Peer Community In Evolutionary Biology

# Sensitive windows for phenotypic plasticity within and across generations; where empirical results do not meet the theory but open a world of possibilities

***Benoit Pujol*** *based on peer reviews by* ***Willem Frankenhuis***, ***Timothée Bonnet*** *and* ***David Murray-Stoker***

Juliette Tariel-Adam; Émilien Luquet; Sandrine Plénet (2023) Sensitive windows for within- and trans-generational plasticity of anti-predator defences. OSF Preprints, ver. 4, peer-reviewed and recommended by Peer Community in Evolutionary Biology.
https://doi.org/10.31219/osf.io/mr8hu

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It is easy to define phenotypic plasticity as a mechanism by which traits change in response to a modification of the environment. Many complex mechanisms are nevertheless involved with plastic responses, their strength, and stability (e.g., reliability of cues, type of exposure, genetic expression, epigenetics). It is rather intuitive to think that environmental cues perceived at different stages of development will logically drive different phenotypic responses (Fawcett and Frankenhuis 2015). However, it has proven challenging to try and explain, or model how and why different effects are caused by similar cues experienced at different developmental or life stages (Walasek et al. 2022). The impact of these 'sensitive windows' on the stability of plastic responses within or across generations remains unclear. In their paper entitled "Sensitive windows for within- and trans-generational plasticity of anti-predator defences", Tariel-Adam (2023) address this question.

In this paper, Tariel et al. acknowledge the current state of the art, i.e., that some traits influenced by the environment at early life stages become fixed later in life (Snell-Rood et al. 2015) and that sensitive windows are therefore more likely to be observed during early stages of development. Constructive exchanges with the reviewers illustrated that Tariel et al. presented a clear picture of the knowledge on sensitive windows from a conceptual and a mechanistic perspective, thereby providing their study with a strong and elegant rationale. Tariel et al. outlined that little is known about the significance of this scenario when it comes to

transgenerational plasticity. Theory predicts that exposure late in the life of parents should be more likely to drive transgenerational plasticity because the cue perceived by parents is more likely to be reliable if time between parental exposure and offspring expression is short (McNamara et al. 2016). I would argue that although sensible, this scenario is likely oversimplifying the complexity of evolutionary, ecological, and inheritance mechanisms at play (Danchin et al. 2018). Tariel-Adam et al. (2023) point out in their paper how the absence of experimental results limits our understanding of the evolutionary and adaptive significance of transgenerational plasticity and decided to address this broad question.

Tariel-Adam et al. (2023) used the context of predator-prey interactions, which is a powerful framework to evaluate the temporality of predator cues and prey responses within and across generations (Sentis et al. 2018). They conducted a very elegant experiment whereby two generations of freshwater snails *Physa acuta* were exposed to crayfish predator cues at different developmental windows. They triggered the within-generation phenotypic plastic response of inducible defences (e.g., shell thickness) and identified sensitive windows as to evaluate their role in within-generation phenotypic plasticity versus transgenerational plasticity. They used different linear models, which lead to constructive exchanges with reviewers, and between reviewers, well trained on these approaches, in particular on effect sizes, that improved the paper by pushing the discussion all the way towards a consensus.

Tariel-Adam et al. (2023) results showed that the phenotypic plastic response of different traits was associated with different sensitive windows. Although early-life development was confirmed to be a sensitive window, it was far from being the only developmental stage driving within-generation plastic responses of defence traits. This finding contributes to change our views on plasticity because where theoretical models predict early- and late-life sensitive windows, empirical results gathered here present a more continuous opportunity for sensitive windows over the lifetime of freshwater snails. This is likely because multifactorial mechanisms drive the reliability and adaptive significance of predator cues. To me, this paper most original contribution lies probably in the empirical investigation of sensitive windows underlying transgenerational plasticity. Their finding implies mechanistic ties between sensitive windows driving within-generation and transgenerational plasticity for some traits, but they also shed light on the possible independence of these processes. Although one may be disheartened by these findings illustrating the ability of nature to combine complex mechanisms in order to produce somewhat unpredictable scenarios, one can only find that this unlimited range of phenotypic plasticity scenarios is a wonder to investigate because much remains to be understood. As mentioned in the conclusion of the paper, the opportunity for sensitive windows to drive such a range of plastic responses may also be an opportunity for organisms to adapt to a wide range of environmental demands.

