LETTER OF RESPONSE TO REVIEWERS

Dear Editor

Biogeosciences

We are here submitting a revised version of the MS “Reviews and synthesis on increasing hypoxia in eastern boundary upwelling systems: zooplankton under metabolic stress”, by Frederick and co-authors. We thank you and the reviewer for the constructive criticism, we have considered all comments, corrections and suggestions from the reviewer, which are now included in the present version.

We appreciate the consideration of our work and thank the reviewer for the very detailed revision, which has considerably improved our work.

On behalf of all coauthors

Ruben Escribano

Review of manuscript egusphere-2024-836 “Reviews and synthesis on increasing hypoxia in eastern boundary upwelling systems: zooplankton under metabolic stress” The manuscript titled "Reviews and synthesis on increasing hypoxia in eastern boundary upwelling systems: zooplankton under metabolic stress" provides a comprehensive and timely synthesis of the effects of hypoxia on zooplankton, with a focus on Eastern Boundary Upwelling Systems (EBUS), a critical area affected by climate change. Zooplankton play a vital role in marine food webs, and understanding their physiological and ecological responses to hypoxia is crucial for predicting broader ecosystem impacts. The review integrates numerous studies and presents valuable insights into the effects of deoxygenation on marine organisms. However, while it addresses relevant and pressing topics, the manuscript suffers from unclear, overly complex, and repetitive writing at times. The scientific argumentation lacks depth in discussing the mechanistic links between hypoxia and ecosystem impacts, and some key ideas are introduced without sufficient clarification or support. After addressing the detailed comments regarding clarification of mechanisms, inclusion of quantitative data, improved structure, and a more thorough discussion of ecosystem consequences, this review will become a robust resource for researchers and policymakers.

Once these revisions are made, the paper should be published, as it will make a significant contribution to the fields of marine ecology and climate change research.

R. We thank the reviewer for the comments and suggestions. We have fully revised the text and deepened the analysis and discussion on mechanisms linking adaptive responses to hypoxia and consequences at population, community and ecosystem level.

Major Points of Critique :

1. Vagueness in Hypoxia Mechanisms: The paper frequently mentions that hypoxia affects zooplankton through physiological and behavioral responses but lacks detailed explanations on how exactly these mechanisms operate at a biochemical or ecological level. For example, the metabolic consequences of hypoxia and oxidative stress are only superficially discussed without clearly linking these processes to specific outcomes for zooplankton populations or ecosystems. Including this information would provide a very strong motivation for the review and provide and excellent resource for future researchers.

R. Thanks for the comment, we have further developed a mechanistic link between the physiological/biochemical responses/adaptions and the changes at population and whole ecosystem level. In this work we are stressing that the major effects of hypoxia on zooplankton are linked to metabolic responses which may depend on species adaptations or lack of adaptation to variable levels of oxygen, and the specific mechanisms result from a trade-off between the adoption of an oxygen-conforming or oxygen-regulating physiology and the energetic demands for other vital processes, such as reproduction, feeding or swimming to avoid predation. Regarding oxidative stress we here emphasize that exposure to severe hypoxia may lead to stressful conditions causing damage due to oxidative stress upon thereafter re-oxygenation. This can occur differently depending on the migration of some species for example, but regardless of that, ocean deoxygenation can certainly enhance this phenomenon with ecological consequences. We have now revised the text to make clear these messages. In the same context, we are adding a new Figure in which we describe in detail the mechanisms underlying hypoxia effects and the connections among different ecological levels.

2. Insufficient Discussion of Data and Citations: Some sections mention important concepts (e.g., "variable tolerance to hypoxia") without referring to specific datasets or detailed empirical evidence. As before, providing concrete examples are needed to motivate the review and support broad claims. Additionally, some of the claims appear to lack references.

R. We agree with the reviewer and now we are providing references and data for some mentions. New references have been added to the work.

3. Lack of Quantitative Insight: The paper rarely provides quantitative information regarding the extent of hypoxia, or the physiological thresholds of different species, which could help readers understand the magnitude of the impact. These could be as simple as providing how the extent of the OMZ has changed globally in the last 20 years, or how it changes from season to season, and providing some species-specific thresholds for key species presented in the manuscript.

