Effects of climate warming on the pine processionary moth at the southern edge of its range: a retrospective analysis on egg survival in Tunisia

Revision 2

Decision for round #2 : *Revision needed*

minor revision before recommendation

Dear Authors,

Thank you very much for submitting a revised version of your work for recommendation in PCI Ecology. I apologize for the delay in evaluating this revision, as two of the former reviewers were not available and it took some time to find another reviewer.

Both reviewers evaluated the revision very positively and have only minor comments that should be easily addressed. Dr Iltis, who did not review the first version of the manuscript, had very interesting suggestions to improve the structure and the clarity of the manuscript, so please try to address these at best.

For my part, I am still convinced that focusing on the longitudinal analysis would be a better option for the manuscript, but I only ask you to consider all my arguments carefully before making your decision.

I am looking forward reading your final improvements on this already very good manuscript. Don't hesitate if I can be of any help, or if you want to discuss my suggestions further.

Best,

Elodie

Élodie Vercken's review:

I thank the authors for the care they took addressing my comments. I reckon you did a really good job justifying your methodological choices, so I don't have any more concern regarding the robustness of the analyses. I still have a divergent opinion though on the inter-cluster comparison, and I will try again to convince you that it might not be helpful here. I would understand if you choose to stood by your initial strategy (and I will recommend the article no matter what), but please hear me out on this because I strongly believe it can improve the readability and impact of the manuscript:

Thank you for this second round of relevant suggestions and for the minor changes recommended, which we hope we have all addressed as detailed below.

- A main strength of your manuscript, as noted by all the reviewers was that you managed to find an elegant way to analyze an unbalanced dataset. As you clearly explain at the end of the introduction, the clustering method was necessary to analyze the longitudinal data, which is your main question (as you state it l. 123- 124). So it would be justified at the end of the clustering analysis to retain only the clusters that include longitudinal data.

- Analyzing different climatic clusters over the same period is quite a different question than the impact of climate warming, and I don't feel like you have any predictions or hypotheses regarding this comparison. Inter-cluster differences might be related to random genetic differentiation or local adaptations regarding other factors than climate, and the absence of predictions make the results uninterpretable in my opinion. Indeed, if you find differences between clusters you can tentatively attribute them to temperature effects ; if you don't find any, you can suggest that the clusters have different thermal tolerance due to their specific adaptive history. It seems to me that if the data can't allow to discriminate between a null hypothesis (absence of impact of climate warming) and an adaptive one, then this specific question should not be addressed with these data.

- On a practical note, results from the inter-cluster comparison are hardly mentioned in the discussion (no mention of cluster 4 at all I think), and do not provide any further insight compared to Analysis (1). The paper is quite long and complex as it is (especially the discussion), and I am convinced there would be no loss of information if only Analysis (1) was reported, and you may still include Analysis (2) in supplementary material. To me the main messages are : (i) mean temperature has increased in cluster 3, but not in cluster 1, and it might be related to a decrease in fecundity; (ii) hatching rate decreased in both clusters, and might be more sensitive to extreme events than mean temperatures. I didn't feel that the specifics of cluster 2 add anything significant to these conclusions.

Following your suggestion, we agree that moving analysis (2) to supplementary material (see SM7) and keeping only analysis (1) in the main body of the text, as it better fits the scope of our study, is a good change that improves the readability of the manuscript and keeps it more focused. Due to this change, the analysis are not referred to as "analysis (1)" and "analysis (2)" anymore, and instead they are both described in their respective section (M&M for the main one, directly in the SM for the second one).

Minor comments:

- I think the track change version of the manuscript was updated twice instead of the “clean” revised version.

There were indeed corrections from two computers and therefore identities in the revised version, this second revision should now show only one set of revisions on behalf of all authors. In case you referred to versions 1 and 2 on the pre-print website, then both versions were actually uploaded before the manuscript was reviewed the first time; version 2 was just fixing minor issues before initial peer review (https://www.biorxiv.org/content/10.1101/2021.08.17.456665v3.article-info). The bioRxiv version to be peer reviewed after this second round of revisions in Peer Community In Ecology is therefore version 4 (uploaded on April 15th, 2022).

- The discussion is still quite long (over 6 pages). I like it though, I think it’s full of interesting ideas and perspectives, but it might help to organize it a bit more explicitly (like, having titles for each of the main parts). Ideally, it should start with two sections focused on the two main results: (i) response of clutch size to increase in mean temperature; (ii) increase of hatching failure, related to extreme events of prolonged high-temperatures

We have added sub-sections to better organize the overall structure of the Discussion (see other answer below).

