VICTORIA UNIVERSITY OF WELLINGTON
Te Whare Wānanga o te Ūpoko o te Ika a Māui

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Dear Dr Steven Lade

We received the comments from two reviewers, which we address below. We thank you and the reviewers for your time in appraising our manuscript, and apologise for the misunderstanding regarding the word count limit we thought we had set on this special issue. Both reviewers provided a positive appraisal of the manuscript but had suggestions for some revisions. We now have thoroughly revised the manuscript and below we detail the revisions that we have conducted and our responses to reviewer suggestions and questions. We detail the reviewer comments in quotations and in italics, followed by our revisions and responses in plain text after each comment. Here in this attached file we address Reviewer #1's comments.

"Reviewer #1:

This manuscript is ambitious and seeks to connect "Physiological and ecological tipping points caused by ocean acidification," as its title indicates. To my knowledge, this question has never been explored in ocean acidification research field and is of crucial importance for understanding the occurrence of tipping points and their consequences for ecosystem and global earth system functioning. This review addresses research requirements highlighted in the latest International Pannel on Climate Change's reports and is of major interest for the scientific community. By connecting tipping points in the biosphere to those occurring in the ecosphere, this study is perfectly in the scope of the journal "Earth System Dynamics"."

We thank the reviewer for this positive appraisal of this manuscript.

"However, despite the critical interest of its research question, this article requires major revisions before being considered for publication. First, despite its title, the article fails to discuss the link between physiological tipping points and their ecological counterpart. To address this problem, efforts must be made to connect the second part, "direct impact on key physiological processes", and the third part, "changes at naturally high CO_2 *locations"."*

We now add further substantial discussion in between the second and third section of this manuscript to address the lack of linkages between section 2 on the physiology of organisms, and its third section on ecological responses (new section 2.4.).

"Key concepts discussed in the article (physiological tipping point, ecological tipping point, reaction norms) are not defined in the introduction, which threatens the understanding of the entire article."

We agree with the reviewer that these points should have been included. We apologise, but the reason they were not included is because we were under the mistaken impression we had a much smaller word limit, and because this submission is part of a special issue on tipping points and we assumed this would be introduced in the summary papers. We now introduce these terms for the reader.

"There is a lack of a whole body of literature (see references in the supplement file) on physiological tipping points to ocean acidification, particularly for molluscs, echinoderms, and pteropods."

We now add these and many others. Apologies, we had mistakenly thought we had to keep these sections very brief, so there is a body of literature on all organisms that is missing, not just those pointed out by the reviewer here.

"Discussion of tipping points is almost absent in parts 2.2 and 2.3, which contrasts with the title of the manuscript."

We now add linking material within these sections back to the tipping point concept.

"Part 3 on "Ecological Tipping Points" lacks important concepts such as "engineer species" to effectively link with Part 2 on "Physiological Tipping Points". A figure is missing to help readers understand the ecological concepts described in Part 3."

We have addressed this concern by adding further material in section 3 that introduces these concepts now, where appropriate.

"Finally, despite its crucial interest for the study, Table 1 is not really discussed even if it could help to resolve some of the problems mentioned above."

Table one is where we obtain the 'tipping points', where they exist, and we previously did not extensively discuss the table because of perceived word counts issues. We now discuss these extensively in each section.

"If significant revision work is done, I believe this review could be of great interest to readers of "Earth System Dynamics" and to the scientific community working on climate change in general.

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Please find suggestions for improving the manuscript in the supplement file"

Thank you for your positive appraisal of this manuscript.

"Attached file:

General comments The term "photo-physiology" has an elusive and very broad meaning. Here, the use of the term "photosynthesis" would be more relevant and would unambiguously qualify the physiological phenomenon mentioned in this article."

We respectfully disagree. Photosynthesis is a process that involves many processes that work in tandem (e.g., CCM operation), and most of the general readership unfortunately will mistake "photosynthesis" with "photosynthetic rates", and we consider this is dangerous to equate Fv/Fm and other related measurements to photosynthetic rates. Hence, we are actually being more specific, rather than ambiguous here in our opinion.

"Introduction Lines 44-46: "However, these analyses rarely assess changes in processes at specific time points or at pCO2 values corresponding to such time points. Instead, they usually determine whether standardised responses are different from zero." Why is it a problem not to analyse changes in processes at specific times or pCO2 values? I think it lacks a bit of context for the reader to understand the importance of studying changes in processes over a wide range of acidified conditions (pCO2, pH, Ω), i.e. reaction norms, in order to identify tipping points. Key concepts for the understanding of the article and its relevance should be defined in the introduction: "physiological tipping point" and "ecological tipping point". What is the definition of a tipping point for a living organism? What is the definition of a tipping point for an ecosystem? What is their relevance for the acclimation/adaptation of species to climate change, the functioning of the Earth system and conservation/mitigation policies? Why is it so important to identify them? These concepts are never clearly defined in the article threatening the global understanding. This reference can help: Carrier-Belleau et al., 2022. "Tipping Points and Multiple Drivers in Changing Aquatic Ecosystems: A Review of Experimental Studies." Limnology and Oceanography 67, no. S1: S312–30."