R. We are now adding more quantitative data regarding hypoxia and responses to it. Variability of the OMZ over temporal scales are now included as well.

Suggested Improvements:

1. Clarification of Hypoxia Mechanisms: The manuscript should go deeper into the physiological responses of zooplankton to low oxygen. For instance, the authors could describe, how oxidative stress impacts key processes such as reproduction or energy metabolism. Afterwards, the authors could describe by how much reproduction decreases as a function of the experienced oxygen concentration. An explanation of how different metabolic pathways (aerobic vs. anaerobic) are triggered in various conditions would also enhance clarity.

R. See response above. We have now illustrated these physiological responses and how they impact energy usage by metabolism in a new Figure, which is also now discussed along the text.

2. Improving Structure and Reducing Redundancy: The manuscript tends to repeat certain points about hypoxia and upwelling processes, especially in the introduction and review of adaptive responses. Condensing these points and organizing the material more logically would improve the flow. For example, after introducing the role of zooplankton in the food web, the physiological responses could be systematically addressed before transitioning to broader ecological consequences.

R. Text has been fully revised, including these suggestions.

3. Addition of Quantitative Examples: Including more specific data points, such as oxygen levels in different EBUS regions and corresponding zooplankton adaptations, would greatly enhance the argument. Graphs or summary tables showing hypoxia thresholds for key species, and how these relate to oxygen availability in upwelling zones, would make the paper more informative.

R. We have revised the text and further looked for more specific and quantitative data. Some references have been added.

4. Expand the Ecological Consequences Section: The section discussing ecological impacts is underdeveloped. The paper should explore the consequences of zooplankton stress for higher trophic levels in more detail, specifically discussing how changes in vertical migration or metabolic rates translate into shifts in predator-prey dynamics or nutrient cycling.

R. A new figure illustrate potential impact at different ecological levels. The issue relating metabolic stress and further consequences in higher trophic levels in discussed along with a new Figure. However, how this can impact prey-predator interactions becomes an issue very difficult to address and we are just mentioning it but avoiding speculations about it.

5. Recommendation for Conceptual Framework: I suggest the authors to develop a conceptual framework or model (perhaps visual) that links hypoxia, zooplankton physiology, behavior, and ecosystem impacts. This would provide structure and clarity, while helping unify the various points discussed.

R. A new Figure has been added which represents a conceptual model on how metabolism, adaptive responses and energy demands from aerobic respirations can interact to ultimately affect at the ecosystem level.

6. Highlight Methodological Advances: I encourage the authors to discuss emerging technologies or methodologies (e.g., genomic studies, real-time oxygen tracking) that could enhance understanding of hypoxia in EBUS systems, making the review more forward-looking.

R. We are now providing some information on the subject in the Discussion section.

Language and Grammatical Errors:

1. Vague Phrasing: Terms like "hypoxia can challenge marine life" and "it is well known that zooplankton are affected" should be made more precise by specifying what kind of challenge (e.g., reduced reproductive success, mortality) and what specific zooplankton groups are affected.

R. Ok. Changed

2. Redundancy: Some sentences, such as the discussion of oxygen minimum zones and upwelling intensification, can be condensed to avoid redundancy.

R. The text has been revised and several paragraphs shortened or removed to avoid redundancy.

3. Unclear Sentence Structures: Sentences such as "The ongoing combined processes, deoxygenation, increasing upwelling, and OMZ expansion will alter the oxygen conditions in upper layers (<50 m) in EBUS" are complex and hard to follow. Breaking them into simpler sentences would improve readability.

R. Paragraph modified and corrected

4. Spelling and Punctuation: o Many times the verb does not fit the subject (has instead of have). o The term “deoxygenation” is repeated redundantly in some sections. Using alternative phrasing like "oxygen loss" could improve readability. o A few commas and conjunctions are missing in sentences, making them unnecessarily complicated.

R. Text revised

5. Technical Terms Without Definition: Terms such as "Pcrit" and "ROS" should be explained more thoroughly when first introduced to ensure clarity for all readers.

