We thank the editors for this opportunity to revise our manuscript, Slow particle remineralization, rather than suppressed disaggregation, drives efficient flux transfer through the Eastern Tropical North Pacific Oxygen Deficient Zone. The reviewers both provided excellent suggestions, which we have implemented. Please consider our revision and response to the reviewers.

Sincerely,

Jacob Cram

**Response to Reviewer 1**

Reviewer #1 Evaluations:

Recommendation (Required): Return to author for minor revisions

Significant: Yes, the paper is a significant contribution and worthy of prompt publication.

Supported: Yes

Referencing: Yes

Data: Yes

Accurate Key Points: Yes

Reviewer #1 (Formal Review for Authors (shown to authors)):

I really enjoyed reading this paper. It was presented extremely clearly, with background on why analyses were done and why different hypotheses were explored, even at a granular level. I took no issue with the conclusions the authors drew from their data. The findings are relevant to a growing body of literature regarding the mechanisms of carbon preservation in ODZs. I think it can be published subject to minor revisions, as described below.

We thank the reviewer for their support of this manuscript and their very helpful comments.

Line 63: This is a particular pet peeve, but the efficiency of the biological pump is really the globally integrated balance of preformed and regenerated nutrients (eg Volk and Hoffert 1985), as opposed to export efficiency, which is what the authors describe here.

The reviewer makes a fair point. We actually mean export flux (flux out of the photic zone and into the mesopelagic), plus transfer efficiency (flux out of the mesopelagic and into water deeper than 1000m) per our introduction section introduction section. We were trying to use “efficiency” as shorthand for this but clearly mis-stepped. We have modified the sentence to the following – to address this comment and a suggestion from the other reviewer:

“Zooplankton modulate carbon flux through the mesopelagic (Jackson & Burd, 2001; Steinberg & Landry, 2017; Turner, 2015), and by extension the export efficiency and transfer efficiency of the biological pump (Archibald et al., 2019; Cavan et al., 2017), in three key ways, each of which could in principle be affected by ocean oxygen concentrations.

Lines 105-106: Incomplete sentence

Removed

Line 159: I got to the introduction hoping to get a teaser of what the answers to the questions laid out here might be. Could a short paragraph synthesizing the most important findings/answers to these three questions be added?

Great idea. We added:

In this manuscript, we report a steepening of the particle size distribution in the upper ODZ, which supports Weber and Bianchi’s first model that microbial remineralization of all particles slows, while disaggregation continues in the ODZ. These disaggregation-like processes did indeed appear to co-occur with acoustic measurements of migratory zooplankton.

Line 160: General methods comment: The collection methods for the acoustics data used to determine zooplankton migration are not described in this section. I'm not familiar with these methods, but it might be helpful to add a brief description of how these data are collected and treated.

We added a section called “Acoustic measurments” which does this.

Line 299: Couldn't chemoautotrophy be a "biological basis" for negative remineralization?

Great point. We have reworded to –

While “negative remineralization” is clearly a simplification of chemoautotrophy and active transport processes which likely have more complex effects on particle size distribution, flux increases in the model tend to be close to zero, and this negative remineralization allows our null model to be robust to flux increases when they occur.

Line 405-406: I had a difficult time determining where this equation came from. It appears to be from an empirical relationship between particle flux from traps and particle size measured by UVP. Is this the case?

The reviewer is correct. We have added the sentence:

“This equation represents an empirical relationship between particle flux from traps and particle size measured by UVP”

Section 6.6: This section is neat, but comes out of the blue a little bit and isn't really returned to in the discussion. Could this be moved to the discussion section, with a more detailed exploration of why the particle dynamics differ?

The reviewer makes a fair point. We have added some discussion of these differences to the discussion section.

We’re loth to move results to the discussion section for philosophical reasons.

Lines 473-474 and 484 (and throughout): Using "small" and "large" to describe binary particle size classes is difficult - especially from those primarily in the in-situ pumped particle community, small and large refer to completely different size classes than the size cutoffs used by the authors here. Could the size bins (<500um and >500um) simply be used in place of small and large in the results and discussion sections?