***References:***


Danchin E, A Pocheville, O Rey, B Pujol, and S Blanchet (2019). Epigenetically facilitated mutational assimilation: epigenetics as a hub within the inclusive evolutionary synthesis. Biological Reviews, 94: 259-282. https://doi.org/10.1111/brv.12453

Fawcett TW, and WE Frankenhuis (2015). Adaptive Explanations for Sensitive Windows in Development. Frontiers in Zoology 12, S3. https://doi.org/10.1186/1742-9994-12-S1-S3

McNamara JM, SRX Dall, P Hammerstein, and O Leimar (2016). Detection vs. Selection: Integration of Genetic, Epigenetic and Environmental Cues in Fluctuating Environments. Ecology Letters 19, 1267–1276. https://doi.org/10.1111/ele.12663

Sentis A, R Bertram, N Dardenne, et al. (2018). Evolution without standing genetic variation: change in transgenerational plastic response under persistent predation pressure. Heredity 121, 266–281. https://doi.org/10.1038/s41437-018-0108-8

Snell-Rood EC, EM Swanson, and RL Young (2015). Life History as a Constraint on Plasticity: Developmental Timing Is Correlated with Phenotypic Variation in Birds. Heredity 115, 379–388. https://doi.org/10.1038/hdy.2015.47

Tariel-Adam J, E Luquet, and S Plénet (2023). Sensitive windows for within- and trans-generational plasticity of anti-predator defences. OSF preprints, ver. 4 peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.31219/osf.io/mr8hu

Walasek N, WE Frankenhuis, and K Panchanathan (2022). An Evolutionary Model of Sensitive Periods When the Reliability of Cues Varies across Ontogeny. Behavioral Ecology 33, 101–114. https://doi.org/10.1093/beheco/arab113

# Reviews

## Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.31219/osf.io/mr8hu
Version of the preprint: 2

### Authors' reply, 31 July 2023

Dear Benoit Pujol, David Murray-Stoker, Willem Frankenhuis and Timothée Bonnet,

We would like to thank you very much for the speed with which you sent us your reviews and for your positive, constructive and interesting comments. We have taken into account our suggestions:

- We have added the effect sizes for the fixed effect "Treatment" following David Murray-Stoker's suggestion.
- We have shifted back to the exact P-values following Timothée Bonnet's suggestion as we agree that it is telling something about the strength of evidence.
- We have added a sentence in the M&M section about a limitation of our experimental design following Timothée Bonnet's suggestion.
- With regard to the effect sizes of the contrasts, the reviewers had divergent opinions on whether or not it would be useful to include them. We decided not to include them because we still believe there is redundant information with the contrasts themselves.

We have responded below in more detail.

We hope you find our corrections accurate and helpful.

Thank again,

Juliette Tariel-Adam, Émilien Luquet and Sandrine Plénet

# Response to Reviewer 1 – David Murray-Stoker

## Point 2.3 – Effect size for fixed effects

### Reviewer Comment Round #1

2.3. Effect sizes for treatment (eta-squared and partial eta-squared for F statistics, Cohen's w for chi-squared statistics, and intra-class correlation coefficient for random effects) will say how much variance is explained by those factors (i.e., strength of the effect).

### Author Response Round #1

We agree that P-values do not say anything about the strength of the Treatment effect but we have not added the effect size for Treatment as it would not bring any information regarding the sensitive windows.

### Reviewer Comment Round #2

As the sensitive windows are within the broader effect of treatment, it is important to know the overall treatment effect size to situate the pairwise contrasts for specific developmental windows. For example, a large effect for single contrast will not matter much if the overall treatment has a weak or negligible effect. Additionally, effect sizes for the contrasts can be quantified as Cohen's d, which would standardize the difference between the control and developmental window of interest (Cohen, 1988; Huberty, 2002; Nakagawa & Cuthill, 2007). Cohen's d takes into account the magnitude of the difference between means and the pooled standard deviation. Effect

sizes for contrasts can be calculated directly in emmeans using 'eff_size()', as noted previously in point 2.4 (below).

### Author Response Round #2

Thank you for your comment, it is true that it is important to know if the predator-cue treatment had a significant or small effect on the expression of defences before digging into the effect of the exposure window. We have added the effect sizes by calculating the partial $\eta^2$ for the fixed effects following the guidelines on the 'effectsize' package (and the pseudo $R^2$ for the glm of refuge use) and added this information into tables 1 and 2 + explanation of the effect size calculation in the M&M.

