

Poitiers, December 5th, 2022

Dear Editor,

In September 2022, we submitted the manuscript entitled "Deleterious effects of thermal and water stresses on life history and physiology: a case study on woodlouse" for consideration as a research paper in *PCI Ecology*.

First, we would want to warmly thank the recommender and the reviewers for their insightful comments on our initial manuscript and for offering us the opportunity to revise this manuscript by accounting for the reviewers' comments.

Please find in the attached file the main text of the revised MS (with all the changes compared to the original submission in red) and below our point-by-point responses (in bold) to the reviewers' comments.

This manuscript has not been published nor is under consideration for publication elsewhere. We certify that all authors have contributed substantially to the project and they have all seen this revised manuscript and agree with its content.

We hope you will find this revised paper suitable for publication in *PCI Ecology* and look forward to hearing from you.

Sincerely,

For the authors

Sophie Beltran-Bech

Our Point-by-point responses (in bold) :

According to the Guide for Authors of PCI Ecology website, we added:

- a "Data, script and code availability" section at the end of the Material and Methods section.
- a "funding" section separated from the "acknowledgements" section, in which we have added our thanks to the two reviewers and to the recommender.
- a declaration of no conflict of interest with the section "Conflict of interest disclosure" on the abstract page.

Reviewer 1:

In this article by Depeux *et al.*, the effects of stressful temperature and humidity regimes are tested on adult terrestrial isopods in order to ascertain what effect these stressful environmental conditions may have on isopod reproductive, growth, immune system, and survival metrics. The study was conducted in two parts in two different years--the temperature experiment in 2019, and then separately, the humidity experiment in 2021. The study found that increased temperature and decreased moisture were generally harmful to the isopods in question; increased temperature resulted in nearly a 2x increase in mortality, and decreased moisture led to a 2.5x increase in mortality. Reproductive success was negatively affected by increased temperature and decreased moisture, as was immune function. The study is putatively aimed at understanding the responses of arthropods to future climate regimes.

This paper is well written, interesting, and timely. As heat and moisture are the most important regulators of insect persistence and distribution, this work is helpful in gauging invertebrate responses to future climate scenarios.

- **Thank you for your positive comments.**

I support the publication of this article, and I have only minor comments:

line 47-48 (**new line 52**): "survival decreases with increasing temperature" makes it sound like a linear function, when surely, it is a quadratic function where there is an optimal temperature where survival is at its peak, with a drop in survival on either side of the curve where temperature is higher and lower than the optimum. I would clarify this phrase.

- **We replaced the initial sentence with "increasing temperature beyond the optimum can have high negative effect on survival".**

line 60 (**new line 64**): "woodlice" (plural) should be "woodlouse" (singular). Change throughout.

- **The reviewer is true. We thus replaced 'woodlice' with 'woodlouse'.**

line 128 (**new line 132**): I would state here that the immune function tests are destructive

- **We added a sentence (lines 132-134) to make explicit that we indeed have to sacrifice the animals in the last box (where the measure of physiological traits took place) because of the protein extraction on nerve chains required to measure β -galactosidase activity. We also added an "Ethical statement" section (in response to reviewer 2) in lines 166 to 177.**

line 234 (**new line 253**): Is "time" age in days? Days in treatment? Please clarify.

- **We clarified: "time (i.e. time after placing in climatic chamber, in days)"**

line 252 (**new line 247**): I would put at the beginning that all analyses were done in R

- **We moved the sentence to the beginning of the paragraph as requested.**

line 257 (**new line 277**): "Survival was" instead of "The survival was."

- **We changed according to the reviewer's suggestion.**

Figure 1. On my black and white printer, all the colors look the same. Specifically, in C and D, I cannot tell the colors apart for the treatments.

- **We have modified the colours of the figure 1 so that, even when printed in black and white, the reader can distinguish the treatments. We used the same colours in Figures 1 and 2.**

Reviewer 2:

I recommend rejecting for the following reasons.

Firstly the paper is not providing anything new. It has been known for at least 40 years since I was at secondary school that woodlice are sensitive to moisture and temperature conditions. Woodlice are used as a paradigm in science classes to demonstrate how animals use taxes and kineses to find suitable habitat. I published a lesson plan for demonstrating taxes in woodlice in the 'journal of Biological education' 20 years ago.