We chose these cutoffs to refer to Simon et al. (2002)’s definitions of microaggregates and marine snow. We have changed to refer to them as such, or by their size cutoffs explicitly.

Lines 533-534: Are the acoustic signals of jellyfish diagnostic? What about, for instance, salps?

Section 7.4. - Is has been a long time in this paper since the H1-H3 hypotheses were described - it'd be nice to explicitly remind the reader what each of these hypotheses are here.

Lines 579-580: This looks like an incomplete sentence

The sentence has been corrected to

The observation that the rate of change in flux changes with depth suggests some day-to-day variability in this transport.

Section 7.8: It'd be nice if the authors mentioned the rich datasets of in-situ pumped particle POC and mass data that are coming from the GEOTRACES program (and others). These data contain size information, but at a different cutoff scale (53um) than what the authors discuss here, and what the UVP measurements are able to detect. There's a bridge to be built between these communities - and the <53um particle class might be particularly interesting for detecting disaggregation to non-sinking size classes.

We have added a few lines about this and other connections to communities that are sampling smaller particles.

**Response to Reviewer 2**

OVERVIEW

This paper presents a novel dataset of UVP images and paired particle flux (from sediment traps) along

with a model of particle aggregation and disaggregation, which are jointly used to test hypotheses about

how regions of very low oxygen influence the processes working on particles in the mesopelagic. This is

an exciting approach as it leverages the datasets of the UVP in quantitative way to infer midwater

aggregation, disaggregation and transport processes. As these are incredibly difficult to measure in situ

(if not currently technologically impossible in a truly quantitative way), it provides valuable indirect

evidence validating our assumptions about how regions of hypoxia influence both microbial and

mesozooplankton behavior and physiology in the midwater. These findings are contrasted with regions

with less profound low oxygen to hypothesize how these different oxic regimes moderate the various

processes. This is important as it provides a method by which our increasingly numerous UVP datasets

can be leveraged to assess particle flux, attenuation, and the processes that dictate them across a broad

geographic range. Application of this method to global datasets will allow us to quantitatively predict

how the processes of flux and attenuation are likely to shift in the face of expanding midwater hypoxia

in a warming world.

We thank the reviewer for their positive and exceptionally thorough review. The reviewer clearly went above and beyond in providing actionable suggestions and edits, which we think substantially improved this manuscript.

The introduction needs some polish and reworking of the flow of ideas.

We are grateful to this reviewer for their specific comments that enabled us to improve the flow of this manuscript and the introduction section in general. We think that the changes, which we have made, have indeed improved the flow of the introduction.

The testing of the diel component needs a few more details before I feel like the conclusion – that time of day does not matter is certain.

We were remiss in implying that time-of day does not matter. Indeed, we are unaware of a statistical technique that can support such a null hypothesis. Indeed there can always be diel variability beyond what our method can detect. We have softened some of our language that may have suggested that there is actually no pattern. Rather, we did not observe statistically significant diel variability in many of our parameters.

I would prefer that the UVP dataset excluded the zooplankton, but I understand that this

first iteration of pairing the model to field data is tricky and was not designed to explicitly explore the

role of zooplankton.

We are eager to dive into image analysis of some of the UVP data in future projects. After some discussion, the authors have regretfully decided that it is beyond scope of this project to add an image analysis component at this iteration. We thank the reviewer for giving us an “out.” We do agree that this method would be improved by excluding zooplankton from the particle counts and suggest doing so as a future direction. We have added a few lines to the “Opportunities for future directions” section to this effect.

Down the road I look forward to seeing how this group refines the model and the

associated analyses to better disentangle the properties working on particle formation and loss as it is a

powerful tool for testing our understanding of midwater biogeochemical cycling.

So do we! Stay tuned for more exciting analyses and models.

GENERAL COMMENTS

If this approach is going to be valuable to the community then the protocol for using the UVP datasets

needs to be made transparent and accessible. Currently the UVP data is processed through “custom

MATLAB scripts (L185). These need to be made available prior to publication. They can be shared in

BCO-DMO, github, or as a supplementary file. Without them, the findings of the paper are not

reproducible. If these are available in the data repository listed (figshare??) that needs to be stated and

the scripts pointed to.