The percentage of variance explained by the predator-cue treatment was medium to large (4 to 22%) at both the parental and offspring generations. The percentage of variance was small only when the treatment has no statistically significant effect (only 1% of variance explained by the treatment for refuge use at the parental generation and 3% for escape behaviour at the offspring generation).

## Point 2.5 – Effect size for contrasts

### Reviewer Comment Round #1

I think the paper would benefit by reporting the treatment and contrast effect sizes. Not only would this show the biological relevance of any effect (something a P-value cannot do), and the authors would then be able to say if specific windows were more sensitive than others (i.e., compare the contrast between the control and each window to see which had the greatest difference).

### Author Response Round #1

We don't really see why effect sizes on contrasts would allow us to do this. A big contrast already means a big difference, and it is thus already a measure of the strength of the difference/contrast. We do not believe that the effect sizes of the contrasts provide more information or tests than the contrasts themselves on which window was more/less different from the Control treatment. But we agre

### Reviewer Comment Round #2

I have touched on this point above (response to 2.3), but I think it is important to put the treatment effects in context. The authors have clarified how they can say if some development windows are more sensitive than others; however, unstandardized effect sizes - such as contrasts - do not represent how much variation is explained by the treatment (treatment effect size) or between the control and a development window (contrast effect size). Without standardizing the treatment and contrast effect sizes (i.e., accounting for the explained variance), a large contrast could just be an artifact of the sample size. Cohen's d on the contrasts would show if windows were more or less sensitive compared to the control and the strength of that effect (Cohen, 1988; Huberty, 2002; Nakagawa & Cuthill, 2007). At present, the contrasts are just the result of a t-test, with the interpretation constrained by null hypothesis significance testing.

### Author Response Round #2

The only benefit we see to adding the effect sizes for contrasts is to have standardised estimates of the contrasts. In our study, all treatments had similar sample sizes (40 snails for each treatment) and thus our contrast estimates are comparable with each other. It would be only useful if someone wants to have the standardised contrast estimates, for instance for a meta-analysis, but the code and data are there if needed and we would be happy to calculate them if asked. In the manuscript, we believe that the contrast and the effect size of contrast are redundant information about the magnitude of the difference between the control and an exposure window, and that adding contrast effect sizes will just complicate the comprehension of the results (that are already a bit tricky to understand for naive readers). We hope you agree with our diagnostic.

# Response to Reviewer 3 – Timothée Bonnet

## Blocking

### Reviewer Comment Round #2

Regarding my preivous comment about blocking, I think I now understand why blocking cannot be taken into account, but given the information provided (response to review, 1.3 "Rearing of the F1 parental generation" and SI "1 Rearing") it looks to me like the experimental design actually contain some blocking and partial

pseudo-replication. If I understand correctly (maybe not, but I tried my best with the available text; it is possible that some more explanations are needed) some individuals which were measured shared some time in a same tank and on a same tray. So their fates were not fully independent and the effective sample size for each experimental condition is probably somewhat less than the number of individual measured. The problem is reduced by shuffling every two weeks, but in the absence of individual identification we cannot keep track of how much time individuals spent with each other and when in their lives. Therefore we cannot include the information about time shared in a same tank/tray in models and check if this was influential at all. I do not think the problem is major, but if I understood the experimental design correctly and the authors agree with my diagnostic they may want to acknowledge the limitation in the article and consider ways to mitigate it further in future experiments

### Author Response Round #2

We agree with your diagnostic and have added this limitation L185-188 in the M&M section.

We had two conflicting constraints in our experimental design: maintaining a constant density among aquariums or taking into account aquarium (block) effects. Density has been shown to highly impact snail growth, which could have potentially masked the effect of predator cues. Maintaining similar density between aquariums and keeping the aquarium (block) effects requires marking individuals to track their identity which is very time-consuming and thus logistically impossible. In our past, ongoing and future experiments, we rear snails individually to avoid any aquarium or density effect when it is possible. It is the first time we have tried to read snails in groups (as we had a lot of treatments, it was easier to manage) but this has led to this limitation.

## Writing of P-values

### Reviewer Comment Round #2

Regarding responses to another reviewer, I personnaly disagree with the suggestions of writing p-values as thresholds (e.g., p<0.001). It is not very consequential, but I do not understand why you would not write the exact p-value and throw away information about the strength of evidence. You can write them in rounded scientific format to save space (e.g., 1.2 x 10^-6 ).

