Review of “Direct and transgenerational effects of an experimental heat wave on early life stages in a freshwater snail”.

Overall comment:

Investigating the role of transgenerational effects in the context of extreme climatic events such as heat waves is certainly an important and currently understudied topic in ecology. A strength of the study is that it compares the magnitude of transgenerational effects versus the direct effects of temperature on offspring performance which is rarely analysed in the context of thermal effects. I appreciated the general idea of the manuscript and the amount of work invested by the authors to produce these interesting results. The Introduction and Discussion are well written. However, I have some concerns about the link with previous studies (Leicht et al. 2013: Leicht et al. 2017), the statistical analyses, the result interpretation and the possible selection effects of temperature on both the parental and offspring generations. I detail these concerns below.

On lines 150-152, the authors refer to two studies published by the first author in which the effects of temperature on egg numbers were analyzed (Leicht et al. 2013: Leicht et al. 2017). As these studies used a similar experimental design, it is not clear if the present study involve another set of “new” experiments or if it analyses unpublished data from these previous studies. In my opinion, this is not an issue but it should be clarified. I suggest adding a sentence to clarify this point.

An important point related to this is the link between egg numbers and egg size. We may expect a trade-off between egg size and egg number: the bigger they are, the less abundant they are. By ignoring egg number in the present study, we miss a part of the story. For instance, producing smaller eggs but a larger number of them could be an adaptive response to warming. It would thus be interesting to analyses the relationship between egg number and egg size at the two temperatures.

The statistical analyses section is very vague and should be improved to better understand which statistical analyses were computed. For instance:
Lines 195-198: if the ANOVA has a random factor, then it is a Mixed Model ANOVA.
Lines 199-205: Why family was not included as a random factor in this analysis? Specify that the interaction between maternal temperature treatment and offspring temperature treatment was included in the model.
Lines 209-217: I have the same questions about the random family effect and the interaction for the MANOVA analysis.
Lines 218-227: a GLM with a random effect is a GLMM. Same comment as above for the interaction term.

Analyses on offspring survival: I was surprise to see that the interaction between maternal temperature and offspring temperature was not significant. Figure 3 suggests that it is significant. In table 2 (GLM for offspring survival), it is not clear if family is a random factor or a fixed one. If it is fixed (as in a GLM) then this might explain why the interaction term appears non-significant.

I also noticed that, in the control group (parents and offspring maintained at 15°C), survival is lower at the offspring generation compared to the parent generation: approximately 80 % vs 94.4%. Why is it the case? Maybe it is because I am comparing survival of juveniles versus adults?

Shell length: Table 3 suggests that family was included as fixed effect in the model. It should be random unless the authors are interested in discussing family effects.
The increase in shell length in response to warm temperature (direct effect on offspring) is spectacular (97.4% increase in size). It is also opposite to the temperature size rule (i.e. smaller individual at warmer temperature). I wonder how much of this result is linked to plastic versus selection effects. The effect of family is very strong (Table 3). Is this because there was a selection for families with large snails? It would be interesting to look at the size of snails that died both at the parent and offspring generations to determine if body size is important for survival at warm temperature (if yes, we expect that the dead snails were smaller). If there is a selection for larger size, this might explains some of your results. This should be clarified, especially because the low survival of adults at warm temperature (66.1 % survival) suggests possible strong directional selection that could strongly contribute to the results.

General conclusion: the conclusion that transgenerational effects and direct effects of temperature are equally strong should be lowered as it is not general but depends on the traits. For instance the direct effect of temperature on shell length is much stronger than the maternal effect (Fig. 4). The conclusion should thus be that transgenerational effects and direct effects of temperature **can be** equally strong **depending on the trait considered**.

Overall, I think that it is an interesting study that deserves to be published after clarifications on the statistical analyses and on the relative importance of the potential selection effect. Investigating the relationship between egg size and egg number would also be an interesting addition to this study.

I hope that my comments will help improving the manuscript.
Best regards,
Arnaud Sentis

Minor comments:
I was surprise that some results and element of discussion were already included within the experimental design section (lines 144-158). I think this information is important and the results should go to the Result section. The discussion on potential selection effects should go to the Discussion.

Line 57: replace “cannot” by “can not”
Line 101, 103, 119, 120: “2015” can be removed as the data are unpublished.
Line 125: “large numbers” How many?

Line 112: define “high temperature”. e.g. >20°C.
Lines 159-160: It would be useful to clarify that temperatures were kept at 15 and 25°C even after the initial exposure to temperature treatments. At the first sight, one may think that temperature treatments stopped here after seven days.
Lines 182-185: specifying that the design was full factorial (2 maternal temperatures x 2 offspring temperatures) would ease the understanding of the experimental design.
Lines 234-235: specify that the interaction between maternal temperature and offspring temperature was not significant.