We thank **reviewer #1** again very much for their constructive comments and suggestions on our manuscript. Please find below details on the changes performed in the revised manuscript version that we are now providing based on the comments of both reviews we received. Our point-by-point response is marked in *blue italics* with the prefix *"Response, Revision #1"* where we have also indicated the respective changes by line numbers referring to the marked-up version of the revised manuscript.

RC1: <u>'Comment on bg-2022-157'</u>, Anonymous Referee #1, 12 Aug 2022

The article entitled "Zooplankton community succession and trophic links during a mesocosm experiment in the coastal upwelling off Callao Bay (Peru)" presents a "Timing" of new or timely research results (of special scientific interest), which have not yet been communicated to the public.

The authors studied micro- and mesozooplankton in mesocosms simulating an upwelling under extreme and moderate conditions of the OMZ (N:P) signature for 50 days. To check for possible differences, they also collected samples at a point in the Pacific Ocean close to the mesocosms (control sampling). They obtained values for the abundances of the main species observed, as well as values for stomach content (gut fluorescence), fatty acids, elemental composition (C, N, O, P) and stable isotopes (δ^{13} C, δ^{15} N).

The results obtained may contribute to the knowledge of these first links of the marine food web of one of the largest eastern boundary upwelling systems (EBUSs) the Humboldt Current System, where small and medium pelagic fishes, main predators of the study organisms, are abundant.

The potential and strength of this work are undoubted, however, one of the great weaknesses found in this work has been the lack of a study scheme carried out with the mesocosms that include the study area (location). Not including this information in the work itself has made it very difficult to understand the procedure carried out.

<u>Response, Revision #1</u>: We regret that the lack of study scheme and location area made it difficult to understand the whole experiment set up and working procedure. In the revised manuscript version, we therefore have included a general schematic of a mesocosm together with a map of the study location and positions of the mesocosms off Callao (taken from Bach et al. 2020)..

Likewise, there is a lack of coherence between the hypotheses, the objectives (practically nonexistent), the results and the conclusions that are vague or do not fit the results obtained. Therefore, I suggest this work for "Major Revision".

<u>Response, Revision #1</u>: Both reviewers see a lack of coherence between results and the conclusion, moreover reviewer #1 sees the need of elaborating towards clearly formulated hypotheses or objectives. Our main objective was actually formulated in L77/78 (original version): "With an experimental approach we aimed at improving our mechanistic understanding of the interplay between upwelling processes and coastal plankton dynamics in the northern HCS." But we understand that our wording of the objectives and our approach was apparently not enough to the point. In the revised manuscript we have therefore revised these sections, formulated our objectives clearly and aligned the conclusions better to be coherent with the objectives and actual results (see our specific response to some more comments of the reviewer on that subject further below).

In the attached PDF you will see my suggestions for improvement, both in the main text and in the figures and tables. You will also see specific comments and technical corrections, in order to encourage the robustness and strength of this work. **Citation**: https://doi.org/10.5194/bg-2022-157-RC1 <u>Response, Revision #1</u>: Thanks again very much for the detailed commenting in the pdf. Below, we have collected the comments and suggestions made by the reviewer in the pdf together with our point-by-point replies as to the changes performed in the revised version of our manuscript:

L1: I suggest "is one of the most..."

<u>Response, Revision #1</u>: In terms of fisheries yield, the HCS is the most productive, therefore we have not changed the wording in the revised manuscript version.

L1: To be consistent with the rest of the document it is necessary to include an EBUS "S".

<u>Response</u>, <u>Revision #1</u>: While revising, we noticed that in this case EBUS is a singular case, therefore the plural "s" would be incorrect and, thus, we refrained from adding "s".

L9: different...

<u>Response, Revision #1</u>: different... has been included in the revised manuscript (L8).

L12: Any statistical evidence to prove it?

<u>Response, Revision #1</u>: The differences in NO_x^- were relatively small (2.2 µmol L^{-1}) but significant (p < 0.05) (Bach et al. 2020). We have changed this part of the sentence to: ",... but differences in nutrient concentrations established through OMZ water additions were only borderline significant (L11).

L22: Please, be more precise with your conclusions. Make them more aligned with the objectives and results achieved.

<u>Response, Revision #1</u>: We have revised our conclusions. Please see also further below for our detailed answer(s) to the further comments made by reviewer #1 to our conclusions. The respective sentence in L22 was rephrased to "Concluding, further mechanistic studies are needed to unravel links between intensification and increased frequency of upwelling and expanding/shoaling OMZs and trophic relations of the pelagic food web more precisely" in the revised manuscript (L23–25).

L26/27: Reference, "one of the", and space

<u>Response, Revision #1</u>: The statement of the HCS being the most productive in terms of fisheries yield refers to Bakun and Weeks 2008 and Chavez et al. 2008 that are cited at the end of the next sentence. To make this clearer, we have connected the two sentences into one: "Among the four major...fisheries yield, that despite only moderate ..., sustains extraordinarily large pelagic fish stocks (Bakun and Weeks 2008, Chavez et al, 2008)".

Accordingly, as the HCS is actually the most productive in <u>terms of fisheries yield</u>, we prefer to refrain from replacing "is the most" with "one of the" but have removed the space after "yield" (L26–28).

L30: an

<u>Response, Revision #1</u>: "an" was deleted in the revised manuscript version (L31).</u>

L37: This reference was mentioned in the first place, it should be "a".