### Author Response Round #2

We agree and have shifted back to the exact P-values in Tables 1 and 2.

## Effect size for contrasts

### Reviewer Comment Round #2

I also agree with the authors that it would be redundant to provide ”effect sizes for treatment / contrasts”.

## Diagnosis of binomial glm assumptions

### Reviewer Comment Round #2

F1-analysis.html and F2-analysis.html:

By the way, it is not useful to look at residual ”verification / diagnostic” plots with GLM(M)s. Those diagnostics are designed for Gaussian assumptions which do not apply for Binomial models for instance. The plot will always look wrong, irrespective of the GLM(M) fit to assumptions. Instead you can assess model fit with residual simulations (analog to posterior predictive checks), as for instance performed by the package DHARMa.

### Author Response Round #2

Thanks a lot, it’s great to learn how to test linear model assumptions for GLMs. We have changed the diagnosis of the GLMs using the DHARMa package and no problem was detected.

## Decision by Benoit Pujol, posted 18 July 2023, validated 18 July 2023

**Very minor revision**

Dear authors, as you will see in their comments, the three reviewers have been very positive about the revised version of your paper. I share their conclusion that your paper deserves to be recommended. Beforehand however, because it is important to allow for constructive exchanges between authors and reviewers, I'd appreciate if you would address their somehow conflicting comments highlighted in the review of Timthée Bonnet, whom I would tend to agree with, and consider the points 2.3 and 2.5 raised by David Murray-Stoker. If your response is clear enough for me to consider without contacting the reviewers, I will not contact them at the next round and will recommend the revised version of your preprint. I expect these very minor points to be addressed easily, and may start preparing the recommendation in advance to speed up the process.

Congratulations on a very interresting paper

Benoit Pujol

## Reviewed by David Murray-Stoker, 15 July 2023

I have attached my comments in a separate PDF. I only have reservations about points 2.3 and 2.5, as detailed in my comments.

Reviewed by:

David Murray-Stoker
Ph.D. Candidate
University of Toronto
dstoker92@gmail.com

**Download the review**

## Reviewed by Willem Frankenhuis, 08 June 2023

The authors have been responsive to my review. The revision has improved based on the editorial letter and the reviews. This fascinating study deserves to be widely known. I have no further comments.

## Reviewed by Timothée Bonnet, 21 June 2023

The authors have mostly clarified the potential issues I and the other reviewers had raised. I find the revised version of the manuscript to be readable, convincing and easy to follow, in part thanks to the excellent figures. I have some minor comments left, but I think the manuscript is ready for recommendation.

Regarding my preivous comment about blocking, I think I now understand why blocking cannot be taken into account, but given the information provided (response to review, 1.3 "Rearing of the F1 parental generation" and SI "1 Rearing") it looks to me like the experimental design actually contain some blocking and partial pseudo-replication. If I understand correctly (maybe not, but I tried my best with the available text; it is possible that some more explanations are needed) some individuals which were measured shared some time in a same tank and on a same tray. So their fates were not fully independent and the effective sample size for each experimental condition is probably somewhat less than the number of individual measured. The problem is reduced by shuffling every two weeks, but in the absence of individual identification we cannot keep track of how much time individuals spent with each other and when in their lives. Therefore we cannot include the information about time shared in a same tank/tray in models and check if this was influential at all. I do not think the problem is major, but if I understood the experimental design correctly and the authors agree with my diagnostic they may want to acknowledge the limitation in the article and consider ways to mitigate it further in future experiments.

Regarding responses to another reviewer, I personnaly disagree with the suggestions of writing p-values as thresholds (e.g., $p<0.001$). It is not very consequential, but I do not understand why you would not write the

exact p-value and throw away information about the strength of evidence. You can write them in rounded scientific format to save space (e.g., 1.2 x 10^-6 ). I also agree with the authors that it would be redundant to provide ”effect sizes for treatment / contrasts”.

F1-analysis.html and F2-analysis.html:
By the way, it is not useful to look at residual ”verification / diagnostic” plots with GLM(M)s. Those diagnostics are designed for Gaussian assumptions which do not apply for Binomial models for instance. The plot will always look wrong, irrespective of the GLM(M) fit to assumptions. Instead you can assess model fit with residual simulations (analog to posterior predictive checks), as for instance performed by the package DHARMa.