Thank you for pointing this out. In the previous version, due to miscommunication, we falsely claimed that we used custom matlab scripts. In fact, we used the publicly available Zooprocess software. We have spelled out our analysis pipeline in the “Particle size measurements” section of our Methods.

Right now the figshare appears to be closed so I could not look through the code.

We were alarmed to hear that the reviewer was not able to access our repositor. It was, and is, to our knowledge, open to anyone at the link provided in the manuscript. Multiple authors have confirmed that the link works. We realize an earlier version of the manuscript accidentally had a version of the repository that was behind the university of Maryland proxy. This version, to our knowledge was flagged by the editors prior to being sent out for peer review. Perhaps the reviewer somehow received this earlier version. We have confirmed that the link in the manuscript that we are returning is indeed correct. If the reviewers or editors have any trouble following it, please let us know.

The definition of zooplankton modulation of carbon in the introduction is not precise or accurate.

Specifically, active transport presented in the introduction is not the standard definition (which includes

DOC, POC, respiratory CO2, and mortality at depth supported by surface consumption of carbon in any

format). The authors have defined active transport as the creation of fecal pellets that are the product

of repackaging. They have then defined the respired component as the metabolism of midwater

particles. Since the focus of this work is the particles I would summarize the midwater processes as 1)

active transport, which would cause particles to “appear” in the midwater - fertilizing the microbial food

web and adding particles from “nowhere”, 2) repackaging (your definition is fine here but should be

interpreted as only the change in particles that are consumed in the midwater), 3) and disaggregation,

which is the breaking of large particles into smaller ones in the midwater. This is done both by swimming

and particle rejection, but also by fragmentation or sloppy feeding (Check Steinberg and Landry 2017 for

some good references in the DOC and fecal pellet fragmentation sections).

Done! These were excellent suggestions that we agree improve the structure of the manuscript. We have implemented them as suggested. Thanks also for pointing us in the direction of excellent coprorhexy references, which we have added. We hesitate to call zooplankton fragmenting particles by feeding “sloppy feeding” as that term is often associated with DOM release, rather than POM release from particles.

The other thing that may contribute to the particle spectra is the DOC signature of the zooplankton – enrichment that could occur as part of any of the three prior processes that would result in more labile carbon available to support a growing microbial community. The DOC related processes will be tricky to pull out of this dataset so I don’t think it is important to mention them.

The reviewer makes a great point, that DOC could have important effects on the midwater microbial community and this might result in in-situ production. Per the reviewer’s suggestion, we are not mentioning this in the introduction.

The flow of ideas in the introduction are not smooth. You need a sentence or two to transition between

Line 77-78 about the effect of low O2 on zooplankton. There is an understanding that super low oxygen

changes the vertical migration depth and also the rate of physiology of the organisms. Look in Seibel

2011 for a fast review with good references and use the Bianchi paper about acoustics + midwater for

the biogeography of DVM. Then maybe also a line later about how the physiology of the microbes is

modified. Alternatively move the zooplankton paragraph later (to after line 93??)

We thank the reviewer for the suggestions about improving the flow of the paper. We have added transition statements and relocated the paragraph about zooplankton as suggested.

I would suggest structuring the hypotheses differently. I think they are better presented as patterns of

particle transformation that can be modeled. For clarity, H2 needs to be restructured into what this

would look like from the particle perspective and then explain that pattern using the sentence

“disaggregation by zooplankton….” With the clarification that this occurs since they may be less

abundant or have slower physiological rates. Additionally – is there a way to distinguish between the H2

an H3 particle transformation profiles (L137)?

The reviewer makes a good point here. We have rephrased H2 as suggested, and pointed out that H2 implies a flatter particle size distribution with fewer small particles than H3.

I would revisit the 3 questions you pose. You additionally address how your very low oxygen region

compares with more oxic regions. I might put Question B first.

Question B is first now! We’ve added a question about how our data compare to the oxic station.

Be explicit about the limitations of the UVP. What is the size range it is optimized for and what was the

range you included (methods).