<u>Response</u>, <u>Revision #1</u>: During preparation of the revised manuscript, we noticed, that the reference is correctly indicated with an "a" in our bib file and also in our textfile, however, the Biogeoscience bibliography style arranges this Ayón paper second in the list of references and accordingly adds a

"b" to it. As we cannot change this, we suggest to ask the Biogeoscience production office for assistance in case of acceptance of our manuscript.

L72-89: Material and Methods section

<u>Response, Revision #1</u>: L72–76 provide some literature information on drivers of zooplankton composition off Peru. We guess these lines were accidentally included in the section marked yellow and the reviewer comment actually refers to L77–89?

Please rewrite the hypothesis, objectives, aligned to the question (title).

Response, Revision #1: We agree and have rephrased the last paragraph in the introduction to better carve out our objectives to read (L92–103): "Within the framework of a collaborative research project, in austral summer 2017, a large-scale in situ mesocosm experiment was performed in the coastal Peruvian upwelling region off Callao to investigate impacts of upwelling on pelagic biogeochemistry and plankton communities. An upwelling event was simulated by addition of two different types of OMZ waters to each four of the mesocosms. Within this collaborative effort, the aim of the present work was to improve our mechanistic understanding between upwelling processes and coastal plankton and food web dynamics. Our two main objectives were 1) to describe how the mesozooplankton (MeZP) community responds to an upwelling event with water masses of different OMZ signatures and 2) to describe trophic links in OMZ influenced waters. To address the first objective, we monitored the temporal development of the MeZP community in the mesocosms over a 50 d period. The second objective was addressed by analyzing the fatty acid composition and gut fluorescence of dominant copepods, by determining the stable isotope (SI) and elemental composition (C:N) of dominant zooplankton taxa, and by correlating dominating zooplankton as revealed in the present study with the occurrence of phytoplankton simultaneously monitored during this mesocosm experiment by project collaborators (Bach et al. 2020, Bernales et al. in prep.)." Furthermore, in the revised manuscript, we have moved the sentence in L81–84 "Our experiment coincided with a concurrent strong coastal El Niño,... at the start of the experiment." to the start of the discussion section 4.1. (L568–570).

L92: To better understand the procedure of this paper it is advantagous to have an scheme about the mesocosm setup and a figure about the location area... if hard to understand where are located any of this mesocosm... Although the information is in another paper, I suggest to include a brief information here...

<u>Response, Revision #1</u>: As requested by the reviewer we have included Fig. 1 from Bach et al. 2020 in our revised manuscript version showing the study site and location of the mesocosms as well as a mesocosm sketch (Fig. 1 in the revised manuscript).

L94: Any specific net? (details, name...)

<u>Response, Revision #1</u>: It is not a specific net with a specific name. It is simply a 3 mm mesh attached to the upper and lower end of the mesocosm bags to exclude larger organisms from the enclosed water column during the time of deployment. These meshes are removed as soon as the mesocosms are closed at the bottom with the sediment trap and the bags lifted above the surface, i.e. when the experiment starts. For better understanding, we have included this information in the revised manuscript version (L110–111).

L97: <u>Response, Revision #1</u>: the comma after i.e. was removed in the revised manuscript (L112).

L98: Station (hereinafter St.)

<u>Response, Revision #1</u>: We introduced the abbreviation "St." in the revised manuscript (L113).</u>

L116: Please be consistent in all your work.

<u>Response</u>, <u>Revision #1</u>: We are not exactly sure what the reviewer means here? This sentence describes that the zooplankton was sampled with an Apstein net of 100 μ m mesh size from the mesocosms (i.e. it shouldn't be confused with the 3 mm mesh in L108/109).

L155/156: The reference should go here.

<u>Response, Revision #1</u>: As suggested, we have introduced the abbreviation "GF" after fluorescence and mention the reference Mackas and Bohrer (1976) after "method" (L176).

L165/166: Include "GF" when you use gut fluorescence in the first time, for example line 155

<u>Response, Revision #1</u>: As suggested, we have deleted "gut fluorescence" here and use the abbreviation "GF" instead in the revised manuscript (L187).

L167: gut fullness or gut fluorescense Consistency in the terms and between the main text and the text of the figure legend

<u>Response, Revision #1</u>: Gut fullness and gut fluorescence are indeed two different things. In this case, we really mean fullness (i.e. the extent to which the gut is filled with food, Fig. 2), because the GF method needs to be applied during times of maximum fullness of the gut. Accordingly, gut fluorescence is the response measured to estimate on the extent of gut fullness.

L167: Results section

<u>Response, Revision #1</u>: In fact, during preparation of this manuscript we considered mentioning this part in the results section, but then had the impression this information was needed earlier to explain why sampling of mesozooplankton for GF determination was performed during the midst of night. For this reason, we would prefer to keep this sentence in the M&M section.

L170: 100 or 200? Please be consistent in all your work.

<u>Response, Revision #1</u>: Please note, we used two Apstein nets equipped with two different net bags of two different mesh sizes: 100 µm for the regular collection of mesozooplankton samples for abundance determination (L134/135), and in case of copepods used for GF determination we used an Apstein net bag equipped with 200 µm mesh size (for most possible gentle collection of organisms and to exclude as many as possible smaller zooplankton as for this purpose we were only interested in the larger adult female copepods). To help the reader recognize this difference, we have rephrased the sentence to: "MeZP samples were collected with the same Apstein net as mentioned above but equipped with a 200 µm net bag..." in the revised manuscript (L191).

L180: Any reference about this procedure?