- Building on the 3rd comment of Dr Ilitis, I wonder whether the results concerning the three potential underlying causes of hatching failures should be detailed fully in the main text (it feels more like supplementary material to me). You can still discuss some of the nuances in the responses of abortion and parasitism to temperature, but it will make the results section (and possibly the discussion) shorter and clearer. For instance, on lines 425-429 (track changes version), the distinction between the 3 internal processes is quite confusing. First, it says that parasitism has increased (though I think it is the opposite, am I wrong?). Then, the relationship between abortion rate and temperature is highlighted, which suggest that abortion rate is the driving factor between the hatching rate decrease. But from the very next sentence to most of the following 2 pages, it is the relationship between extreme heat and sterility rate that is mostly discussed. So the whole logic behind the different arguments is quite difficult to follow, and it could benefit from a bit of further structuring, as suggested from my previous comment. In the same line, Fig 5 could be lightened by including only fecundity and hatching rate.

You are right that parasitism rate has decreased indeed, thank you for mentioning this mistake in the text. It has now been fixed (see line 386).

Regarding the discussion, we added titles and subsections to clarify the structure as follows: i. Hatching failure and heatwaves (see line 384), ii. Thermal tolerance and phenology among populations (see line 482), iii. Host-parasitoid interactions and outbreaks in a warming climate (line (544), and iv. Other factors influencing distribution (line 588), v. Conclusion (line 602).

As we modified the structure of results and first presented mortality factors based on Corentin Iltis’s third comment, we believe it is still relevant to keep other factors in Fig 5. We are also afraid that removing most egg phenotypes variables from Fig. 5 may go against comments received during the first round of reviews of the MS, since other reviewers were interested in those other phenotypes, although they are admittedly discussed to a lower extent in the manuscript.

Review by Matt Hill

> The authors have handled all comments expertly and this new version reads well. The issues raised by the other reviewers have been well covered too I feel.
>
> Thanks especially for the clarity around the data resolution as well, that response makes sense and I'm glad it was just an error in the way it was written in the text. Figure 3 is nicer to read in that single column too.

Thank you, we greatly appreciate the feedback. We committed the minor changes you suggested.

> A couple of small changes:
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> Line 59: Is the Parmesan and Yohe reference still relevant / needed here?

This reference was deleted.

> Line 186: parentheses changed to Démolin (1969)

Done.

Review by Corentin lltis

> In this study, the authors investigate the potential impacts of climate change on reproductive (fecundity) and survival traits (egg hatching success/failure) of local populations of the pine processionary moth at the southern edge of its distribution (Tunisia). They test the hypothesis that local facets of climate change (overall warming and increased incidence of extreme high temperatures) should negatively impact the pest population dynamics and thus explain the recent trends toward northward expansion/southward retraction of its distribution range. They take advantage of a large (albeit fragmented) dataset of biological and climatological records across both time (roughly three decades) and space (22 localities). They examine the impacts of temperature on the insects along these spatio-temporal axes through two distinct analyses: (i) comparing time periods for climate-clustered localities where data have been regularly collected over the studied time series, or (ii) comparing climate-clustered localities for a given time period (also interesting to infer the potential impacts of climate change through space-for-time substitution).
>
> I really appreciated how the authors manage to make sense of the disparate (but highly valuable) body of data through climate clustering, and how they justified this procedure based on biologically relevant thermal parameters (diurnal extremes, occurrence of temperatures above or below certain thresholds). The manuscript is well-written, with an overall good flow of ideas, and I liked how the authors integrated many detailed and synthetic meteorological data to support their choices and statements, testifying to the scientific merit of their study. Unifying climatology and ecology is key to forecast the fingerprint of climate change on biological/ecological systems, and I applaud the authors for the high levels of care given to the handle of meteorological data, and cautious biological interpretations.
>
> From my understanding, this is the second round of revision. I was not involved in the previous round and noticed that many comments made pertained to the statistics and sampling methods employed. When reading the revised manuscript and response letter, I found the methodological/statistical choices made by the authors well justified and have no particular queries on these matters. I still have several comments of minor substance, some are just points of discussion, and two (comments 1 and 3) relate to the structure and clarity of some paragraphs I found a bit confusing.

Thank you very much for the positive and encouraging comments on this work and for the helpful suggestions to improve the manuscript structure. Below are detailed answers to your comments.

> **(1)** L56-81: I think the flow of ideas would be improved if the authors first describe the different facets of climate change (increase in both mean temperature and variability), and then the biological responses to this disturbance. Besides, I would suggest breaking down the long sentence L57-66 to improve readability (avoiding point-by-point listing). The main message remains that the concomitant and interactive facets of climate change affect all fitness components (phenology, morphology, behaviour, physiology) of living organisms as well as their persistence and distribution (e.g. Vasseur et al. 2014, doi: 10.1098/rspb.2013.2612).