We have spelled out the theoretical range (60 μm and larger -- in principle particles that take up the entire field of view of the camera could be seen). We also indicate that we actually focus only on the 100um and larger range, with the upper bound of particles considered a function of their scarcity. We now explicitly indicate that we did not truncate an “upper bound” for our particles in this analysis, because our negative binomial based statistics are robust to under sampling of large particles.

We write in the Particle Size measurements section

“The instrument is capable of observing smaller particles (down to 60 μm), but these tend to be underestimated and so are excluded from the analysis. The instrument can in principle also measure larger particles (up to the field of view of the camera), though these tend to be scarce enough to be not detected. In this paper, we do not have an upper size cut-off for our analysis and rather implement statistics that are robust to non-detection of scarce large particles (section 5.5.1)”

Additionally, although 5% of the particles are identified as zooplankton,

they are likely not normally distributed across the dataset (they are going to be skewed to the larger end

of the size spectra).

I would actually consider excluding the zooplankton from the dataset because the

behaviors of the larger organisms WILL have a diel vertically migratory component that could modify

statistical analyses of diel and depth associated patterns, and the smaller organisms don’t sink. This is

going to influence your particle size distribution calculations and your sinking speeds. This is mentioned

in the discussion, but I wonder whether it would not be worth doing the analysis. Additionally, in the

future directions section, the authors claim that since there are minimal diel and day-to-day variations

then we should use the same analysis going forward. I think going forward these analyses SHOULD be

done all the time. I love them and I think they are a fantastic way to quantitatively analyzing what I hope

to soon be abundant UVP data. I would, however, encourage the removal of zooplankton from the UVP

datset in future work. I know this is annoying because it means that some minimal annotation must be

done on the datasets and that the step is time intensive and requires someone to know the difference

between a copepod and a particle. Although machine predictions of the various types of particles is

notoriously tricky, sorting between “live and dead” is actually not as bad. And even if you have a 10%

error rate, you still have done a decent job of removing the zooplankton. From my experience, the error

on live/dead sorting tends to be in the smaller size classes, and those are not the particles you need to

be worried about messing up your day/night characterization. Go ahead and suggest that everyone do

this, but emphasize that if they chose not to it will be important to note the time of day (solar, since that

is what the zooplankton care about), and to consider how live migratory animals will matter (they likely

matter even more in other regions). Alternatively apply an upper bound on the size cutoff on the

dataset.

This is a fair point. While we were not able to add image analysis to this project, including simple live-vs-dead analysis, we agree with the reviewer sentiments. We have added a paragraph to this effect to the Opportunities for Future Directions section.

The descriptions of the various trap deployment protocols was extremely confusing. L 199-206 might

need to be visualized with a diagram

We used combination incubators and particle traps. While their function will be described in future manuscripts, after some discussion, we decided here to just point out that they are traps that can incubate, and we just used the trap capability in this manuscript. We hope to confuse fewer readers this way.

When you are looking at day and hour variability, you need to state what times of day you did your casts

in a quantitative way. I don’t have a good sense about how appropriate the spacing and the replication

is to truly address the diel/hour question. Without this information it is tricky to assess the conclusion

that time of day does not matter.

We have added indicators to Figure 2 (Acoustic measurements) to show when the casts were carried out. Per this reviewer’s suggestion, that figure now shows day and night. We hope this will be a clear way to communicate when casts occurred. In brief. Casts occurred at day, night and during twilight.

Please state what you used as your “hour” (GMT, local, solar?). From your plots it seems like you used

GMT since the acoustic backscatter patterns do not seem to follow the DVM solar dark/light cycles. On

that note, in your diel plots i.e. Fig 2 and the supplemental acoustics ones, could you please add a bar at

the top showing periods of light and dark? If you are going for diel variability that is due to zooplankton

activity, contrasting day versus night makes a lot more sense should zooplankton be retained in the

dataset and you should avoid profiles taken at dawn and dusk. If you remove zooplankton from the UVP

dataset then cyclic spline hour makes sense and the effects of aggregation and disaggregation likely

buildup in different layers over the course of the day (sort of like a sin wave that relates to the solar day

– just like SC6).