R. Agreed. We have done so

6. Inconsistent Citations: Some important statements lack citations, while others rely on the same sources repeatedly without diversifying evidence. Consider diversifying references, especially when dealing with recent studies.

R. We have fully revised references and added cites

Detailed comments: L 17-18: Revise punctuation and phrasing. For instance: These effects, however, depend on specific adaptations of organisms that have evolved in habitats that are permanently or episodically subjected to low oxygen waters.

R. Ok. Corrected

L 20: the oxidative stress is derived from the exposure to highly fluctuating oxygen conditions, rather than from the conditions themselves.

R. Corrected. We modified the paragraph a separated these sentences.

L 28-29: Stating that increases in mean global sea surface are a consequence of the warming of Earth’s surface and ocean feels redundant. Please rephrase.

R. Corrected. We deleted the second part

L 31-32: Use active voice as this is easier to understand. For instance: The warming of the upper layers of the ocean also drives a greater stratification of the water column, which reduces vertical mixing and affects ocean ventilation. A warmer ocean also lowers oxygen solubility, which further challenges marine life.

R. Agreed. Now modified

L 33-34: Do you mean by about 2%? Also, you specify “a decline in oxygen” and “oxygen loss” in the same sentence, which feels redundant.

R, Corrected

L 37-38: The authors should explain why the decrease in oxygen is more critical. If OMZs are already present, them expanding might not necessarily be worse than a lowering of oxygen at a place where oxygen was previously at a high concentration.

R. Agreed. We removed this paragraph to avoid redundancy

L 39: I encourage the authors to be more quantitative here. For instance, they could specify the area and provide specific locations.

R. Paragraph has been removed

L 42: It is not clear what is meant by "intensifying the OMZ". Do you mean the extent gets larger, or that the oxygen concentration is lowered, or both?

R. This paragraph was deleted to avoid redundancy

L 43-44: Remove "the action of". Name in the citation is wrong according to the bibliography at the end (same error in L 57)

R. OK. corrected

L 57: Should be "…takes place, which compresses the …" Figure 1: It should be raises rather than rises, as the upwelling is performing the action.

R. This sentence was removed.

Figure 2: I encourage the authors to describe the figures in the captions with more detail. As such, the authors could name both modes by name and describe the critical oxygen tension in this caption, including the colors used. In addition, I encourage the users to avoid using green and red in the same plot as this is the most common type of color vision deficiency.

R. Agreed. The Figure was modified as suggested and the caption too to describe it in more detail.

L 109: Only short timescales rather than short-term timescales L 113: Comma before which

R. Corrected

Figure 3: Consistent labels Figure 3 instead of Fig. 3. Also avoid using red and green in the same plot, and a description of the shading is missing.

R. Corrected

L 128-131: Here the authors describe a lot of mechanisms and the interactions with the metabolic rate. However, they are not specific on how everything changes and makes it difficult to understand. It might be easier to say how MR and Pcrit specifically change as oxygen increases/decreases.

R. Agreed. This paragraph has been fully modified.

L 133: Rephrase “does not allow taking advantage of …”. Furthermore, the response itself cannot take advantage of favourable periods. Rather the organisms with this adaptive response can do so.

R. Agreed. Modified as suggested

L. 133-135: Rephrase. The verb does not match the subject. It might also be better to rephrase it from the point of view of an organism. For instance: “Organisms with the second adaptive response are able to exploit ….”.

R. This sentence was removed.

L. 137: Please rephrase. For instance: “Pcrit is constant within a given fish species.”

R. Agree. Done

L 141: Punctuation before “Furthermore” missing and it should be “each” rather than “along”

R. Now corrected

L. 142-143: Rephrase and use the active voice. The verb in the second half does not match.

R. Ok. Corrected

L 162: Please rephrase and use the active voice.

R. Ok. the sentence has been changed

L 164-166: This sentence stands in contrast with the previous one where the authors describe that M. norvergica is able to resist prolonged periods of low oxygen conditions.

