Review of the article “Direct and transgenerational effects of an experimental heat wave on early life stages in a freshwater snail” by Leicht & Seppälä for PCI Ecology.

General comments:

The article demonstrates that both direct and maternal effects of temperature are involved in determining traits in a freshwater snail. Authors used a proper factorial design by switching two different temperatures to test such effects. Although they remained overall weak, maternal effects were identified on hatching success, onset of hatching, survival rate and size of the offspring.

The abstract would benefit from adding more details on the experimental design and results so readers could better perceive what was done in the study. I believe the abstract is not clear enough as it stands for now.

Overall, the structure of the manuscript is a bit confusing. Some information is not provided in the right section, to my opinion (see comments below). It is often difficult to identify parts refereeing to experiments on direct effect, maternal effects, and offspring effects. A summary figure of the experimental design in the material and methods would greatly help!

I have spotted several grammar and syntax mistakes throughout the text, but as I am not myself a native English speaker, I would just suggest the authors to revise carefully the use of English in the manuscript.

Specific comments:

L28: I would replace “completely” by “often” as it is not true to affirm that transgenerational plasticity has been completely neglected in the context of climate change.

L28-31: This sentence is not clear, please rephrase. Do you already know that high temperatures reduce adult performance, or is it something that you will test? Also please write it at the past tense.

L30: “which traits are affected” in the offspring, the maternal generation or both? Precise what kind of traits you are measuring (morphological, physiological ...).

L31: “with direct effects of high temperature” Here you are talking about the offspring, right?

L37: “Direct effects of high temperature on offspring” from both maternal origins?

L38-39: I am not sure it is worth insisting on this similarity in magnitude as the relationship between direct and maternal effect is in one case negative (hatching rate) and in the other case positive (survival).

L39-41: I would reverse the order of this sentence so the focus would be made on the importance of transgenerational effects. It could read “This indicates that heat waves cannot only impact natural populations through direct effects of temperature, but that such effects can be equally strong to maternal effects”, or something similar.

L42: Replace climate change by climate warming as you focus on temperature only

L59-61: This is what evolutionists do. It would require specifying that you are studying transgenerational plasticity (and not long-term evolution).

L73-74: This is only true if the offspring environment is predictable by the mother environment. Otherwise, bet-hedging strategies could appear, or the "predictive" maternal effect would not be fully adaptive.

L86: It would be interesting to have information (if available) on the relationship between temperature and egg size in invertebrates and/or in the study species. What are the known factors determining egg size and embryo development in this species?

L93: Please give the authority and (Order: Family) for the species the first time you mention it.

L91-103: Most of the information here should be moved to the material and methods section. Instead, hypotheses are missing at the end of the introduction and should be clearly stated. What do the authors expect to see on direct and maternal effects and on which traits? Why?

L99: Is there any more recent reference than Vaughn (1953) describing the thermal optimum of this species? In 65 years, it is highly probable that selection would have acted on thermal optima of *Lymnaea* populations. If nothing is known about current thermal optimum in this species, this is a point that should be discussed in the manuscript. To the same extent, artificial selection could happen in the laboratory, as snails were maintained at constant 15°C for 2 years before the study. It was shown in some (insect) species that thermal plasticity can be highly reduced when maintained over a long time or over several generations at constant temperatures (i.e., it has a narrowing effect on the thermal optima curve).

L99: "reduce life-history" is not very informative. Which traits were affected? Also it should read "reduce the value of life-history traits" or "of life histories".

L103-107: Please remove this part from the introduction. It is a summary of the results and is already mentioned in the abstract. It can be moved to the beginning of the discussion, if needed, to briefly summarize your findings.

L111-115: This part should actually be in introduction.

L152-158: Again, I find it a bit awkward to discuss results and potential experimental bias before exposing the results *per se*. I would move this part to the discussion section. It has to be discussed in regards to results from the offspring generation.

Figure 1, 2, 3 & 4: Please display on figures results of statistical analyses so we can see significant differences among treatments without referring to the text or to the tables.

L218 and Figure 3: If daily survival data is available, it would be better to analyze and represent this data using Cox-regression models and survival curves. Using a GLM will only compare mean survival rates among treatments but cannot capture any time effect. Using a Cox model should not alter the conclusions. Survival % at 5 weeks can still be given in the text.

L221: The “family” effect in the model represents a mother ID effect, correct? Why did you chose to nest this effect within the interaction effect?

L235-236: Please provide precise data on how much hatching success was increased/decreased by increasing temperatures?

L236: What about the non-significance of the interaction term (M x O)? What does it mean biologically speaking? Same remark at L239-240.

L241: What about differences in median and end of hatching between 25 and 15°C? Are there no significant differences? Please precise.