Yes, we accept that this should be introduced as part of a standalone paper on the topic. We now add this material, but leave this to the editor, as the manuscript is part of a special issue on this topic. We do not define reaction norms, as this phrase is perhaps unhelpful in understanding complex responses to ocean acidification, and we do not use it here.

"…Moreover, I think the article would benefit from defining the concept of "reaction norms" in the introduction. Indeed, establishing reaction norms (modelling physiological processes over a wide acidification range) is essential to identify tipping points. Reaction norms can

take many different forms and can for example be non-linear, e.g. showing tipping points, or linear. This explains the different reaction norms observed in corals mentioned by the authors in lines 7983. Therefore, defining the concept of "reaction norms" would greatly improve clarity for the reader. This should be done in the introduction when the authors discuss the different methodologies used in ocean acidification research."

We use the term reaction norm to explain the effects on molluscs, but for other taxa we respectfully disagree on including this phrase/topic. There is no reaction norm for the response of more complex organisms to ocean acidification, and we consider using this phrase to mislead junior readers. For example, concentrations of H^+, CO_2, HCO_3^- and even saturation state itself can all impact the physiology of taxa that both calcify and photosynthesize. Thus, while for organisms where few physiological processes are being impacted by ocean acidification (e.g. molluscs) this concept is useful, understanding of these processes could be hampered by using such terms here. Additionally, at least in the organisms we study, environmental contexts and individual variability often modify responses beyond the usefulness of such terms.

"Part 2. Direct impact on key physiological processes Lines 56-57. "However, there are also instances of resistant species or entire taxa (Kroeker et al., 2013b; Leung et al., 2022)." Please specify the names of these taxa."

Added, as per table 1 that outlines some of these (e.g., Crustaceans), a well as examples of resistant species (e.g., some *Porites* spp. corals).

"Lines 77-78. "These three taxa are also identified as the most at risk from the effects of ocean acidification." Please add a reference."

Added "Table 1".

"Line 80. "relationship." Replace with "reaction norm". Same comment for the rest of the article."

Replaced.

"The paragraph, lines 94-105, on tipping points in mollusc calcification, omits references available in the literature referring to this issue. The following references must be included: Bednaršek et al., 2019. "Systematic Review and Meta-Analysis Toward Synthesis of Thresholds of Ocean Acidification Impacts on Calcifying Pteropods and Interactions With

Warming." Frontiers in Marine Science 6. This meta-analysis identify tipping points for the whole taxon of pteropods including for calcification and shell structure. Lutier et al., 2022. "Revisiting Tolerance to Ocean Acidification: Insights from a New Framework Combining Physiological and Molecular Tipping Points of Pacific Oyster. Global Change Biology 28, no. 10. This study describes tipping points in the calcification and shell parameters of the pearl oyster.""

It was a mistake to omit Bednaršek et al. 2019 in our table, and it is now included. However, the more recent Leung et al. paper completely overlaps its findings. Bednaršek et al. 2019 also compares saturation state and not more easily comparable and physiologically relevant pH/pCO_2. Additionally, the difficult issue with that paper is that they separately analyse growth and calcification, and that the median "ocean acidification" treatments used had an aragonite saturation sate < 1 (i.e., much lower than the tipping point already identified previously for molluscs in our manuscript).

The Lutier et al. manuscript only examines one species, and they also find extremely low pH tipping points, beyond which will occur under ocean acidification (pH ~7). Thus, it is of limited utility here for our analysis. That being said, a good paper to add to our discussion, thank you for point this out. We now add a citation to this and other papers to our discussion.

"Lines 120-135. This paragraph regrets the lack of studies determining the physiological tipping point in the response of species/taxa to ocean acidification, and in particular the lack of metaanalyses. Although rare, such studies does exist. There is, for example, two meta-analyses (Bednaršek et al., 2021, 2019). It is therefore necessary to cite more references so as not to give a false impression to the reader, for example (this list is non-exhaustive): Bednaršek et al., 2021. "Synthesis of Thresholds of Ocean Acidification Impacts on Echinoderms." Frontiers in Marine Science 8. Dorey et al., 2013 "Assessing Physiological Tipping Point of Sea Urchin Larvae Exposed to a Broad Range of pH." Global Change Biology 19, no. 11. Lee et al., 2019. "Tipping Points of Gastric pH Regulation and Energetics in the Sea Urchin Larva Exposed to CO2 -Induced Seawater Acidification." Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 234 (August 1, 2019): 87–97. Lutier et al., 2023. "Pacific Oysters Do Not Compensate Growth Retardation Following Extreme Acidification Events." Biology Letters 19, no. 8 Ventura et al., 2016. "Maintained Larval Growth in Mussel Larvae Exposed to Acidified Under-Saturated Seawater." Scientific Reports 6, no. 1. Bamber, 1990. "The Effects of Acidic Seawater on Three Species of Lamellibranch Mollusc." Journal of Experimental Marine Biology and Ecology 143 Bamber, 1987. "The Effects of Acidic Sea Water on Young Carpet-Shell Clams Venerupis Decussata (L.) (Mollusca: Veneracea)." Journal of Experimental Marine Biology and Ecology 108, no. 3"

We now cite some of these papers where appropriate. We were under the false impression we had a much tighter word count than we did and we already had around 100 references. However, some of these papers discuss individual instances, and others find tipping points that are much lower than will occur due to ocean acidification (i.e., pH 7.2, pH 6.9). Thus, there is a difference between a "pH tipping point" that will occur below pH 7, and a tipping point that will actually be caused by ocean acidification in the next 100 years.