<u>Response, Revision #1</u>: The procedure follows recommendations by Båmstedt et al. (2000) in "ICES Zooplankton Methodology Manual", p322–330. We have added a sentence at the start of this paragraph stating that determination of gut clearance rates followed Båmstedt et al. (L194).

L181: Consistency throughout the work, check, in some cases you use "sp" and in other cases "spp".

<u>Response, Revision #1</u>: We have corrected this issue in the revised manuscript. We have deleted "spp." in L181 as this sentence refers to the copepod taxon (L204).

Fig. 1: GF or Gut fullness to be included in the axis.

<u>Response, Revision #1</u>: We have included GF to the y-axis title in the revised manuscript.

Fig. 1 caption: include units

<u>Response, Revision #1</u>: We have included "(ng Pigment Ind⁻¹) after Gut fluorescence and corrected "spp." to "sp." However, we did not remove the full stops as the first one abbreviates "sp" and the second one ends the sentence.

L191: <u>Response, Revision #1</u>: We have included "GF" and deleted "fluorescence" in the revised version (L214).

L192: Are these units correct?

<u>Response, Revision #1</u>: In the literature gut fluorescence is often given as "ng pigment per individual". However, the unit "ng pigment per μ g DM" is the standardized version that better reflects size variation and possible variation in the body constitution among the female copepods, which we prefer to use.

L198:

<u>Response, Revision #1</u>: We have replaced "gut fluorescence" with "GF" (L221).

L251:

<u>Response, Revision #1</u>: "O" was included in the heading (L255)

L264: Consistency of section title and main text.

<u>Response, Revision #1</u>: We agree and have included "O" in the section heading.

L291: And how does it affect the results and the study? By putting this sentence, it seems that something is not right, don't you?

<u>Response, Revision #1</u>: This simply means that for the mentioned taxa, samples were not taken on each day or in each mesocosm. This is often the case in biological experiments, and the statistics used does account for that. We have added this information to the respective sentence in the revised version: "For the two polychaete species as well as the microzooplankton, the sampling design was incomplete, i.e. samples were not taken on each day or in each mesocosm, but the statistics used does account for this fact." (L314/315).

L294: I suggest removing the full stop at the end of "Ind". Indivuduals per Liter; Ind L-1

<u>Response, Revision #1</u>: The full stop was removed here and throughout in the revised version.

L310: ... to the values found in the mesocosms, also after day 18 (Fig. 3).

<u>Response, Revision #1</u>: As suggested be the reviewer, we have added "compared to the values found in the mesocosms, also after day 18" to this sentence (L334).

L310: The difference seems negligible (has a statistical test been carried out, to see if there are significant differences?)

<u>Response, Revision #1</u>: This statement relates to the lower curves of the upper graph of Fig. 3 (now Fig. 4). "Others" in the Pacific (black triangles) have some higher percent contribution than "Others" in the mesocosm treatments also after Day 18 (black triangles fall clearly above the red and blue triangles of the mesocosm treatment means). We didn't do a statistical test because we have only one Pacific sample per time point. For the mesocosm treatments, confidence intervals are shown along with the means. To make this clearer, we have added "(a)" and "(b)" to the two subfigures of Fig. 3, and changed the figure references accordingly.

Fig. 3: text missing on x-axis

<u>Response, Revision #1</u>: Thanks for making us aware of this shortfall. We have added the x-axis text to the revised Fig. 3 (Fig. 4).

Fig. 3 caption: Very confusing to understand without information on the location scheme. Suggestion, why not create three columns or rows and put in one Pacific (control sampling) Second column or row (Mesocosms in extreme conditions) Third column or row (Mesocosms in moderate conditions) In this way we can still achieve a better interpretation of the results in the two subjected conditions.

<u>Response, Revision #1</u>: In the revised manuscript, we have rearranged Fig. 3 as suggested (Fig. 4b).

Fig. 5: cursive

<u>Response, Revision #1</u>: Species names have been formatted in italics (Fig. 6).

Fig. 5 caption: Consistency

<u>Response, Revision #1</u>: Inconsistencies in the legend have been eliminated ("sp." is used consistently) and the strip text was confined to only the species name (Fig. 6).

Fig. 6: is cursive? Cursive

<u>Response, Revision #1</u>: Italics have been changed to normal font (L⁻¹, please note: the " μ " is not cursive) (Fig. 7).

For a better interpretation I suggest first, to put the axis with the same range as the "Copepoda" figure, and second, to see the curve better, you can put the "Copepada" figure in the upper panel and the "Polichaeta" figure in the lower panel.

<u>Response, Revision #1</u>: The maximum copepod biomass is twice as high as the polychaete biomass. Putting them on the same scale will make the second panel rather illegible. Therefore, we preferred not to change the scaling of the figure, but have rearranged the two subplots to have Copepoda above the Polychaets.

Fig. 6 caption: I suggest modifying by "Copepoda" as shown in the figure. Consistency, please check throughout the work. I suggest modifying by "Polychaeta" as shown in the figure.

<u>Response, Revision #1</u>: Copepods have been changed to Copepoda and polychaets to Polachaeta.

L396: Include figure 7 after it is indicated in the text.

<u>Response, Revision #1</u>: The layout of a final manuscript would be the responsibility of the Biogeoscience production office. In general, we would also prefer to have Figures as close as possible to the referring text passage. Of course, we could try to place Fig. 7 closer to its text in a revised manuscript, but it will always depend on the remaining format how well this can be realized (and will not be the final layout anyways).

L401: I suggest including as supplementary material.

