variation (biological/statistical). The phylogenetic models are cutting edge and further reveal interesting insights that help understand the key sources of variation that may arise. I can find no major faults in the logic employed, methodology or results presented. The manuscript should be of broad interest to the readership of the journal.

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The authors may wish to see and cite this paper online early now: Jorgensen et al. How to assess Drosophila heat tolerance: Unifying static and dynamic tolerance assays to predict heat distribution limits. Functional Ecology doi: 10.1111/1365-2435.13279. I think it relevant and would be useful to include a line or two as it supports the author's view of the Rezende tolerance landscapes study too.

*Thank you for pointing out this study. We saw it shortly after submission and recognized its relevance and value immediately. We appreciate the opportunity to include reference to it and now do so. Specifically we raise this work in the following parts of the ms:*

*1. In the Introduction we now write: 'Several studies have now considered the thermal tolerance landscape approach, the most comprehensive of which is a recent analysis of 11 Drosophila species focussing especially on knockdown time [22].'*

*2. In the Discussion we now write: 'Indeed, assessment of the method used by Rezende et al. [2] revealed that the parameters z (z'), a constant characterising the sensitivity to the temperature change, and $CT_{max}$ ($CT_{min}$), redefined as a knockdown temperature at 1 min of exposure, are not always highly correlated as originally proposed. These outcomes are in agreement with a recent comprehensive assessment of the thermal landscapes approach, which demonstrated that heat tolerance parameters, z and $CT_{max}$ at 1 min of exposure in 11 Drosophila species are not correlated [22]. The lower correlation of z and $CT_{max}$ than the one found in the study by Rezende and the colleagues [2] may have several explanations. One of these may be variation of species' $CT_{max}$ response patterns to the ramping rate, since two thirds of the species show the decline in thermal tolerance with the longer exposure time (i.e. slower ramping rate), while the rest of the species yield different responses. The other explanation, supported by evidence from modelling analyses, is that experimental noise, autocorrelation and unwarranted extrapolation, are responsible for the initial finding of a strong relationship between z and $CT_{max}$* [22]. *Perhaps unsurprisingly, therefore, we also found no correlation between z' and $CT_{min}$, a result while different to the original thermal landscapes idea [2], is in keeping with the growing body of literature testing it [22].'*

**Minor comments**

L. 79 – 'the expectation is that critical thermal limits should improve' – is it worth adding that this is a 'theoretical' expectation? Or possibly another type of expectation.

*We have added the term "theoretical", as suggested, to clarify that we refer to the model proposed.*

L. 273- 'not universally observed pattern among ectotherms' – 'the' is missing?

*Thank you, we have made the correction.*

L. 279 – "Similarly, ontogeny, body size, and sex also have varying effects on heat and cold tolerance [40-42]." Perhaps also worth adding reference to nutritional or desiccation stress which has been the focus of several ramping rate discussions too?

*Thank you. We have now included both nutritional and water balance stress and have included appropriate citations to support the additions:*

*'Similarly, ontogeny, body size, and sex, along with nutritional status and the extent of desiccation stress have varying effects on heat and cold tolerance [36-40].'*

L 324 – 'we obtained different best-fitting model' – missing 'a' before 'different'?

*Yes, we have corrected the error as suggested.*

L 349 – 'high stressful temperature' – perhaps rephrase the sentence so it works for both upper and lower thermal limits? E.g. 'high' replaced with 'extremes' of stressful temperature.

*Rephrased the sentence as suggested to include both upper and lower thermal limits as follows: "This response potentially reveals an optimal ramping rate at which maximum tolerance gain is achieved due to acclimation to the rapid change in temperature, while an organism simultaneously becomes exposed to the deleterious effects of temperature extremes."*

Figure 4 – symbols are not explained but should be. Presumably they match taxonomic affiliation of those in Fig. 1 but it is presently unclear.

*Thank you for noticing this. The legend is inserted within each figure panel and this has now been fixed.*

Figure 5 – I found this figure hard to follow despite the accompanying text in the ms Discussion being quite clear. I think I get what the authors are trying to convey although my confusion probably arises from the way it is illustrated. For example, broken (separated) arrows of the thermal tolerance arrows and lack of an axis legend on the y-axis. Can the authors revisit this and the legend to make the figure standalone more clearly? Is it possible that one of these patterns be considered the null model or null expectation?