The structure of the introduction has been modified accordingly, we have also added the reference Vasseur et al. 2014, while retaining those specific to each of the fitness components (L57-69 and L79-80).

(2) L215-220: Here and throughout the manuscript, the authors stress the importance of maximal daytime temperature (namely TX) in driving the biological responses observed. If I understood correctly, trait variation is explained as a function of TX in the analyses (L227-230), and so is the evolution of climate over time (Figure 4). Results are discussed in light of exposure to high maximal temperatures during the day, and I fully agree that these set the intensity of the stress incurred by organisms in fluctuating environments. That being said, I think the importance of nighttime temperatures (namely TN) should not be excluded. Nights are warming faster than days in many parts of the world as a consequence of climate change, thereby shrinking diurnal thermal range (e.g. Higashi et al. 2020, doi: 10.1111/1365-2656.13238). In fluctuating thermal environments, nighttime temperatures are biologically meaningful because nocturnal cooling offers the physiological opportunities for buffering of injuries sustained during daytime heat. Thus, nocturnal warming may be more biologically impactful than daytime warming by preventing such physiological mitigation if organisms are heat-stressed throughout the day. I note that climate clusters were defined based on both TX and TN. I am not saying that the authors should rerun analyses with TN, just that this point could be worth-mentioning in the discussion (e.g. L492-511) regarding the thermal biology/ecology of the focal species, with a couple of sentences and appropriate references (e.g. Higashi et al. 2020, Zhao et al. 2014 doi: 10.1111/1365-2656.12196). Is there any information available about the moth susceptibility to nighttime minimal thermal thresholds?

Following your comment, we realized that it would be relevant to mention in the Materials and methods that TX weigh more than TN in the clustering, just like summer months weigh more than winter months due to higher absolute values (as we used a covariance matrix, not scaled data) (see lines 215-218). The susceptibility of the PPM to nighttime temperatures and suggested references about the DTR are now discussed (L464-481).

(3) L352-369: While I found the paragraph on clutch size particularly clear and well-structured, I had more trouble following the description of the result and carve out the main findings for traits related to egg survival. I can see the paragraph has been fully rewritten (possibly to answer another comment during round 1). To further improve readability, I would suggest switching the order for result description: first come all the statistical results for hatching rates, then those for the potential causes of egg mortality that may explain such differences in egg survival (sterility, abortion, parasitism). In my view, overall changes in hatching rates are the most meaningful for population dynamics (whatever the underlying reasons) and should, as such, constitute the main message conveyed by this paragraph. I notice hatching rate appears before the factors of egg mortality in Tables 2 and 3, and so should they in the text in my opinion.

We switched the order of all the statistical results accordingly (lines 322-352). To be consistent with the other studied variables, we also added the results on main factors and their interaction term for hatching rate.

(4) Table 3: On my screen there is an ‘Abortion’ written in bold before ‘Hatching rate’, in the second line of the ‘Variable’ column. Please remove it if necessary.

Fixed.

(5) L403-406: I also found this reference to consolidate the author’s statement: Jactel et al. 2019, doi: 10.1016/j.cois.2019.07.010. Perhaps worth-citing?

We agree, it is a relevant reference and it is now cited (L372).

> **(6)** L421-428: Just a point of discussion here. This pattern (decrease in hatching rate over time) is only true for cluster 1, if I understood correctly (L360-362). On the reverse, meteorological data reveal that the summer climate characterising cluster 1 did not warm significantly over the last three decades (Figure 4). Attributing this biological trend to warming sounds like to a counterintuitive reasoning to me, but I might have missed something. As explained in comment 3, I had some trouble teasing out the main statistical conclusions from this paragraph, some points of clarification should help here.

In this section, we actually discussed the steep decrease observed in 2017 only, a year characterized by 10 consecutive hot days (see figures 6 and SM10) and not an overall warming trend during the whole period of three decades.

> **(7)** L483: Shouldn't it be 'showing' instead of 'showed'?

Fixed (line 434).

> **(8)** L585: This is very interesting. The authors may wish to add this recent and comprehensive review on the topic (how insects deal with predictable/unpredictable temperature fluctuations, including bet-hedging strategies): Le Lann et al. 2021, doi: 10.1242/jeb.238626

This is definitely a relevant reference and it is now cited too (see L533).

> **(9)** L612: I would add melanisation as well (the humoral component of immunity in insects), which usually follows encapsulation as part of the immune response, especially against microscopic intruders like parasitoid eggs.

Melanisation is now mentioned in the text too (L562).