<u>Response, Revision #1</u>: GF data are available via the PANGAEA database. Therefore, we have included the PANGAEA doi link (L426/427).

L408: Insert Table 1 after this paragraph.

<u>Response, Revision #1</u>: Usually, reference to Tables and Figures, respectively, follows after the first mention of any of their content in the text. Therefore, we would prefer to refer to Table 1 here together with the first mention of results summarized in Table 1.

Fig. 7: correct

<u>Response, Revision #1</u>: The "L" has been changed to normal font (Fig. 8).

Fig. 7: I suggest placing the "Pacific" curve on top of the other curves, as the dots are not visible.

<u>Response, Revision #1</u>: The Pacific curve is now on top of the others (Fig. 8).

Fig. 7: Please, include the units here.

<u>Response, Revision #1</u>: Units have been included in the revised manuscript (Fig. 8).

L424: Insert Table 2 after this paragraph.

<u>Response, Revision #1</u>: As mentioned above, we prefer to refer to Tables (and Figures) at first occurrence of any of their contents in the text. This approach is also coherent with Bach et al. 2020 and other papers published in this special issue.

Table 1: Please correct the units to be the same as in the main text. Include the units in the text of the table legend.

<u>Response, Revision #1</u>: The unit has been corrected to be the same as in the main text and was included in the legend.

L437: spp., Sp.

<u>Response, Revision #1</u>: In the revised manuscript, we use sp. consistently throughout the document.

L466: ?

<u>Response, Revision #1</u>: Thanks for spotting this wrong Table reference. Of course, this should be Table 2 and we have corrected for this issue in the revised version (L492).

L476: I suggest including as supplementary material.

<u>Response, Revision #1</u>: We agree and have prepared a supplement with pair plots and a table showing Pearson correlations between measured GF of copepods as well as their total abundances and phytoplankton/microzooplankton protist groups (pooled per mesocosm and for data pooled per OMZ treatment) that we have submitted along with the revised manuscript.

L476: This is the first time "protist" is used in main text, please include in parenthesis the groups of protists, already referenced in the text, consistency.

<u>Response, Revision #1</u>: Protist groups have been included in this text passage (L503/504).

L480: P value?

<u>Response, Revision #1</u>: As mentioned above already, we provide now Pearson correlations as supplementary material and have included reference to Table S1 (with significant p-values and correlation coefficients) in the respective text passage. We only mention here that p-values were < 0.05 as including all different p-values would be too confusing (L509).

L485: statistical information should be included

<u>Response, Revision #1</u>: We included reference to Table S1 at the end of this sentence and mention that p-values were < 0.5 (including all different p-values here would be too confusing, L514).

L495: Why not include as supplementary information?

<u>Response, Revision #1</u>: These data will be publicly made available on PANGAEA and the doi link was included in the revised manuscript version (L526).

Fig. 8 caption: Include what the grey dashed lines mean.

<u>Response, Revision #1</u>: According to a comment by reviewer #2, we have added green dashed lines to all figures (3–9) to indicate the days of OMZ water additions to the mesocosms and removed the gray lines indicating phases (not to overload the figure). This further corresponds with Bach et al. (2020).

L508: Include Tables 3 and 4 before discussion.

<u>Response</u>: This is again more a matter of the final typesetting with Biogeoscience production office. If we would change it for a revised manuscript version, it wouldn't be final anyways.

L509-511:

The first paragraph of the discussion is information that is not observed or presented in the results of this work, it is information that has already been published previously. It should be rewritten for the avoidance of doubt.

In the results you have not mentioned "phytoplankton" so, although we can understand what you mean, we have to use the same line throughout the paper.

Please try to modify the text of the discussion to be in line with the results obtained.

<u>Response, Revision #1</u>: We are not exactly sure, if we understand correctly what the reviewer means here, especially with "we have to use the same line throughout the paper". Although we have referred to Bach et al. 2020 several times in this paragraph it is apparently not clear without doubt that what we summarize here to introduce some of the key results of the entire experiment is results presented in Bach et al. To enhance clarity, we have rephrased this text passage starting with the mention that in this paragraph we report results by Bach et al. to provide some basic information on the main outcome of the entire study for the following discussion (539–542). With respect to the phytoplankton, in the revised objectives (last paragraph of the introduction, L92–103), we mention now correlation with phytoplankton. We think, this will help the reader to see the recurrent theme.

Table 3: [µg Ind⁻¹]

<u>Response, Revision #1</u>: We have corrected the unit to make it consistent throughout the document.

Table 3: I suggest including which are polychaetes and which are copepods, at least between parentheses or in a new column on the left, for better interpretation.

<u>Response, Revision #1</u>: We have revised Table 3 and indicated which are copepods and which the polychaets.

Table 4: Isn't it "molar"? Please check consistency with the main text, lines 505.

<u>Response, Revision #1</u>: Yes, correct, it is molar and we have added "... as well as their molar ratios."

L516: Result section

<u>Response</u>: This summarizes results from Bach et al. to describe some wider context of the entire study for the following discussion, i.e. it represents not our results but is introductory information to help put our results into context with the entire study. Therefore, we would like to keep this passage here.

L538: say which group.

<u>Response, Revision #1</u>: We have replaced "on this group" with "on copepods" in the revised manuscript.

L558: There is no mention of "eggs" in the results section.

<u>Response, Revision #1</u>: Egg production was not measured in our study, but as mentioned in this sentence, we frequently noticed egg carrying females of Oncaea and Hemicyclops during processing of zooplankton samples. We have included "(personal observations)" at the end of the sentence for clarification (L593).

