Peer Community In Ecology

Escargots cooked just right: telling apart the direct and indirect effects of heat waves in freashwater snails

vincent calcagno based on reviews by Amanda Lynn Caskenette, arnaud sentis and Kévin Tougeron

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A recommendation of:

Katja Leicht, Otto Seppälä. **Direct and transgenerational effects of an experimental heat wave on early life stages in a freshwater snail** *(2019), bioRxiv, 449777, ver. 4 peer-reviewed and recommended by Peer Community in Ecology.* 10.1101/449777

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Amongst the many challenges and forms of environmental change that organisms face in our era of global change, climate change is perhaps one of the most straightforward and amenable to investigation. First, measurements of day-to-day temperatures are relatively feasible and accessible, and predictions regarding the expected trends in Earth surface temperature are probably some of the most reliable we have. It appears quite clear, in particular, that beyond the overall increase in average temperature, the heat

waves locally experienced by organisms in their natural habitats are bound to become more frequent, more intense, and more long-lasting [1]. Second, it is well appreciated that temperature is a major environmental factor with strong impacts on different facets of organismal development and life-history [2-4]. These impacts have reasonably clear mechanistic underpinnings, with definite connections to biochemistry, physiology, and considerations on energetics. Third, since variation in temperature is a challenge already experienced by natural populations across their current and historical ranges, it is not a completely alien form of environmental change. Therefore, we already learnt quite a lot about it in several species, and so did the species, as they may be expected to have evolved dedicated adaptive mechanisms to respond to elevated temperatures. Last, but not least, temperature is quite amenable to being manipulated as an experimental factor. For all these reasons, experimental studies of the consequences of increased temperature hit some of a sweetspot and are a source of very nice research, in many different organisms. The work by Leicht and Seppala [5] complements a sequence of earlier studies by this group, using the freshwater snail *Lymnaea stagnalis* as their model system [6-7]. In the present study, the authors investigate how a heat wave (a period of abnormally elevated temperature, here 25°C versus a normal 15°C) may have indirect effects on the next generation, through maternal effects. They question whether such indirect effects exist, and if they exist, how they compare, in terms of effect size, with the (more straightforward) direct effects observed in individuals that directly experience a heat wave. Transgenerational effects are well-known to occur following periods of physiological stress, and might thus have non negligible contributions to the overall effect of warming. In this freshwater snail, heat has very strong direct effects: mortality increases at high temperature, but survivors grow much bigger, with a greater propensity to lay eggs and a (spectacular) three-fold increase in the number of eggs laid [6]. Considering that, it is easy to consider that transgenerational effects should be small game. And indeed, the present study also observes the big and obvious direct effects of elevated temperature: higher mortality, but greater propensity to oviposit. However, it was also found that the eggs were smaller if from mothers exposed to high temperature, with a correspondingly smaller size of hatchlings. This suggests that a heat wave causes

the snails to lay more eggs, but smaller ones, reminiscent of a size-number trade-off. Unfortunately, clutch size could not be measured in this experiment, so this cannot be investigated any further. For this trait, the indirect effect may indeed be regarded as small game : eggs and hatchlings were about 15 % smaller, an effect size pretty small compared to the mammoth direct positive effect of temperature on shell length (see Figure 4 ; and also [6]). The same is true for developmental time (Figure 3). However, for some traits the story was different. In particular, it was found that the (smaller) eggs produced from heated mothers were more likely to hatch by almost 10% (Figure 2). Here the indirect effect not only goes against the direct effect (hatching rate is lower at high temperature), but it also has similar effect size. As a consequence, taking into account both the indirect and direct effects, hatching success is essentially the same at 15°C and 25°C (Figure 2). Survival also had comparable effect sizes for direct and indirect effects. Indeed, survival was reduced by about 20% regardless of whom endured the heat stress (the focal individual or her mother; Figure 4). Interestingly, the direct and indirect effects were not quite cumulative: if a mother experienced a heat wave, heating up the offspring did not do much more damage, as though the offspring were 'adapted' to the warmer conditions (but keep in mind that, surprisingly, the authors' stats did not find a significant interaction; Table 2). At the end of the day, even though at first heat seems a relatively simple and understandable component of environmental change, this study shows how varied its effects can be effects on different components of individual fitness. The overall impact most likely is a mix of direct and indirect effects, of shifts along allocation trade-offs, and of maladaptive and adaptive responses, whose overall ecological significance is not so easy to grasp. That said, this study shows that direct and indirect (maternal) effects can sometimes go against one another and have similar intensities. Indirect effects should therefore not be overlooked in this kind of studies. It also gives a hint of what an interesting challenge it is to understand the adaptive or maladaptive nature of organism responses to elevated temperatures, and to evaluate their ultimate fitness consequences.