Timothée Bonnet (I sign all my reviews)

## Evaluation round #1

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Version of the preprint: 1

### Authors' reply, 29 May 2023

**Download author's reply**

### Decision by Benoit Pujol, posted 09 February 2023, validated 09 February 2023

**Revise**

Dear Dr. Tariel-Adam and collaborators,

Thank you for submitting your preprint to PCI Evolutionary Biology for recommendation. I have read your preprint entitled “Sensitive windows for within- and trans-generational plasticity of anti-predator defences” and I have read the comments of the three reviewers who have complementary expertise on your research topic.

The three reviewers and I agree about the quality of your research and the value of this preprint. The paper is clearly written, straight to the point, and Figures participate actively to make experimental approaches easy to understand. The reviewers and I found that your research targets a valuable question on phenotypic plasticity and its adaptive significance when considering that it may be restricted to specific developmental windows that are themselves, as you state in the abstract: “highly sensitive and responsive to environmental changes”. You will see in their reviews that although the three reviewers appreciated your work, they raised some concerns that I would like you to address. I have an additional request, which is to adda a paragraph at thei end of discussion on the contribution of your findings to the theory. In the introduction, you present the state of art with strong bibliographic support and outline the hypotheses that are tested in this paper. As a result, we understand what is at stake in terms of contribution of your work. In the discussion, you discuss the proximal conclusion that can be drawn from your results but do not discuss the implication of your findings to the actual theory. Such discussion would add substantially to the scope of this work.

Looking forward to reading the next version of this promising paper.

Regards,

Benoit Pujol

### Reviewed by David Murray-Stoker, 20 January 2023

I have provided my comments in the attached PDF file.

David Murray-Stoker
Ph.D. Candidate

University of Toronto
dstoker92@gmail.com
**Download the review**

## Reviewed by **Willem Frankenhuis**, 22 January 2023

**Download the review**

## Reviewed by **Timothée Bonnet**, 17 January 2023

This manuscript presents an experiment trying to identify sensitive windows for plastic responses to predator cues, within and across generations, in a freshwater snail. Overall the experiment and data analyses appear neatly done, while the interpretation seemed fully appropriate. I had only two somewhat substantial comments that require clarifications. The writing was mostly clear, to the point and enjoyable, although I pointed to a few questionable choices of vocabulary or syntax.

Main comments:

* How where the different treatments organised in space and time (and with respect to any other potential experimental factor)? I did not find any information about this, but I think it is crucial to properly interpret the results. Some non-random experimental factor correlated with treatment could explain some of the apparently idiosyncratic results (e.g., fluctuation of direction of the effect along treatments on figure 4 F). If there is information about experimental factors, those could also be included in models in order to account for blocking and perhaps decrease noise and reveal more of the treatment signals.

* I think individual random effects are necessary to analyse the ”refuge use” variable which has four measurments per individual. The authors should modify their models or explain why they think it is not necessary to account for repeated measurements.

Specific comments:

L.111 A short summary of the species reproductive system could be useful.

L.116 ”bowled” -> ”boiled”?

L.147 ”We wanted” -> ”We waited”?

L.167-170 and L.201-202. From what I understand, each individual was measured four times for ”refuge use”. It seems necessary to account for individual repeated measurement (most likely using a random effect). Using the package lme4 that GLMM be done with glmer, but I would recommend glmmTMB (which tends to perform better with binary GLMMs; it is also compatible with emmeans).

L.231 ”at both generations and removed from the model” -> ”for either generations so we removed it from the models”?

Figure 3 caption. ”confidence interval” at which level? 95% as is the default in emmeans?

L.305 ”Contrary to what expected” -> ”Contrary to what we expected”?

L.323 ”snails during embryonic” -> ”snails exposed during embryonic”?

L.333-336 Alternatively, the decreased escape behaviour could be a maladaptive change, either as a correlated side effect of another response, or an idiosyncratic plastic response (plasticity is often not adaptive!).

L.336 ”may have orient snails to a developmental pathway” -> ”may have directed / lead snails toward a developmental pathway”?

L.338 ”orient snails in” -> ”directed / lead snails toward”?

L.366 ”infirm” -> ”contradict / disprove / refute” (infirm = cripple)

Timothée Bonnet (I sign all my reviews)