L658: The actual text that is in the conclusion should be in the discussion session. Please list 3 or 4 conclusions you draw from your work in this section.

<u>Response, Revision #1</u>: We agree and have included the conclusion text in the discussion (L659–668 of the original version following L628 of the revised version, and L669–675 of the original version following L706 of the revised version). With respect to the second part of this comment, please see our response to the comment in L675 below.

L663: Egg production has not been studied in this work, therefore this sentence cannot be a conclusion of the results achieved.

<u>Response, Revision #1</u>: We have rewritten the conclusion (L730–742) as requested by the reviewer and, as mentioned in response to the previous comment, have moved this sentence to the discussion.

L675: Any conclusions on "Zooplankton community succession and trophic links during a mesocosm experiment in the coastal upwelling off Callao Bay (Peru)"?

Response, Revision #1: We have reconsidered our conclusion section and suggest the following text in our revised manuscript version: "Our experiment provided some interesting insight on the response of a zooplankton community and their trophic relations to an upwelling event with OMZ waters. In particular grazing rates (gut fluorescence) and fatty acid compositions revealed that feeding of dominant copepods on autotrophic food (diatoms) was insignificant and the question arose whether copepods were starving. However, our methodical approach does not allow conclusion on the potential importance of omnivorous feeding. Likewise, the question whether starvation was responsible for reduced fecundity of copepods or whether the loss of eggs (of broadcasting copepods) to the oxygen minimum layer in the mesocosms was the main reason for the low numbers/lack of copepod nauplii found in the mesocosms remains open. To answer this question, future studies need to better constrain the nutritional condition of the copepods (body size/mass, grazing experiments: importance of omnivorous feeding?), the importance of microzooplankton grazing (dilution experiments) versus copepod grazing in the food web, and determine copepod fecundity through determination of instantaneous in situ egg production rates and hatching success. Measures of nutritional condition would also help a profound interpretation of isotopic signatures in relation to starvation of organisms. In this way, trophic links in a plankton community under OMZ influence could be investigated in much more detail and more profoundly, but implementation of further methodical approaches would also require much more time and manpower." (L730–742)

L684: Last author

<u>Response, Revision #1</u>: We think this is more a matter of personal taste and would prefer to keep it as it is (also it is coherent with other papers resulting from this study published in this BG special issue).

L783–788: The order of these two references by Franz et al. should be changed

<u>Response, Revision #1</u>: References were automatically ordered by the Biogeoscience bibliography style according to the journal specific style.

We thank **reviewer #2** again very much for his careful consideration of our work and the time spent commenting on our manuscript. Please find below details on the changes performed in the revised manuscript version that we are now providing based on the comments of both reviews we received. Our point-by-point response is marked in *blue italics* with the prefix *"Response, Revision #1"* where we have also indicated the respective changes by line numbers referring to the marked-up version of the revised manuscript.

Review of Dejo et al.

General comments:

The reviewed paper, "Zooplankton community succession and trophic links during a mesocosm experiment in the coastal upwelling off Callao Bay (Peru)" is an interesting experiment using impressive mesocosms to determine the effect of upwelling and shoaling OMZ on a zooplankton community. The research topic is pertinent, given the predicted increases in upwelling and OMZ shoaling in the region due to climate change. The paper is well written, and I commend the authors on an impressive amount of work. While the experiment was thorough and the resulting samples carefully analyzed, a number of issues remain to be addressed. Based on the below comments, I recommend the paper for Major Revisions.

- The experiment did not have a true control, as all of the mesocosms had deep water added to them. While the surrounding Pacific waters can be used as a partial control, it would have been much more convincing to have several untreated mesocosms throughout the course of the experiment. This makes it difficult to interpret the results and attribute them directly to the treatment, as the containers themselves could have had a large impact on the zooplankton. Zooplankton are known to behave differently when confined in containers and frequently encountering walls, and this could have led to the reduced feeding seen in the results.
- <u>Response, Revision #1</u>: We totally agree with the reviewer with regard to a true control consisting of untreated mesocosms and the potential artificial behavior of zooplankton in closed systems that may have impacted our results. However, it is logistic, budget and time constraints that unfortunately drastically limit the number of mesocosms and resulting samples (chemical, biogeochemical, biological, etc.) that can be effectively handled during such an experiment. However, we believe the impact of the bags on the zooplankton can be considered equal for both treatments, thus allowing for between treatment response comparison. To consider this valid criticism of the reviewer we have to included a sentence in L681/682: "However, captivity of the zooplankton in the mesocosms could also be a reason of reduced feeding".
- Most of the results (abundance, copepod community composition, biomass, fatty acids, isotope ratios) did not show any significant differences between treatments or across the experiment. While this is understandable and often occurs in mesocosm experiments, the authors draw conclusions that are not fully supported by the results or highly speculative. Specifically, the authors argue that the presence of a shallow oxycline led to decreased reproduction of copepods, but their results could have been from lack of reproduction due to starvation or container effects. They do point this out somewhat in the conclusions, but I believe that the evidence presented is too variable and unclear to make any strong assertions about the causes of low nauplii and egg abundances. The main basis for the assertion that shoaling OMZ reduces egg survival seems to be the observations outlined in Lines 345-352 and 586-591. However, the authors do not provide statistical tests or strong support for this observation. It certainly is worth mentioning, but it is not strong enough evidence to base the bulk of the conclusions on, especially given the high variability of the observations.

