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Reply on RC2

Patricia Ayón Dejo et al.

Author comment on "Zooplankton community succession and trophic links during a mesocosm experiment in the coastal upwelling off Callao Bay (Peru)" by Patricia Ayón Dejo et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2022-157-AC2, 2022

We thank reviewer #2 very much for his careful consideration of our work and the time spent commenting on our manuscript. We appreciate his overall positive evaluation and helpful suggestions. Below we provide our detail point-by-point answers in *italics*.

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.
- *Response: 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 suggest to include a sentence in L634: "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.
- *Response: 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. For a revised version we suggest to restructure our conclusion and refrain from basing the bulk of it on these rather speculative aspects. (This would be also in accordance with some criticism of reviewer #1 who also suggested to reconsider our conclusion points).*
- *and 2) to rephrase the discussion in L556–585 and balance the different aspects that might have contributed to low nauplii occurrence/low reproduction better (low reproduction due to starvation and/or container effects, slowed/hindered egg development due to low oxygen concentrations at deeper depth).*
- 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?
- *Response: 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 (gut 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 will be made available as supplemental material). Hence, we think we have no further means to analytically explore what role starvation might have played. But for a revised manuscript, we suggest to stress the aspect of starvation more in the discussion (L589–591): "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: 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?

Response: 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 µmol 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). For a revised manuscript version, we suggest to include this information in L38: "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?

Response: The mesocosms are not made of a net but of polyurethane bags. The specification and dimension of the bags is mentioned in in L93 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)?

Response: 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 would include this information in L100/101 in a revised manuscript.

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

Response: We regret this unclarity and would suggest rephrasing this sentence in a revised version of this manuscript to read: "In each mesocosm ~20m^3 of water were exchanged with deep water from St. 3 (mesocosms M2, M3, M6, M7) or St. 1 (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)."

L119 – On each tow in the mesocosms, the net sampled 0.77 m^3 of water. Two tows per sampling day and 10 sampling days means that you could have removed zooplankton from up to 15.4 m^3 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?

Response: 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"?

Response: 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 could rephrase 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 µm) and the net and cod end were subsequently rinsed to also wash zooplankton attached to the mesh into the sample bottle."

L173 – split using a Motoda splitter?

Response: We are not exactly sure what the reviewer means here. As explained in L173 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?

Response: 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.

Response: Yes, we would add the x-axis label of the upper figure in a 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.

Response: 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 (Bach et al. 2020). The results on fish development will be presented in a different manuscript within this special issue. For clarification, we suggest to refer to this study at the end of the sentence in a revised manuscript.

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

Response: We discussed this point in L631/632. A 100 µm net probably missed the younger nauplii stages but should have captured the older stages (compare with L559). 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?

Response: 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 6.4%. For a revised manuscript we would correct Fig. 4 accordingly and delete the respective sentence mentioning the "…exceptional peak of nauplii…".

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?

Response: 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.

Response: Yes, we would surely include a vertical line indicating the deep-water additions in a revised manuscript.

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

Response: Yes, we would include some explanation of the different phases in the heading 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?

Response: 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 would include in the table heading the information "per treatment" 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: 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 L532 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) 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. For a revised manuscript we therefore suggest to include a figure as supplemental material showing single mesocosm total abundance as a function of experiment day. Accordingly, we would delete the text passage in L531–535: "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).

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.

Response: We do understand the reviewers' point and agree to remove this section. For a revised manuscript version, we would only like to keep the last sentence that would than follow after "…Criales-Hernández et al. (2008). During our study, Hemicyclops sp. regularly occurred in the surrounding Pacific with different developmental stages including older copepodids."

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

Response: The oxycline in the surrounding Pacific was at similar depth (5–15 m, Bach et al. 2020). We would add this information to the sentence in L572.

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

Response: We mean the max. depth of the mesocosms. To clarify, we would rephrase this sentence to: "…over the entire mesocosm water column and all mesocosms..."

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?

Response: Paracalanus is a broadcast spawner (L575/576), 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.

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

Response: 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. For a revised manuscript version, we would add this sentence at the end of the discussion.

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.

Response: Yes, we agree with the reviewer. As mentioned in reply to some comments above, in a revised manuscript, we would restructure our discussion and in particular our conclusions to reflect better on potential starvation impact on copepod reproduction in our mesocosms. This would also be in accordance with some of the comments made by reviewer #1 who suggested to rewrite the conclusion and move the current text to the dicussion.

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

Response: 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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Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2022-157/bg-2022-157-AC2-supplement.pdf