"There is almost no discussion of "tipping points" in subsections 2.2 and 2.3 on "photophysiology" and "internal pH regulation" even though this concept is emphasized in the title of the article. I understand that this is due to a lack of literature on the subject. However, I think it is possible to make assumptions about the existence of potential tipping points in these key physiological processes based on all the information presents in literature. For example, some thresholds seems to exist in the functioning of photosynthesis in organisms exposed to ocean acidification (Chen et al., 2014. "A Red Tide Alga Grown under Ocean Acidification Upregulates Its Tolerance to Lower pH by Increasing Its Photophysiological Functions."). For tipping points in "internal pH regulation", data are presents in Bednaršek et al. (2021) and Lee et al. (2019)."

Physiological tipping points in internal pH cannot be found for larger groups of logical physiological groups of seaweeds and coral. Thus, we now explicitly state this for the reader.

There are hundreds of papers examining the impacts of ocean acidification on photosynthetic rates/photo-physiology (including 50+ species assessed by the author team), so unfortunately we cannot use this cited example to help the reader. We clarify that we mean there is no meta-analysis that could find tipping points. Nor could there ever with the literature the way it is. This is because photo-physiology is extremely complex, and most studies do not define the affinity of their species for DIC (nor how the CCM changes or not), so we are left with hundreds of studies with responses of organisms that physiologically would be expected not to respond to ocean acidification, and others who would be expected to respond positively. However, these responses are also strongly mediated by light, water motion and other factors often not measured.

"Part 3. Changes at naturally high CO2 locations I think the title of this part should be changed to "ecological tipping points" for clarity. Indeed, the study of changes in an ecosystem naturally rich in CO2 is only one of the methodologies for determining tipping points, another being modelling…"

We thank the reviewer for their comment and change the title of this section now.

"*Further efforts should be made to define what ecosystem tipping points are, i.e. the transition from a stable to an unstable state, and why it is important to identify them (conservation policies, etc.). A conceptual figure would benefit the reader and greatly improve clarity. Additional efforts should also be made to connect physiological tipping points with ecosystem tipping points. Indeed, for the moment part 3 is not really linked to part*

2. The questions are: "What happens to a species when ocean acidification reaches its physiological tipping point?" "How does this translate into tipping points for the ecosystem? ». The authors are already exploring the modification of competition between species by ocean acidification in an attempt to link the physiology of species to ecology."

We now add material addressing this in an entire new sub-section 2.4 as well as wording within section 3, and now add a conceptual figure. We also further clarify that the smaller, almost non-existent, physiological tipping points combine to impact all species within an ecosystem, that manifests in more easily defined and measured ecosystem shifts. Thus, while tipping points in the physiology of most species truly do not exist (outside of the effects on calcification), they do at ecological levels, where small, difficult to measure and variable effects add up to alter ecosystem functioning.

"However, this needs to be explored further, for example by discussing the key concepts of "engineer species", "trophic food webs" and "cascade effect"...."

We consider all readers should have learned this in their undergraduate training by now. We understand the merit of including such terms, and particularly stable state hypotheses. However, it is technically difficult to properly prove the existence of stable or unstable states in most existing ocean acidification work due to the lack of long-term time series in field studies.

"Some mesocosm experiments could also provide clues to the existence of ecological tipping points, for example: Legrand et al., 2017. "Species Interactions Can Shift the Response of a Maerl Bed Community to Ocean Acidification and Warming." Biogeosciences 14, no. 23 Wright et al., 2018. "Ocean Acidification Affects Both the Predator and Prey to Alter Interactions between the Oyster Crassostrea Gigas (Thunberg, 1793) and the Whelk Tenguella Marginalba (Blainville, 1832)." Marine Biology 165, no. 3 This reference could also help: Monaco & Helmuth, 2011. "Chapter Three - Tipping Points, Thresholds and the Keystone Role of Physiology in Marine Climate Change Research." In Advances in Marine Biology, Academic Press, 2011"".

A good point raised by the reviewer. We now add additional material that involves attempts to determine change in mesocosm experiments, or at least discusses information gained and the caveats.

".   Conclusion Lines 326-328. "Quantitative projections of the tipping points at which CO2 will have negative (or positive) impacts is also required for most taxa, where here we generally rely on semi qualitative assessments for all taxa except corals and coralline algae." Incorrect, see reference list for echinoderms and molluscs above."

We agree that for echinoderms and other taxa this does exist, but for "most" taxa it does not, as we state originally. Additionally, some find tipping points far below environmentally relevant seawater pH/pCO_2 values, or assign subjective points during linear declines. We now alter our manuscript accordingly.