Reviewer comments: (all recommended changes/suggestions in quotation marks below)

The study uses *Drosophila subobscura* as a model to study the metabolic rate responses to thermal stress and does so using slow- and fast-ramping temperatures and compares them to a control treatment. These designs are important and give us a snapshot into how species will respond to future climate change. However, there are a few points to clarify and a major revision of the text so that the study is received well and is consistent in its presentation. The language needs to be checked thoroughly as in places they are not fully formed sentences. I have highlighted a few and did not want to go through all of them, please could you take some time and look at the entire manuscript for consistency.

The other issue with this is that it is not clearly defined in terms of the importance of the context of this work. For example, why not compare thermal plasticity and its impacts on insects within fluctuating and constant environments, providing a solid basis for the framework of this design (like for

e.g., https://www.annualreviews.org/doi/abs/10.1146/annurev-ento-010814-021017).

Title:

• Typo- change heat tolerant to 'heat tolerance'

Abstract:

- Typo- # 2 change which allows to organisms to 'allows organisms to'
- Reword- #5 sentence needs rewording (L44-46). For example: 'should be taken into account for future studies to understand and predict adaptive responses to continued climate change'

Introduction:

- Typo- L49 change led to 'lead'
- Reword- L52 change organisms cause that fitness to 'causes fitness declines, their abundance and distribution is likely to be affected exposing them to current and future increases of temperature'
- L54 change to understand the capacity of...'to explore how ectotherms will withstand global warming'
- L58 change responses of to 'responses at'
- Throughout the manuscript, please check the use of replicated lines and change it to 'replicate' lines.
- L96 'an' emergent property
- L114-L115, the references provided are quite limited and recent systematic empirical work shows the extent of damage of reproduction from thermal/heat stress (Sales et al. 2018; <u>https://www.nature.com/articles/s41467-018-07273-z</u>
 Parratt et al. 2021; <u>https://www.nature.com/articles/s41558-021-01047-0</u>
 Van Heerwarden and Sgro 2021, <u>https://www.nature.com/articles/s41467-021-22546-w</u>)

Methodology:

- L138 change feed to 'fed' (make changes throughout the manuscript for grammatical inconsistencies)
- L139 population, mention 'population size like you do in L136'

- L143 and elsewhere throughout the manuscript, replicated = 'replicate'
- L145 what is 'positive oviposition?'
- L149 delete 'flies belong to the' and follow this style elsewhere in the article
- L165 change it to 'four-day old virgin' maintain a consistent style!
- L170 size of the cotton mesh? Is this for ventilation?
- L184 change from 27th generation to generation 27 (keep it consistent throughout)
- L237-238 poisson (link=?) also mention it everywhere you have used a distribution family!
- Results L257-262, why is it important to run tests on the mean ramping of the temperatures- to me this makes little sense, but perhaps you have a better idea? Make it clearer for the reader here please.
- L285 and throughout the results P=0.04 is only a marginal significance, so be careful on over emphasis of low P values and report it responsibly!
- L285 significatively? A typo?
- L288-291 is hard to follow, please simplify this.

Discussion:

- The entire discussion needs restructuring and having a better context on the findings of the study, then link it to the findings of other studies that support or fail to support what you find
- Then, also speculate on the reasons why this might be because of the less harsh temperatures that were used in this experiment and that metabolic rate could be evolutionarily conserved as changing it means serious consequences for an organism like an ectotherm. Use the Colinet et al. 2015 paper to construct some of the ideas! As metabolic rates at higher temperatures remain constant for maintenance purposes etc. (pg. 129)
- Within the area where you discuss adaptive responses, you could refer to a study that looked at adaptive thermal plasticity in reproduction in an insect, especially when temperatures were drastically changes, sperm and reproductive output changed too in order to improve male fitness (see, Vasudeva et al. 2019; <u>https://doi.org/10.7554/eLife.49452.001</u>)
- Your conclusion is good and provides an importantly open ended question on how organisms will respond in the future to climate change!