- <u>Response, Revision #1</u>: We agree with the reviewers' view of being a bit too speculative and one-sided with the conclusions we draw from the finding of low nauplii occurrence/egg abundance. In the revised version we have restructured our conclusion and refrain from basing the bulk of it on these rather speculative aspects. (This was also in accordance with some criticism of reviewer #1 who also suggested to reconsider our conclusion points). Moreover, we have included a sentence in the discussion in (now L600/601) to balance the different aspects that might have contributed to low nauplii occurrence/low reproduction better: "However, starvation or container effects could also have contributed to low reproduction." (L602/603).
- It seems much more supported that the copepods were simply starving throughout the experiment. It would be interesting for the authors to explore more fully why that may be. Was it a change in phytoplankton composition, low phytoplankton abundances, reduced feeding rates due to container effects, or something else?
- *<u>Response, Revision #1</u>: This comment follows the previous line of the reviewers'* • argumentation well and we agree that starvation maybe was the primary aspect to explain low reproduction/nauplii occurrence. The feeding rate measurements (aut fluorescence) we performed largely suggest that autotrophic food sources did not play a major role. It is known, that many zooplankton (respectively copepods) are not restricted to phytoplankton (i.e. are not strict herbivores) but feed omnivorously. Grazing on heterotrophic food was not assessed in this study. Thus, we cannot further conclude whether reduced feeding rates occurred. Pearson correlations also did not suggest for any particularly strong relation between protist groups and adult copepods (note, as requested by reviewer #1, Pearson correlations have been made available as supplemental material together with the revised manuscript version). Hence, we think we have no further means to analytically explore what role starvation might have played. But in the revised manuscript, we have stressed the aspect of starvation more in the discussion (L627/628): "Slightly higher oxygen concentrations at the end of the study resulted from a phytoplankton bloom event facilitated through guanotrophication (Bach et al. 2020), and hence, this concomitant increase in food availability and oxygen concentrations may have supported an increase in eggs and nauplii in both mesocosm treatments."
- It would also be informative for the authors to describe what was present in the sediment trap material at the bottom of the mesocosms. If the copepod eggs and nauplii were indeed being produced but dying due to low oxygen, they would be present in the sediment trap material in high concentrations.
- Response, Revision #1: Yes, we agree, theoretically, the sediment trap material could give valuable information on what zooplankton sank out. In practice, the sediment trap material wasn't analyzed for zooplankton organisms or eggs for the following main reason: time constraints. As standard, the sediment trap material is analyzed for biogeochemical parameters (TPP, BSi, PON, POC, Bach et al. 2020). This requires rapid processing of the freshly collected sediment trap samples. Any prior analyzes of containing zooplankton organisms could only be done in a short time frame usually not sufficient to quantitatively look through the sediment trap samples of eight mesocosms (compare with Lischka et al. 2018, Front. Mar. Sci. 5:379). Moreover, at the prevailing temperature of around 20°C, small and fragile organisms (like nauplii and copepod eggs of Paracalanus and Hemicyclops) decay very fast and usually cannot be detected/recognized in the sediment trap sample anymore (that usually consists of lots of fluffy brown detritus). So, it is a combination of time, available personal, and conflict of methods that prevented analyzes of the sediment trap material for zooplankton organisms or their reproductive outputs, respectively.

Specific comments:

Line 38 – How shallow and intense is the OMZ?

<u>Response, Revision #1</u>: Bakun & Weeks 2008 mention the existence of an intense and extremely shallow OMZ (without providing depth ranges) in the HCS off Peru. Oxygen minimum waters can reach very close to the surface (< 10 m, i.e. into the euphotic zone, Graco et al, 2017, BG 14:4601–4617). During the course of our mesocosm experiment, hypoxic conditions (dissolved oxygen < 25 μ mol L⁻¹) almost consistently prevailed in the surrounding Pacific from 10 m downwards (Bach et al. 2020, introductory paper to the mescosom campaign off Peru 2017). In the revised version, we have included this information in L39–41: "Moreover, the HCS is characterized by a uniquely shallow and intense (acidic) oxygen minimum zone (OMZ) (Bakun and Weeks 2008), where hypoxic waters may reach very close to the surface (< 10 m, Graco et al. 2017), and prevailed already below 10 m depth during our study (Bach et al. 2020)."

Line 93 – What is the size of mesh that makes up the walls of the mesocosms? Can water move through the mesocosm walls?

<u>Response, Revision #1</u>: The mesocosms are not made of a net but of polyurethane bags. The specification and dimension of the bags is mentioned in in L107 in parenthesis.

L100-101 – For clarity, is station 3 considered your "extreme OMZ addition" (M2, M3, M6, M7) and station 1 your "moderate OMZ addition" (M1, M4, M5, M8)?

<u>Response, Revision #1</u>: No, station 3 provided the deep water for our moderate OMZ signature addition, and station 1 the deep water for our extreme OMZ signature. For clarity, we have included this information in L118–121 in the revised manuscript.

L101-102 – I'm not sure what you're referring to here when you say "from/into corresponding depth ranges". Please clarify.

