

# ***Interactive comment on “Ideas and Perspectives: When ocean acidification experiments are not the same, reproducibility is not tested” by Phillip Williamson et al.***

**Phillip Williamson et al.**

p.williamson@uea.ac.uk

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We appreciate the constructive comments provided by Solan, with five grouped suggestions for additional content. Solan confirms that there are many fundamental methodological issues involved, affecting the full range of scientific process – not just relating to experimental design, but also the analysis and interpretation of results. We fully recognise the importance of the issues raised for consideration. Comment-specific responses are provided here, and we also (briefly) cover several of these points in additional text of our manuscript.

COMMENT: 1. The opinion reinforces the argument about the need for proper ex-

perimental design, both within and across experiments, but doesn't explicitly provide solutions or minimal requirements. I have emailed you the recent Haddaway perspective (<https://doi.org/10.1038/s41559-020-01295-x>), maybe some of the styling from that would be good, but either a Boxed flow diagram or take home message at the end of each section of what readers should do would be good.

RESPONSE: Haddaway et al. (2020) address issues relating to (the lack of) rigour in systematic evidence syntheses in ecological disciplines, providing many key insights in that regard. We appreciate that their paper is primarily brought to our attention as an example of styling, with aspects that could be reflected in our manuscript. However, the approach and scope of Haddaway et al. (2020) is very much broader than our own. In particular, we need to avoid "mission creep" (one of the pitfalls they identify), by trying to change what was intended to be a focused commentary on one replicability case study into a much more comprehensive best-practice guide for ocean acidification, or even for experimental biology as a whole. For the former, we acknowledge that the overview guidance of a decade ago (Riebesell et al., 2011) now requires updating, to take account of methodological advances and greatly increased understanding during the past decade – in particular, the increased emphasis on multifactorial studies (Boyd et al., 2018), rigour in experimental design (Cornwall and Hurd, 2016), and linkages between experimental studies and modelling (Ullah et al., 2020). Whilst our manuscript was clearly not meant to fill that gap, we now include the following additional text to show our recognition of the importance of such issues:

"Future ocean acidification experiments would also benefit from an updating of Riebesell et al. (2011), to provide improved best practice guidance on the key parameters that can affect results".

COMMENT: 2. Three things are missing for me. (i) one glove doesn't fit all, and what is high variability in one system (e.g. pelagic) is within the noise in another system (e.g. benthic), and that needs to be recognized, especially in review. Variability in carbonate chemistry between systems has not been summarised anywhere, although

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could be now that there are so many papers, and then these need to be couched within temporal variation (e.g. diurnal) for the same system. (ii) the detection of treatment effects is valid, even if the carbonate chemistry is different to other experiments. You hint at this, but this would benefit from some elaboration. (iii) reading most ocean acidification papers, the narrative is that OA is ‘bad’ which is not necessarily true. I take the point that calcifiers are affected, but would challenge the statement that they are most sensitive as this is only one parameter and there is a bias in the literature (people have picked calcifiers). You highlight other examples, including behaviour, in non-calcifying species which could be equally devastating to that species. I key message is that this literature base needs to move on from documenting effects, and think about what the consequences of those effects are for species interactions, fitness, reproduction/growth and the rest of the foodweb etc etc.

RESPONSE: We recognise the importance of these three issues, and minor edits and additions to our manuscript have been made to indicate that awareness. For example:

“This [laboratory-based] approach has the advantage of enabling statistical testing of cause and effect for single factors, yet necessarily omits many of the complexities of natural conditions, that may involve temporal as well as biotic and abiotic environmental factors.”

“Effects on the production of shells and skeletons have been a major research focus, but reduced calcification is not the only impact; there is also strong evidence for low pH affecting many other physiological processes (Pörtner et al., 2014; Baumann, 2019; Hurd et al., 2020), including vertebrate and invertebrate behaviour (Clements and Hunt, 2015; Cattano et al., 2018; Zlatkin and Heuer, 2019).”