R: Agree. We have modified the paragraph

L 170: Rephrase. “…behavioural adaptations have been observed…”

R. Done

L 170-172: This sentence is convoluted and difficult to understand. Please rephrase. Section 3: I encourage the authors to structure this section into several subsections, as this would increase readability and clarity.

R. Paragraph modified

L 174: Start with “Oxidative stress…” as this would make the sentence much clearer.

R. Agree. Done

L. 176-179: The explanation should come at the beginning of the paragraph. L 179: It is unclear what is meant by “stressful”

R. We have fully rephrased these paragraphs

L 184-185: Please rephrase. Rather than saying whether this is surprising or not, I encourage the authors to directly tell the reader why this is important. For instance: “Since all signaling molecules are highly dependent on the oxygen availability for mitochondrial functioning, their regulation is likely impaired by the exposure to variable oxygen levels near the OMZ”.

R. Agree. Done

L 191: O2 was introduced before already L 218: POS was presented previously already. L 225. Remove the first sentence or integrate in the second one, as it does not add any information to the manuscript.

R. Weh have checked all the text to avoid repetition.

L 225-226: Please specify less stressful than what and better than what or under which conditions.

R. Ok. Corrected.

L 234-235: Please rephrase. This sentence is difficult to understand.

R. Paragraph modified

L 239: I encourage the authors to explain what the timing of the development of antioxidant defences is important. Here it would be important to link the development of antioxidant defences to population dynamics and compare the life-stage compositions of key species and potential exposure to deoxygenated waters across the seasons.

R. We used the word "timing" mistakenly, because we meant "time". Now changed.

L 251: Remove the sentence "The interplay between …" or incorporate into the previous sentence as (see Figure X).

R. Sentence removed

L 251-254: Please rephrase. This sentence is rather long and has a lot of information that could be split into several sentences.

R. Sentence removed

L 267: I would rephrase to something along the lines of "likely led to the adaptation" or "likely selected for the adaptation" rather than "required the evolution".

R. Agreed. Changed

L 270-273: I encourage the authors to include some references in this sentence. L 271: SDA is only used once.

R. This sentence has been removed (no reference required)

L 274-276: Please rephrase. For instance: "However, not all zooplankton perform DVM where for instance copepods, which significantly contribute to the bulk of zooplankton biomass in the global ocean, remain within the upper 50 m of the water column."

R. Agree. Done

L 279-280: Remove "Also, as mentioned above" and combine with the previous sentence. In addition, remove occasional, as extreme events by definitions are not frequent.

R. Paragraph has been modified

L 281: Rephrase to "has a strong seasonal signal" or "has a strong seasonal variation" L 285: Verb does not match subject. Do you mean Autumn-Winter winds/conditions exhibit…?

R. Agreed. Changed

L 285: Please rephrase. In my opinion, “depressed upwelling” is not necessarily the same as downwelling.

R. Agreed. We deleted depressed upwelling

L 287-288: Please rephrase. For instance: “before the upwelling spin-up phase” or “during the transitions phase between downwelling and upwelling”

R. Ok. Rephrased

L 290: What is meant by projected conditions? I was under the impression that this paragraph was about upwelling vs. downwelling.

R. We deleted this sentence

L 290: Capitalize to match the previous use of “Autumn-Winter” (L 285) L 291: In my opinion it should be “zooplankton are”

R. Done

L 292-294: Please rephrase: “This oxic condition promotes the existence of ROS production, whereas strong upwelling and the shoaling of the OMZ into the photic zone can expose nonmigratory zooplankton to changing hypoxia-normoxia conditions, which result in POS+ROS.”

R. Done

L 295: Use “by further compressing the vertical extent of the oxygenated habitat” Figure 6: Spell out OMZ in the caption.

R. Done

L 304-306: This paragraph feels out of place here. L 315: The fact that zooplankton tend to have short life spans should be introduced sooner in the introduction. This way, the reader would be able to follow the argumentation here better.

R. Agreed. Sentence removed and idea presented before

L 344-347: Please rephrase. This sentence has a lot of information and is difficult to understand.

R. Agreed. The paragraph has been modified and shortened.