- **We respectfully disagree with the reviewer's view here. While we value the use of woodlice in terms of teaching taxes and kineses in relation to habitat requirements, our study does not address at all the question of the ability of woodlice to use taxes and kineses to find suitable habitats in relation to their ecological requirements (need for moisture in particular). On the contrary, we specifically built on this knowledge gained on woodlice habitat ecology to investigate the effects of stress and moisture on the life history and physiological traits of these animals. Our study highlights that the modulation of these factors (temperature and moisture) affects the life history and physiological traits of these animals in a controlled environment (identical in all our conditions) simulating the natural environment with soil in which the animals can bury themselves and dead leaves *ad libidum* allowing possible hiding places. The novelty of our work lies in quantifying the effect of these two different stresses on these animals. At the best of our knowledge, such a work is no part of any teaching program and has not been even investigated from a research viewpoint before. As our experimental conditions are otherwise strictly identical, the responses of life history and physiological traits of "stressed" vs. "non-stressed" animals we recorded allowed revealing and quantifying the deleterious effects of both stressors that were tested independently. The next step will be to carry out experiments on field animals (as we discussed in lines 383-386). The present study will therefore serve as a reference for such future field studies.**

Secondly, the researchers used a laboratory grown line of woodlice that had been reared under constant conditions of daylength, temperature and humidity 40 years. This strain would have very different characteristics from a wild strain and would be less likely to be adaptable to any temperatures or humidity deviating from the range they are used to.

- **The reviewer's statement is not based on any firm theory or observation and, while plausible, is totally subjective. Contrary to what happens in nature, our experimental study permits a rigorous test of the effect of temperature and moisture stresses in absence of any confounding factor. It is the value of performing well-designed experiments. For instance, controlling the age of animals is far from an easy task in the wild and age is most often assessed from the size of the animals, which is at best an imperfect approximation of age because of well-established sizer variation in relation to variation in access to resources or population density). In this context, the use of our controlled laboratory line on which we developed our physiological**

markers allows accounting for the exact age of the animals (which is itself linked to life history and physiological traits (Depeux et al. 2020b)) and for their genetic origin. We indeed compared groups of the same origin (and our controlled crosses guarantee the genetic diversity of our line) and of the same age. This allowed us to limit confounding effects as much as possible and to quantify the effects of the two tested stressors independently.

- We agree with the reviewer that this line of animals has been present in the laboratory for many years under conditions that are not stressful (i.e. low variation in temperature and moisture but variation in the photoperiod, which is natural (lines 91-107)). It is why, as we proposed in discussion (lines 384-386), future studies should test and quantify the effect of temperature and moisture changes on populations along a wide continuum of variation in moisture and temperature in the wild.
- A paragraph has been added in the Discussion section to emphasise better the points mentioned above (lines 387-395). More specifically, we wrote: “Unlike what happens in nature, our experimental study on a laboratory line of woodlouse allowed us to test the effect of the temperature and moisture stresses while controlling for potentially confounding factors such as individual age. Indeed, it is highly challenging to control for individual age in the wild. In this context, the use of our controlled laboratory line on which we developed our physiological markers allowed us to account for the exact age of the animals (which is itself linked to life history and physiological traits (Depeux et al., 2020b)) and for their genetic origin. We compared groups of the same origin (and our controlled crosses guarantee the genetic diversity of our line) and of the same age. This allows us to limit confounding effects as much as possible and to quantify the effects of the two tested stressors independently.”

Thirdly, I question the ethics. Animal experimentation is becoming less acceptable. As we learn more about animal sentience, more animal taxa are being included in the circle of moral concern. The New Zealand Animal Welfare Act includes crabs and shrimps as animals showing 'sentience'. Experiments on these animals need ethics committee approval, and can be rejected if the costs to the animals outweigh any benefits.

Woodlice are not decapods, but they are crustaceans, with the same advanced nervous system as decapods. It is therefore likely that they feel pain, which makes lethal experiments on them ethically questionable, especially with no ethical oversight.

- **Thanks to the reviewer for this comment. We agree that it is obviously important to clarify the ethical statement related to our work. The Decree n°2003-768 from 01/08/2003 and the European Directive 2010/63/EU regulating animal research does not require ethical evaluation prior to research on arthropods (including woodlice). However, we complied with the ethical 3R rules (Replace/Reduce/Refine). We added an “Ethical statement” section in lines 166 to 177.**

We hope that our responses and the substantial changes we performed to get this revised version will satisfy both reviewers and the recommender.

Sincerely Yours,

For the authors

Sophie Beltran-Bech