2023 PCI Evolutionary Biology: Willem Frankenhuis review of "Sensitive windows for within- and trans-generational plasticity of anti-predator defences".

My expertise is well-matched to theoretical aspects but not to empirical aspects of this paper. Hence, before accepting to review, the editor and I have discussed limiting the scope of my review to theoretical aspects of the paper.

I enjoyed reading this well-written and well-structured paper describing an experimental study, with a rich variety of measures, that addresses an interesting question: how does the developmental timing of exposures to predator cues influence the development of anti-predator defences in the freshwater snail *Physa acuta*?

In the study, the F1 parents (parental generation) were randomly assigned to one of 6 treatments: 5 treatments with exposures to predator cues at different developmental windows: in the embryonic stage, in the early/mid/late post-embryonic stages, and lifelong (embryonic and all post-embryonic windows). Treatment 6 is a control condition with no exposure to predator cues in all of these windows. Following these treatments, the researchers quantified “Within-Generational Plasticity” by measuring 6 defences: 2 behavioral (refuge use, time to reach the refuge) and 4 morphological (snail mass, shell length and width, shell thickness, shell crush resistance). The F2 offspring (offspring generation) were reared in water without any predator cues during all windows; so, all of the offspring received the same treatments. The researchers then quantified “Trans-Generational Plasticity” by measuring the offspring’s defences.

Despite being very positive, I have some questions about conceptual framing, rationale for experimental design, and data interpretation. I have provided a few suggestions that the authors may consider to strengthen their excellent paper. I think all of my questions can be addressed. My comments about the conceptual framing are largely about the semantic fit with other theoretical and empirical reports in the field and may be easily addressed. My question about rationale for an aspect of the experimental design may also be easy to address. My suggestion to consider conducting an additional statistical analysis, if the authors choose to incorporate it, would require more substantive work, yet is feasible. I think it would be a worthwhile addition. Per the reviewer guidelines, I should add that I have not identified any flaws in the study.

1. Definition of sensitive windows

The term “sensitive period” is often, perhaps typically, used to refer to a developmental window in which the impact of a given experience on a particular phenotype is greater than the impact of that same experience on that phenotype in other developmental windows. The authors use a similar definition: “the same environmental change may generate different phenotypic effects depending on whether it was experienced early or late in development. Certain developmental windows are particularly sensitive to environmental changes, i.e. environmental changes during these sensitive periods generate particularly strong effects on phenotype” (lines 34-37). Whereas the former definition applies generally to any cue (i.e. any observation that reduces uncertainty

about the environment or the organism), the latter definition focuses specifically on cues reflecting environmental changes. However, cues may also indicate that environmental conditions have remained the same (e.g. predator density is high from one time period to the next). I wonder whether this focus on a subset of sensitive periods is deliberate; if not, it can be easily addressed by in the authors' definition replacing the word "changes" with "cues", which is more general. This difference is subtle and perhaps it is unlikely to confuse readers, but I would like to mention it just for the authors to consider.

2. Uniform plasticity

More important is the question how to describe cases where the evidence does not reveal differences in plasticity across developmental windows. Based on the widely used definition I provided earlier, I would describe such cases as providing a lack of evidence for a sensitive window (consistent with uniform plasticity). By contrast, the authors write that the sensitive window is very wide: "Overall, none of the exposure windows was particularly sensitive compared to the others. The sensitive window for within- and trans-generational defence induction was thus very wide, which could be the result of the strong selective pressure imposed by predation at all developmental stages" (lines 11-12). This wording is fine, in principle, yet I worry that it may confuse some readers, if they would not refer to such cases (of uniform plasticity) as 'a sensitive window'. The authors may consider slightly rewording, for instance, as follows: "We find no evidence for a sensitive window; rather, snails responded to a similar extent across all developmental periods." That said, the authors' and my own wording do not capture one important way in which the data "do" support the existence of sensitive windows, discussed next.

3. Global and specific patterns

Irrespective of whether one prefers to summarize the overall patterns as providing evidence for a "wide" sensitive window or as providing "no support" for the existence of a sensitive window (see comment 2), as the authors note, the impact of predator cues depends on their developmental timing. The Discussion section summarizes well: "the defences expressed were different depending on the window of exposure to predator cues for both WGP and TGP. At the parental generation (WGP), all exposure windows induced the expression of morphological defences; but two morphological strategies can be differentiated between exposures at embryonic or post-embryonic stages and only post-embryonic exposures altered behavioural defence. At the offspring generation (TGP), all exposure windows induced offspring to use refuge, but only certain windows altered morphological defences. These results confirm that the developmental windows at which environmental cues were perceived is an important factor driving both WGP and TGP" (lines 306-313).

In my view, if researchers employ the widely used definition I described earlier, these findings "do" support the existence of sensitive windows: in F1, "only post-embryonic exposures altered behavioural defence" (line 310); in F2, "only certain windows altered morphological defences" (line 311). The impact of a given experience (predator cue) on

a particular phenotype (behavioral and morphological defences) is greater in some developmental windows than others. I think this finding will be of much interest to the community and deserves to be made more explicit, particularly in the abstract, which currently states, in my view less clearly: “Although all exposure windows impacted on the expression of offspring defences, they did so differently and the response patterns were complex for morphological offspring defences. This complexity in the temporal dynamics of transgenerational induction could result from the transmission to offspring of both cues about predator presence and parental somatic condition” (lines 14-18).

When I first read the abstract, I mainly took away that all predator exposure windows induced within- and trans-generational responses, without a clear view of the interesting fine-grained patterns—that the impact of a given cue on a particular phenotype depends on the developmental window in which exposure to the cue occurs (as summarized well in the above quote, lines 306-313, from the Discussion section). My hope is that this will be more clearly reflected in the abstract. I should add that I very much appreciated the authors’ thoughtful reflections on potential adaptive reasons for these fine-grained patterns (described in the paragraph beginning on line 331).