*We have modified the figure, legend, and accompanying text to match the clarity of discussion as suggested so that the message figure provides stands well on its own. Indeed, pattern 'c) no effect' could be considered the baseline or the null model, since several empirical results suggest that regardless of the rate of the temperature change, we can observe no change in CTs. Therefore, the other patterns could present deviations from this baseline. However, we don't want to overstep our current understanding and define it as a null model because we don't yet know the underlying mechanistic reason for this outcome where rate has no effect.*

*Changed Figure 5 legend as follows:*

*'Hypothetical relationship between failure rate and recovery rate and their effect on thermal tolerance. a) Thermal tolerance declines with exposure time if recovery rate cannot catch up with the failure rate; b) Thermal tolerance increases with exposure time if recovery rate improves over time and overcomes failure rate; c) Thermal tolerance is not affected by exposure time when failure rate and recovery rate are closely matched; d) Thermal tolerance initially shows an increase (or a decrease) in thermal tolerance, reaches a peak high (or low) temperature at a certain ramping rate (i.e. time of exposure), followed by a decrease (or an increase) in thermal tolerance. This response potentially reveals an inflection point or ramping rate, which either decreases or improves recovery rate relative to the failure rate.'*

**Referee: 2**

Comments to the Author(s)
Review of RSPB-2019-0174, entitled "Rate dynamics of ectotherm responses to thermal stress" submitted to Proceedings B.

Based on existing literature the study analyses and discuss the effect of rate (ramping rate) for estimates of critical thermal limits, and specifically discuss two current frameworks for predicting and explaining these effects.
The paper is generally well written and well argued, and as a thermal biologist I see a lot of merit to the general discussion and premise of the study. However, I am less convinced that the paper in its current form really achieve its full potential for providing novel knowledge or set a clear direction for future research (the statement in the abstract that rate cannot be ignored is probably not surprising to many researchers in the field).

Main topics to consider are:
Analyses are done in the framework of time versus CTmax, and here time is used as a proxy for rate. However, rate and starting temperature are confounded and it is unclear how starting temperature were handled in the analyses. Thus, while the main patterns are likely quite solid, the more specific (e.g. intra-specific patterns) are likely very sensitive to laboratory assay differences or differences in starting temperature.

*As we pointed out in our response to the Associate Editor, very few studies (one we could find) examine interactions between starting temperature (ST) and rate. As we also show in that response, ST was (and remains) included explicitly in the interspecific analyses and we have now included in the supplementary materials a figure showing minimal effects of an alternative, but we think less appropriate, approach.*

*The intraspecific analyses generally only included a single ST because that is what most authors have done. Thus, we write in the Methods: 'It is important to highlight that we tested these models using the reported mean values of CTLs for each ramping rate and a single experimental starting temperature per species, with the analyses focusing on the effect of ramping rate at the intraspecific level.'*

*Our view is that doing so avoids confounding ST and rate. We have, however, now examined the likely effect of ST using the comprehensive dataset of Terblanche et al. (2007 Proc B). As we show (see the Table above) ST has no effect on the outcomes. Thus, we do not think that the analyses are confounded. Nonetheless, the reviewer raises an important point that we think deserves further consideration and we now raise this matter in the more general discussion, noting:*

*'However, as an early study showed [9], so too is the starting temperature of the process, since this may determine the extent to which an organism is already outside the zone of tolerance [48], which precedes the onset of damage. Just what the effect is of starting temperature on experimental outcomes is not yet well resolved. The proposed framework suggests that future work should focus on three main areas. First, determining whether starting temperature has as large effect as ramping rate on outcomes, as a single study suggests it might [9] and whether a threshold effect, indicating that differences in starting*

*temperatures inside or outside the organism's zone of tolerance (i.e. on either side of the incipient lethal temperature [48]) are important.'*

Furthermore, here noise likely drives patterns derived from extrapolation (log time 0 where no data usually exists). The recent paper in Functional Ecology from the Overgaard laboratory might be useful to look up for a take and some data on e.g. the relationship between z and CTmax (or z' and Ctmin) and why such a relationship exists.