L241-243: How much earlier? Please provide data in days for onset, median and end of hatching in the main text for as it is not precisely displayed in Fig. 2.

Figure 2: This figure has a standard display for representing mean±SE data and it could be confusing at first sight. It actually represents onset, median and end of the hatching period. Although the authors’ choice makes sense when reading the figure caption, I wonder if a clearer way to display this data could be imagined. Maybe just adding text in the graph, or dashed lines to show onset and end of the hatching period would help. It would also help the reader seeing differences among treatments more clearly.

L267-268: By how long survival was reduced in days and in percent? Did they die faster over the five weeks of experiment? A survival curve would allow showing this information better than barplots.

L269-270: Please move this sentence after Fig. 3 as it presents another type of results.

L267-270: What about the M x O effect and the family effect? Please add a sentence for the biological significance of these factors according to the presented results. It is important because it is the part that allows saying that the magnitude between direct and maternal effects is similar.

Tables 1, 2 & 3: add “interaction effect” in the table legend.

L274 and 288: Please briefly precise what is the “family” effect in the legend so readers don’t have to refer to the material and methods.

L305: “largely negative”. I think it is worth to precise here which traits were negatively affected by direct effects of high temperature.

L306-307: “early life stages”: what traits do you consider to be beneficially affected by high maternal temperature? “later stages”: same remark, please precise which “late” traits you found to be negatively affected.

L309: What is the rationale of using these references here? Pettay *et al.* is on humans, Heath *et al.* is a case study on salmons, and Mousseau & Dingle is a review focusing on insects. I suggest removing references from this part of the text and adding specific examples later in the discussion if and when relevant.

L309-311: Although the direction effect was reversed for hatching rate but not for survival levels, correct?

L316: Eggs were significantly smaller at 25°C, but only by 0.20 mm² on average. Is this difference biologically meaningful? What does it imply in terms of fitness?

L317: replace “benign” by “optimal”?

L318: Hatching success is affected, but is it really biologically important (a matter of 9% maximum)?

L323-328: Would faster development also be associated with lower risks of predator attack at the egg stage? Exposure to parasites? Would snails access to reproduction faster? Also please try to refer to literature on aquatic invertebrate systems, as references on vertebrates and homeothermic organisms may not be relevant for pond snails.

L329-330: Is there any evidence of increased metabolic rate within eggs at high temperature in the literature?

L331: Thus, could the effect of temperature on egg size simply be a plastic response to temperature constraints and not be adaptive? Is it a “maternal decision” or a response to temperature constraints? Are mothers able to lay different quality/type of eggs depending on the conditions they encounter (including temperature)? This is why we need information about determination of egg size and development in this species in the introduction of the paper.

Also, egg size is often a good indicator of egg quality because it correlates well with energetic reserves. Here you show that you also have to consider potential trade-offs involving response to high temperature. I think there is a bit more to discuss about the advantages of developing in a small egg at high temperatures (resistance to heat shocks? More parsimonious energy consumption? ...).

L334: What do you mean by “resource level”?

L337: This second hypothesis would require females to have the choice in laying high quality versus low quality eggs depending on the temperature or mortality risks. Can they?

L351: Is egg size correlated with offspring size? It would be expected. If so, reduced offspring size at high temperature could be explained by reduced egg size.

L369: Higher temperature usually fastens metamorphosis rate (or organ development rate) but not growth rate, which leads to smaller adults in arthropods, or smaller individuals hatching from eggs. Does this temperature-size rule (see Atkinson, 1994) also apply to aquatic snails?

Concerning survival rates, increasing metabolic rates and faster use of energetic reserves could also explain high temperature effect.

L381-384: This paper also show unexpected similarities in the magnitude of direct and transgenerational effects, for example hatching success increases at high temperature but decreases when mothers were exposed to high temperatures. Therefore, and as mentioned for the abstract of the paper, I would be more parsimonious in insisting on this “equally strong” effect.

L385: In this paragraph, the authors should temper a bit their claim. Indeed, it is unlikely that climate warming change pond and lake temperatures by +10°C in a short period of time (one generation). Thus, transgenerational plasticity is not the only factor to take into account, but also genetic evolution over several generations.

Could it be a mother/offspring conflict on fitness in the context of climate change? If mothers encounter conditions that will not be those that offspring will develop on, the maternal response could not be adaptive.

L397-398: “none of the observed direct effects of temperature depended on the maternal environment”. It is not clear what this sentence refers to. No interaction effect? Please detail a bit more, as it is interesting.

L400: Could it be that water environments are way more buffered than terrestrial environments, as you mention? Thus, maternal effects are unlikely to evolve if maternal and offspring environments have a high probability to be similar. It is also possible that other environmental factors fluctuate more than temperature does in such environments. Maternal effects could thus be much stronger when looking at resource availability, pH, ...