But there is risk of being diverted, with discussion of any one of these topics (that each could justify separate monographs) resulting in our manuscript being very much longer than the “few pages” specified in Biogeosciences’ guidance for an Ideas and Perspectives article. Even including brief mention could result in the criticism that our coverage

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of such issues is superficial, not reflecting their importance, and should therefore be expanded.

COMMENT: 3. Is there a standard checklist, or could you provide one in this article, of what authors should be reporting in every paper, e.g. in a table in supplementary? The carbonate chemistry, but what else? Which of these are necessary, and which are nice to have?

RESPONSE: We recognise that the suggested checklist could be a key component of a comprehensive, updated good-practice guide – and very much hope that the preparation of such material might be stimulated by the current discussions (as indicated by our response and manuscript edit already given above). However, the drafting of such a checklist is not a trivial issue; it will require thorough consideration of all the quantitative and qualitative issues involved, on a global basis. We therefore consider that such effort, whilst highly desirable, is outside the scope of our manuscript.

COMMENT: 4. It would be good if you could add some commentary about being pragmatic. Alkalinity, in particular, is expensive so an experiment with hundreds of replicates cannot hope to achieve regular samples from all units on a daily basis. You need some, but there are ways to achieve something sensible (e.g. a set number of aquaria within each treatment, once a week or something). There has to be some common sense, but also some indication of what the acceptable minimum is. The point I am making, and have witnessed at several OA meetings, is that the conversation about chemistry can go way further than is needed when discussing accuracy, repeatability and reproducibility. All of these depend on the system you are in, what the question you are asking is, and what is practically possible, i.e. the requirements are context dependent. A related point is that what works in one system should not dictate what is acceptable in another system. An analogy is the US water quality standards – when they were brought in some states were automatically above threshold as the ground composition was markedly different to the areas where the standards were formulated.

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RESPONSE: We recognise the need for pragmatism in achieving an appropriate balance between perfectionism (controlling and measuring every conceivable influence, to three decimal places) and real-world resource limitations. We also recognise that chemists and biologists may have different priorities in that regard, and that the adopted approach for any replication study should be context dependent. Such ideas are covered in the following additional text:

“Since a very wide range of factors are potentially important, pragmatism will be needed with regard to associated issues of resource deployment and measurement accuracy, recognizing that chemists and biologists may have different priorities on such matters”.

These issues are already covered, to some degree, in the existing best-practice guidance for ocean acidification studies (Riebesell et al., 2011). We consider that such guidance would benefit from updating, as already mentioned; nevertheless: i) inconsistencies in alkalinity measurements per se were not identified as a reason why Clark et al. (2020) did not find the same effects reported in previous studies; ii) the fluctuating and unstable pCO<sub>2</sub> conditions in Clark et al. (2020) that were considered important did not require particularly sophisticated nor expensive techniques for their detection and control; and iii) differences in carbonate chemistry were only 1 out of 16 factors identified by Munday et al. (2020) as potentially influencing the outcome of the experiments.

COMMENT: 5. Variability – there are more sophisticated statistical methods available to look at variability and outliers. There is a danger that trying to make everyone confirm to a very controlled set of conditions means that you lose the insights from the variability that you have factored out. Part of the answer has to be embracing variability and using appropriate statistical approaches to account for and/or explore them. Meta-analysis is one way to look at multiple experiments, but not the only way and much could be done with mixed modelling, GLMM, GAMM and then specific analyses that analyse outliers (rather than account for them).

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RESPONSE: We agree that environmental variability should be embraced, rather than factored out, and that there are opportunities for innovative methods (both statistical and model-based) to investigate its effects. The take-home message from our manuscript is intended to be fully consistent with that approach: there needs to be a spectrum of 'replication' experiments from those that are intended to be as similar as possible to those that are known to radically differ, with results interpreted accordingly. Acknowledgement of such a spectrum goes some way to resolving disputes on whether the conditions for a valid test of reproducibility have been met. There is also a second fundamental issue: the need for interpretation of any single study to take account of the wider body of relevant evidence.

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