<u>Response, Revision #1</u>: We regret this unclarity and have rephrased this sentence in the revised version of this manuscript to read: "In each mesocosm ~20m³ of water were exchanged with deep water from St. 3 (moderate OMZ signature: mesocosms M2, M3, M6, M7) or St. 1 (extreme OMZ signature: M1, M4, M5, M8). Deep water was injected on Day 11 and Day 12 to similar depth ranges as water had been removed from each mesocosm before (14–17 m and 1–9 m)." (L118–121)

L119 – On each tow in the mesocosms, the net sampled 0.77 m³ of water. Two tows per sampling day and 10 sampling days means that you could have removed zooplankton from up to 15.4 m³ of water total or 28% of the total volume of the mesocosm. Do you think this could have had an effect on your experiment, or is it a small enough volume to not make a difference?

<u>Response, Revision #1</u>: This is an important point and at the same time addresses a general problem with mesocosm experiments (closed systems). Indeed, the number of net samples taken from each mesocosm during a study can always only be a compromise between resulting data resolution (that should ideally be as high as possible) and the effect net sampling has on the density of the zooplankton (that should ideally be as small as possible). For this reason, we always limit the number of nets allowed to be taken from each mesocosm to a maximum of 1/3 of the mesocosm volumes, and – as the number of nets taken is equal in all mesocosms – assume a constant effect on the zooplankton density independent of the upwelling treatment.

L122-123 - What is meant by "quantitatively rinsed"?

<u>Response, Revision #1</u>: We mean that the collected zooplankton was emptied from the cod end of the Apstein net by opening the valve and subsequent rinsing of the cod end through the mesh window to obtain a quantitative sample. For more clarity, we have rephrased the sentence to: "As soon as the abundance net haul was retrieved onboard, the zooplankton sample was emptied into sample bottles with filtered seawater (100 μ m) and the net and cod end were subsequently rinsed to also wash zooplankton attached to the mesh into the sample bottle." (L142–144).

L173 – split using a Motoda splitter?

<u>Response, Revision #1</u>: We are not exactly sure what the reviewer means here. As explained in L158 the zooplankton sample was split applying the HML beaker technique. This technique is the standard splitting method used in the zooplankton lab of Dr. Ayón at IMARPE and is explained in van Guelpen et al. 1982.

L301-304 – What was the zooplankton community abundance and composition in the different deep waters and how did it differ between the two different deep waters and the existing mesocosm community? Was this difference quantified?

<u>Response, Revision #1</u>: Unfortunately, no net samples were taken from the collected deep water (see also our response to another comment on that line further below).

Figure 3 – Should label the x-axis in the upper panel.

<u>Response, Revision #1</u>: This figure is now Fig. 4. We have added the x-axis label of the upper figure (a) in the revised manuscript. (This was also mentioned by reviewer #1).

Line 311-317 - The variability in the "other" zooplankton abundances between days 1, 8, and 10 is perplexing. Why did you find lots of euphausids and Mollusca on day 8, but not day 1 or 10? You mention that the numbers of Chordata increased, but do you think that they hatched and grew, or is the sampling volume too low to accurately measure them? How many individual ichthyoplankton did you count in these samples? It may be more informative to give actual abundances (ind. m⁻³) in this paragraph instead of percentages of the total.

<u>Response, Revision #1</u>: On Day 8 we had a comparatively high number auf euphausiid nauplii in the samples that must have been in the appropriate size range to be collected with our relatively small net on that day. Larger euphausiid larvae and older developmental stages escape from the net. On the contrary, the Mollusca in the mesocosms were mostly meroplanktonic larvae that also only appear in the samples for short, and as soon as they (would) settle to the benthic are lost to the sediment traps of the mesocosms. Chordata (ichthyoplankton) increased because fish eggs were added to the mesocosms on Day 31, however they did not stay for long in the mesocosms (Bach et al. 2020). For clarification, we have added the sentence on fish eggs to the revised manuscript version (L341/342).

Line 345 – Do you think that all nauplii were retained by a 100 um mesh net?

<u>Response, Revision #1</u>: We discussed this point in L678/679. A 100 µm net probably missed the younger nauplii stages but should have captured the older stages (compare with L593–559). I.e. our data should adequately reflect relative changes in (copepod) nauplii abundances.

Line 349-350 – Here and throughout, you say there was an "exceptional peak of nauplii", but was that due to an increase in the abundance of nauplii or a decrease in the abundance of other copepod categories?

<u>Response, Revision #1</u>: Thanks for pointing out this issue, it made us realize an error in the depiction of nauplii in the extreme OMZ treatment that caused the (wrong) peak in mean contribution on Day 36. The correct mean %-contribution of nauplii is actually only 6.4%. We have corrected Fig. 4 (which is now Fig. 5) accordingly and deleted the respective sentence mentioning the "…exceptional peak of nauplii…" (L373–375).

Line 393-395 – Are the generation times of the dominant taxa short enough to allow for observable changes in abundance and biomass over the 50 days of the experiment?

<u>Response, Revision #1</u>: Yes, generation times of the dominant copepods (Paracalanus, Hemicyclops) are quite short at the prevailing temperatures during our study. Generation times of Paracalanus sp. at ±20°C are around 20 days (Liang & Uye 1996, Mar Biol 127:219–227), and at 18°C about 18 days (Checkley 1980, see L580). Generation times for Hemicyclops sp. are not well described, but duration times of copepodid stages (CI–CVI) of species with a symbiotic life style at 25°C vary between 20 to 30 days (Itoh & Nishida 2007, Plankt Bent Res 2:134–146). Acartia sp. develops within a week to the adult stage at 20°C (Miller et al. 1977, L&O 22:326–335). To our knowledge, generation times of Oncaea sp. for low latitude regions are not available, but year-round reproduction is reported (de Melo Júnior et al. 2021, JPR 43:751–761).