References

[1] Meehl, G. A., & Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. Science (New York, N.Y.), 305(5686), 994–997. doi: 10.1126/science.1098704 [2] Adamo, S. A., & Lovett, M. M. E. (2011). Some like it hot: the effects of climate change on reproduction, immune function and disease resistance in the cricket Gryllus texensis. The Journal of Experimental Biology, 214(Pt 12), 1997–2004. doi: 10.1242/jeb.056531 [3] Deutsch, C. A., Tewksbury, J. J., Tigchelaar, M., Battisti, D. S., Merrill, S. C., Huey, R. B., & Naylor, R. L. (2018). Increase in crop losses to insect pests in a warming climate. Science (New York, N.Y.), 361(6405), 916–919. doi: 10.1126/science.aat3466 [4] Sentis, A., Hemptinne, J.-L., & Brodeur, J. (2013). Effects of simulated heat waves on an experimental plant–herbivore–predator food chain. Global Change Biology, 19(3), 833–842. doi: 10.1111/gcb.12094 [5] Leicht, K., & Seppälä, O. (2019). Direct and transgenerational effects of an experimental heat wave on early life stages in a freshwater snail. BioRxiv, 449777, ver. 4 peer-reviewed and recommended by PCI Ecology. doi: 10.1101/449777 [6] Leicht, K., Seppälä, K., & Seppälä, O. (2017). Potential for adaptation to climate change: family-level variation in fitness-related traits and their responses to heat waves in a snail population. BMC Evolutionary Biology, 17(1), 140. doi: 10.1186/s12862-017-0988-x [7] Leicht, K., Jokela, J., & Seppälä, O. (2013). An experimental heat wave changes immune defense and life history traits in a freshwater snail. Ecology and Evolution, 3(15), 4861–4871. doi: 10.1002/ece3.874

Revision round #2

2019-03-19

Dear authors, Thank you for th eeffort you put into revising your preprint. The reviewers were satisfied and so am I. I am willing to recommend this preprint and I've almost finished writing up a recommendation text. In the process I have picked a few typos. I list them below and perhaps you could fix them in the final preprint. All the best

-- Line 84: the sentence starting with "Especially..." is strange. Perhaps you want to start it with "In particular, trasngenerational..."

-- Line 109: Remove "of" after "despite"

-- In legend of Figure 1 :"Hatchlings" is mispelled

-- In the references : journal name is BMC Evolutionary Biology, with BMC capitalized

-- The paper by Wadgymar et al in Am Nat is no longer in press: please update the reference

Preprint DOI: 10.1101/449777

Reviewed by arnaud sentis, 2019-02-26 17:53

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

First of all, my apology for the delay.

I think that the authors did a great job in considering reviewer comments. I feel that the modifications and the new figure 1 clarify the manuscript and strengthen the manuscript.

I appreciate the new paragraph on the potential selection effect on the parent generation even if it is unfortunate that the authors were not able to assess the strength of this potential selection effect. Is it not possible to measure the size if the dead parents if samples were conserved?

For the relationship between clutch size and egg size, I understand the author response and the limits of this analyze given the experimental design. Although only one egg clutch was collected per parental snail, I still think that the relationship between egg size and clutch size could be analyzed at the population level (i.e. by pooling data from all females together for each experimental treatments). This would provide us some information on whether the allocation to egg numbers versus egg size was affected by the thermal treatments. That being said, I feel that the study could be accepted for publication without this

analyze (although this would be very interesting). I suggest to at least mention this point in the discussion.

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

Reviewed by Amanda Lynn Caskenette, 2019-02-13 16:07

The changes outlined in the response to reviewers and the tracked changes on the document are sufficient to deal with my previous issues.

Author's reply:

Dear Dr. Calcagno, This letter accompanies our revised preprint “Direct and transgenerational effects of an experimental heat wave on early life stages in a freshwater snail”. We have revised the preprint according to your final comments. Detailed responses to your and the reviewer’s comments are listed below.

Sincerely, Otto Seppälä

Dear authors, Thank you for th eeffort you put into revising your preprint. The reviewers were satisfied and so am I. I am willing to recommend this preprint and I've almost finished writing up a recommendation text. In the process I have picked a few typos. I list them below and perhaps you could fix them in the final preprint. All the best

-- Line 84: the sentence starting with "Especially..." is strange. Perhaps you want to start it with "In particular, trasngenerational..."

Response: Corrected accordingly.

-- Line 109: Remove "of" after "despite"

Response: Corrected accordingly.