*Thank you for raising this point and for the useful insight. We have now made this exact point in the Discussion and refer explicitly to the Jørgensen et al. (2019) paper on the likely effects of extrapolation, autocorrelation and noise (which actually means low sample size). We write:*

*'These outcomes are in agreement with a recent comprehensive assessment of the thermal landscapes approach, which demonstrated that heat tolerance parameters, z and $CT_{max}$ at 1 min of exposure in 11 Drosophila species are not correlated [22]. The lower correlation of z and $CT_{max}$ than the one found in the study by Rezende and the colleagues [2] may have several explanations. One of these may be variation of species' $CT_{max}$ response patterns to the ramping rate, since two thirds of the species show the decline in thermal tolerance with the longer exposure time (i.e. slower ramping rate), while the rest of the species yield different responses. The other explanation, supported by evidence from modelling analyses, is that experimental noise (or small sample size), autocorrelation and unwarranted extrapolation, are responsible for the initial finding of a strong relationship between z and $CT_{max}$* [22]*. Perhaps unsurprisingly, therefore, we also found no correlation between z' and $CT_{min}$, a result while different to the original thermal landscapes idea [2], is in keeping with the growing body of literature testing it [22].'*

The paper contains some discussion of the mechanistic background for different responses, however, this is not something the data really contribute, so such a discussion really needs to cautiously considered.

A paper like this ought to (in order to be of more general interest for the Proc B readership) make general conclusions, discuss the wider the perspectives and set the direction for future research (new hypothesis, work that needs to be done) – which it does not really achieve.

*We have now entirely modified the Discussion. We note that our framework is quite new, but agree that we did not explore its benefits sufficiently well. Thus, we have removed the small statement on specific mechanistic matters (which was simply in support of Figure 5 and as the Reviewer noted, not especially pertinent). We have also deleted the final paragraph and replaced it with a section outlining both the general implications of our findings and the areas future work should consider. In doing so, we should note that finding independent support for the failure rate approach is of considerable significance, so we have further highlighted this, which did not come through in our original draft. We appreciate the Reviewer's guidance here. Thus, we now write:*

*'Based on the four response patterns found, we propose a set of hypothetical relationships between failure rate [23], recovery rate, and critical thermal limits that may explain the variation in species' responses (Fig. 3). If recovery rate cannot catch up with the failure rate*

*during prolonged time of exposure (Fig. 3a), thermal tolerance declines with exposure time. On the other hand, if recovery rate improves with time due to a rapid physiological response, thermal tolerance increases with exposure time (Fig. 3b). In the case of the no effect response, time of exposure (i.e. ramping rate) might have no effect on thermal tolerance (Fig. 5c). Alternatively, failure rate and recovery rate could be matched due to some form of beneficial physiological response by an organism. Finally, a mixed response could reveal an inflection point [8], with a pattern of declining thermal tolerance from an intermediate ramping rate towards faster and slower rates (Fig. 3d). This response potentially reveals an optimal ramping rate at which maximum tolerance gain is achieved due to acclimation to the rapid change in temperature, while an organism simultaneously becomes exposed to the deleterious effects of temperature extremes.*

*What these four relationships provide is a framework for further exploration of the way in which differing damage accumulation rates and organismal-level physiological and biochemical response rates interact to determine thermal tolerance. Clearly, time of exposure (given different ramping rates) is an important component thereof, especially given the high $Q_{10}$ of the process [22]. However, as an early study showed [9], so too is the starting temperature of the process, since this may determine the extent to which an organism is already outside the zone of tolerance [48], which precedes the onset of damage. Just what the effect is of starting temperature on experimental outcomes is not yet well resolved. The proposed framework suggests that future work should focus on three main areas. First, determining whether starting temperature has as large effect as ramping rate on outcomes, as a single study suggests it might [9] and whether a threshold effect, indicating that differences in starting temperatures inside or outside the organism's zone of tolerance (i.e. on either side of the incipient lethal temperature [48]) are important. Second, further considering the outcomes of the failure rate approach in the context of the framework proposed here to determine the extent to which simple failure rate models may afford the null expectation for thermal limits in the absence of biological responses [23]. The failure rate models provide good fits to the available data and are readily interpretable both in a statistical and physiological context. Finally, investigation of whether differential rates of damage and repair really are responsible for variation in the rate-thermal limit response. Very high rates of $Q_{10}$ for thermal limits [22] suggest that, at least at the highest temperatures, any form of repair will be rapidly overwhelmed given generally lower thermal sensitivities of routine physiological functions including, for example, protein synthesis [49]. Of course, even in the absence of investigation of these questions, it is clear that rate variation cannot be ignored in compiled comparative studies, either of critical thermal limits or of their implications for environmental change.'*

Minor corrections

How can the 95% CI nor cover the median (e.g. line 229-230 & 240)?

*Thank you for noticing this, we had made an error here and have rechecked the data and corrected this. Corrected 95% CIs now read:*

*'median $r^2$ = 0.872, 95% CI between 0.656 and 0.976'*
*'median $r^2$ = 0.858, 95% CI between 0.694 and 0.956'*

What is an adult species (line 304)

*Changed to "species of beetles in the adult life stage"*