Figures throughout – It would be nice if you added a vertical line to each figure or x-axis at day 10/11 denoting when you added nutrients to the experiments.

<u>Response, Revision #1</u>: We have added green dashed vertical lines indicating the days of OMZ water additions to each figure of the revised manuscript.

Table 2 – You should remind the reader what the difference phases correspond to in the table caption.

<u>Response, Revision #1</u>: In the revised manuscript, we have included explanation of the different phases in the heading of Table 2 as a reminder for the reader.

Table 2 – Are the confidence limits from the pooled copepods across all mesocosms in each treatment, or is it from the average differences between mesocosms within a treatment?

<u>Response, Revision #1</u>: We show here the mean %-contribution (as % of total fatty acids) of fatty acids per phase and treatment (moderate, extreme) with their confidence limits. We have included in the table heading the information "per treatment and phase" after "(mean contributions (%) and confidence limits (CI)...." for better clarity.

L534-535 –What was the zooplankton abundance in the water that was added? In the water that was removed? If you calculate the difference between the zooplankton additions and subtractions, is it comparable to the change in abundance you see within the mesocosm? If the dilution effect disappeared after a single day, what caused it to disappear? How could numbers of zooplankton change so quickly?

Response, Revision #1: Unfortunately, the zooplankton removed/added was not quantified (mostly for logistical/manpower reasons during deep-water exchange that is a physical highly demanding, exhausting and time-consuming operation). Moreover, reconsideration of this passage, made us aware of some flaw: The statement in L564–566 of a larger water volume added to the extreme treatment is not correct (apologize for the confusion!), the water volumes added to all mesocosms were equal. But looking at the total zooplankton abundance plotted separately for each mesocosm over the experiment duration, shows that the difference in average abundance between the moderate and extreme treatment on Day 18 (Fig. 2 which is now Fig. 3) is due to some higher abundance in mesocosm 7, whereas the abundance in the remaining mesocosms was much more similar on Day 18 and later on. The higher abundance in M7 could be simply due to patchiness. In the revised manuscript we therefore have included a supplemental figure (Fig. S1) showing single mesocosm total abundance as a function of experiment day. Accordingly, we have deleted the text passage in L564–566: "The short period of noticeable differences of ..., when abundances were back to similar numbers as in the moderate-treatment mesocosms" and add some text mentioning that the higher abundance in the moderate treatment is due to M7, thus the relatively large confidence intervals (with reference to the supplemental figure S1, L563/564).

L542-555 – How specifically did your Hemicyclops differ from descriptions of H. thalassius? This whole section really is mostly new results that were not referenced in the prior results section. A description of a potentially new species warrants its own paper, and this is not particularly relevant to the current paper. I suggest removing this section and submitting it as a separate paper, as it needs much more background description of Hemicyclops taxonomy, anatomy, and ecology to support the authors' conclusions.

<u>Response, Revision #1</u>: We do understand the reviewers' point and agree to remove this section. In the revised manuscript version, we only kept the second last sentence that now follows after "...Criales-Hernández et al. (2008). During our study, Hemicyclops sp. regularly occurred in the surrounding Pacific with different developmental stages including older copepodids." (L588/589).

L572 – What depth was the oxycline in the surrounding Pacific?

<u>Response, Revision #1</u>: The oxycline in the surrounding Pacific was at similar depth (5–15 m, Bach et al. 2020). We have added this information to the sentence in L608.

L574 – Is the "entire water column" to the max depth of the mesocosms or to the bottom of the ocean?

<u>Response, Revision #1</u>: We mean the max. depth of the mesocosms. To clarify, we have rephrased this sentence to: "...over the entire mesocosm water column and all mesocosms..." (L610).

L580-585 – Did you observe eggs attached to adults? Where the females actively producing eggs? Or were they not producing eggs at all due to starvation?

<u>Response, Revision #1</u>: Paracalanus is a broadcast spawner (L612), i.e. we cannot say whether they produced eggs because any egg would have been released in the water column, respectively lost to the sediment traps.</u>

L635 - Can starvation also influence isotopic signatures? If so, how?

<u>Response, Revision #1</u>: Thank you for raising this point. Indeed, starvation would lead to loss of body mass and preferential metabolism of the lighter isotope, with a resulting increase in delta values for

both tracers. In the revised manuscript version, we have added this sentence at the end of this paragraph (L705/706).

L663-665 – It seems that the evidence presented in the paper more strongly suggests that the copepods starved, which led to lack of reproduction. At the very least, it's difficult to disentangle the two potential factors leading to lack of copepod reproduction.

<u>Response, Revision #1</u>: We agree with the reviewer. In the revised manuscript, we have restructured the discussion (i.e. moved most of the conclusion text to the discussion) and in particular rewrote the conclusions to reflect better on potential starvation impact on copepod reproduction in our mesocosms. This matched well with some of the comments made by reviewer #1 who suggested to rewrite the conclusion and move the respective text to the discussion.

L666-667 – How shallow is the OMZ predicted to shoal? Is it close to the ~10-15m depth used in this experiment?

<u>Response, Revision #1</u>: Hypoxic waters occurred in the surrounding Pacific almost constantly throughout our study period from 10 m downwards (Bach et al. 2020). To our knowledge, no studies are available making predictions on the shoaling of the oxycline in the shallow coastal area off Peru. For offshore regions, studies are available on temporal shoaling trends over the last years/decades. Possibly, in the future we may find a similar shallow oxycline offshore as we find today already in the coastal upwelling?

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