4. Rationale for predictions

The authors note: “For WGP, we predict that embryonic and early post-embryonic windows should be particularly sensitive. For TGP, we predict that (1) one sensitive window of the TGP should be the late parental development; and (2) other sensitive windows of TGP should be those of WGP and so should also be early-life windows” (lines 104-107). I recommend making the grounds for each of these predictions more explicit. As it stands, the introduction discusses a relevant selection of theoretical and empirical research, but does not seem to make the link between this research and these two predictions explicit. Are both predictions based on both the theoretical and the empirical research? Or is prediction 1 based primarily on theory and prediction 2 on the empirical record?

5. Statistical power

I am not a statistical expert, but to my understanding, the authors have conducted null hypothesis tests that afford the following inference about the overall pattern: the data provide no evidence for differences in the extent to which the phenotype responds to cues across developmental windows. In addition, it would be interesting to quantify the extent to which the data support the hypothesis that the phenotype responds equally to cues across developmental windows. A Bayesian analysis can address this question and may have merit for two reasons. First, theoretically, it is valuable to know whether the data are more likely under the hypothesis that the phenotype responds equally to cues across developmental windows (H0) versus the hypothesis that the phenotype responds differently to cues across developmental windows (H1). If such an analysis would show, for instance, that the data are 100 times more likely under H0, this would constitute evidence for the absence of a sensitive window (or, for a wide sensitive window). Second, an analysis may not lead us to reject the null hypothesis—i.e., the

data provide no evidence for differences in the extent to which the phenotype responds to cues across developmental windows—for different reasons: the null hypothesis may be a good model, or the study may not be adequately powered to detect anticipated effect sizes. The current study included 240 F1 and 240 F2 adult snails (40 snails x 6 treatments). I do not have the expertise to evaluate whether this sample size implies adequate power for the current design. Regardless, I think it would be interesting to know whether, and if so to what extent, the data support H0.

6. Duration of predator cues

Whereas the F1 parents were exposed to predator cues for 5 days (out of 7 days) in the embryonic stage, they were exposed to predator cues for 14 days (out of 42 days) in the early, middle, and late post-embryonic stages. There may be good (biological) reasons for this variation in the duration of exposure to the predator cue, but I did not see these stated in the paper (I'm sorry if I missed them). I think it would be good to explain why this design is preferable over, for instance, one where the F1 parents were exposed to predator cues for 5 days (out of 7 days) in the embryonic stage, 5 days (out of 14 days) in the early post-embryonic stage, 5 days (out of 14 days) in the middle post-embryonic stage, and 5 days (out of 14 days) in the late post-embryonic stage. If the global pattern supports uniform plasticity (see comment 4) "despite shorter exposures" to the predator cue in the embryonic stage compared with the post-embryonic stages, one could even argue that the embryonic stage is more sensitive to the cue than the other stages.

7. Rate of environmental change

Mathematical theory on the evolution of sensitive windows suggests that the rate of environmental change, relative to generation time, may influence the width of sensitive windows (see Walasek et al. 2022, below). From this perspective, it would be interesting to have estimates of the rate of environmental change in predator density (or a related measure, like abundance) in the wild population from which the snails in the study were sampled. If such information is currently not available, this may be a valuable direction for future research. This information would also be relevant to the authors' theoretical claim in the Introduction section that "Late-perceived cues are indeed the most reliable about offspring environment because of the short time lag between cue exposure by parents and phenotypic selection in offspring, implying a low likelihood of environmental change if the environment is auto-correlated over time" (lines 53-55).

8. Figure 1 is great

Figure 1 is offers a very clear and informative overview of the experimental design! My only suggestion would add to write the number of days of exposure to predator cues directly below the thick red line (e.g., in the embryonic stage, you could add "5 days"). Though this information can be read from the x-axis, it will be helpful to have it directly in view. I don't think adding this information will clutter the figure (e.g. if the font size is similar to that of the text in the figure stating "No exposure to predator-cues"), but the authors can make this call.

9. Additional literature

I would like to end my review by suggesting some additional literature that the authors may consider incorporating. These papers may have the potential to enhance the paper but are not essential to include. Thus the authors should feel entirely free to include, or not include, these papers as they see fit:

Groothuis, T. G., & Taborsky, B. (2015). Introducing biological realism into the study of developmental plasticity in behaviour. Frontiers in Zoology, 12, 1-14. https://doi.org/10.1186/1742-9994-12-S1-S6

Smallegange, I. M. (2011). Complex environmental effects on the expression of alternative reproductive phenotypes in the bulb mite. Evolutionary Ecology, 25, 857-873. https://doi.org/10.1007/s10682-010-9446-6

Stamps, J. A., & Luttbeg, B. (2022). Sensitive period diversity: Insights from evolutionary models. The Quarterly Review of Biology, 97, 243-295. https://doi.org/10.1086/722637

Uller, T., Nakagawa, S., & English, S. (2013). Weak evidence for anticipatory parental effects in plants and animals. Journal of Evolutionary Biology, 26, 2161-2170. https://doi.org/10.1111/jeb.12212

Walasek, N., Frankenhuis, W. E., & Panchanathan, K. (2022). Sensitive periods, but not critical periods, evolve in a fluctuating environment: A model of incremental development. Proceedings of the Royal Society B, 289, 20212623. https://doi.org/10.1098/rspb.2021.2623

Signature

I hope my review will help the authors to strengthen their manuscript.

For accountability and transparency, I would like to sign my review.
Willem Frankenhuis