-- In legend of Figure 1 :"Hatchlings" is misspelled

Response: The misspellt word in the figure is corrected.

-- In the references : journal name is BMC Evolutionary Biology, with BMC capitalized

Response: Corrected accordingly.

-- The paper by Wadgymar et al in Am Nat is no longer in press: please update the reference

Response: The volume and page numbers of the article are given now.

Reviews Reviewed by arnaud sentis, 2019-02-26 17:53 Review of “Direct and transgenerational effects of an experimental heat wave on early life stages in a freshwater snail”. First of all, my apology for the delay. I think that the authors did a great job in considering reviewer comments. I feel that the modifications and the new figure 1 clarify the manuscript and strengthen the manuscript.

I appreciate the new paragraph on the potential selection effect on the parent generation even if it is unfortunate that the authors were not able to assess the strength of this potential selection effect. Is it not possible to measure the size if the dead parents if samples were conserved?

Response: It is possible to measure the shell length of dead snails. However, comparing those measures to the size of snails that survived all the way to the end is problematic because the time period they were able to grow is different. Thus, the size of snails that died during the treatments could appear smaller even if their growth rate was the same when compared to individuals that survived to the end. This is the reason why we did not measure the size of snails that died. Further studies could follow snail growth over time with high resolution to get estimates of their growth trajectories. Such data could help to address the issue raised by the reviewer.

For the relationship between clutch size and egg size, I understand the author response and the limits of this analyze given the experimental design. Although only one egg clutch was collected per parental snail, I still think that the relationship between egg size and clutch size could be analyzed at the population level (i.e. by pooling data from all females together for each experimental treatments). This would provide us some information on whether the allocation

to egg numbers versus egg size was affected by the thermal treatments. That being said, I feel that the study could be accepted for publication without this analyze (although this would be very interesting). I suggest to at least mention this point in the discussion.

Response: As stated in our earlier response letter we have two reasons why we think our data is not suitable for such an analysis. First, snail fecundity should be estimated from several egg clutches laid over a longer time period rather than from one clutch. This is because the size of egg clutches varies a lot even within individual snails. Second, egg clutches with low egg numbers were not suitable for examining our main study question and thus not collected. Data from such clutches would be highly important for the suggested analysis.

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

Reviewed by Amanda Lynn Caskenette, 2019-02-13 16:07 The changes outlined in the response to reviewers and the tracked changes on the document are sufficient to deal with my previous issues.

Revision round #1

2018-12-04

I have read the preprint and had it evaluated by three expert reviewers. All reviewers expressed concerns related to the presentation of the results and some aspects of experimental or statistical methods that could not easily be parsed from the manuscript. Importantly, all reviewers pointed that egg number/size is not shown and very little analyzed, and that it would be important to do so because egg size and egg number might show correlated responses in the context of a size-fertility tradeoff. This may relate to a, possibly adaptive, shift in the egg-laying strategy of females as a function of temperature, and deserves consideration.

There were also several concerns regarding the statistical procedures and interpretations. These should be clarified (possibly, as suggested, with the help of an additional Figure). Similarly, the comparison of effect sizes between direct and indirect effect should be moderated somehow, since not all traits were not equally affected.

I agree with the reviewers. Personally,I recommend the authors to homogenize the different result figures: whilst several follow the exact same pattern, some are presented as barplots, some as simple dots, some show mean+-SE, some show median/quartiles. This is confusing. I even think that figures are pretty small and contain one panel only, and that they would benefit from being lumped (at least, Figs 1&2 and 3&4 constitute pairs of companion panels). This way, they might get some extra space for the reporting of the additional results and figures that reviewers have asked for.

Last, the manuscript needs some restructuring, and the reviewers made several suggestions to this end. For instance, some parts are found in the Material & Methods section while they really are Results material (I am thinking for instance of survival and fertility values, provided on page 7 in the Methods, and that one reviewer found missing in the Results). An additional figure highlighting the general experimental setup would certainly help.

Considering all these elements, I cannot recommend the preprint as it is, even though it addresses an interesting topic and reports some nice results. If the authors can, and are willing to, address all the points in the present letter and in the review documents, the preprint may certainly become recommendable. In any case, I hope these reviews will be of some help to the authors.

Preprint DOI: 10.1101/449777

Reviewed by arnaud sentis, 2018-11-15 16:26

Download the review (PDF file)

Reviewed by Amanda Lynn Caskenette, 2018-11-20 18:01

Download the review (PDF file)

Reviewed by Kévin Tougeron, 2018-11-08 20:18

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 mm^2 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, ...

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Author's reply:

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