Our figure 2 is confusing in part because there was (and the reviewer caught) a time-zone conversion issue. We have corrected that issue (this figure, like the others, is now in local-standard time). Per the reviewer’s suggestion, added a bar to indicate day and night to this figure. Per the reviewer’s earlier comment that we need to clarify when our casts happened, so we also added time and depth of UVP casts to this figure as well.

In sections 6.7 and 6.8 be clear about which site and dataset you smoothing and averaging. I found the

transition from section 6.6 confusing because thereafter I was never certain which dataset we were

discussing. Maybe section 6.6 needs to be somewhere else?

Great suggestion. Both of these sections discuss both sites. We have now specified, rather than implied when we are talking about the ETNP-ODZ site, and added paragraph breaks when we switch sites in these section.

DETAILED COMMENTS

L57-58: It is important to be specific about how you are dividing your water column – by light, by depth,

by oxygen, etc (different communities have VERY different working definitions of “deep” or

mesopelagic). Please be specific in your definition of the mesopelagic, and passing through (out?) of the

mesopelagic. Since you contrast it to the photic zone (which is variable), it is unclear what you mean

quantitatively. I \*think\* you mean 200-1000 m.

We added a statement to this effect. “While definitions of vary between studies, we define “mesopelagic” as the region between the base of the photic zone, and 1000m.” In this study, we define the base of the photic as 160m at the ETNP Section 6.1. and 200m in the oxic site (Figure Legend S1), because that is where the chlorophyl fluorescence signal attenuates.

L85: “Understanding the driving mechanisms of these patterns is important because the oxygen

content….”

Changed as suggested

L90: you need a transition thought between the two sentences.

Modified to:

… (Gilly et al., 2013). Models and chemical data are providing clues about the ongoing and potential impacts of these changes. Recent data informed models suggest that ODZs may enhance carbon transport to the deep ocean, by inhibiting microbial degradation of sinking marine particles (Cram et al., 2018).

L93: “…only beginning to be quantitatively explored”

Done

L94: “Current models of …”

Done

L105: remove fragmented sentence “UVPs…”

Removed

L110: “measurements of zooplankton”

Done

L114: MOCNESS (no k, all caps)

Done

L156: You actually demonstrate two locations.

True. Though only one location within the ETNP. This should be less ambiguous now with question D added, per this reviewer’s earlier suggestion.

L191: “for between 21-96 hours”

Removed, as this is redundant with the later 21-91 hour statement.

L196: here you say 21 and 93 hours. This is inconsistent with L 192 and with the 91 hours in L206

Both were wrong. The trap was actually deployed for 91 hours. Corrected.

L201: Move the sentence L201-202 further up in the paragraph (to 196?)

This section was removed in favor of a simpler explanation.

L215: Sentence is fragmented. I assume samples were above the detection limits?

This was indeed a run-on sentence. The mass spec didn’t see any carbon in four traps. These traps were excluded. Modified to clarify

L221: “which is within the ODZ, and 1000…”

Done

L252 what are Cf and A? Is this the same A as L256?

We refer to the coefficients in Eqn. 2. “A” is indeed the same as in L258 which we have now pointed out.

L274: “of the form:”

Done

L288: “indicates the role of”

Done

L344: “that reverse migrated…”

Done

Figure 2: I don’t think the arrow bar for D is in the correct space. As previously mentioned, explain

day:hour (GMT? Local?) and add light/dark cycle bars at the top of the image.

L394: define the surface (80? Above the ODZ?)

This figure has been modified as requested. Some text was added to the legend as well –

Times are local Mexican standard time. The black and white bar at the top indicates day and night periods, with day defined as times when the center of the sun is above the horizon, per the OCE R package. Diamonds indicate the depths and times of UVP casts, with casts below 1000m shown as diamonds on the 1000m line.

L397: it is actually a more even distribution of particle sizes (yes it is a higher proportion of larger

particles compared to the other slopes, but there are still more smaller particles than large ones).

Fair point. We have reworded this sentence to

Steeper, more negative, slopes indicate a higher proportion of small particles relative to large particles, while flatter, less negative, slopes indicate a more even particle size distribution. Flatter distributions still have more more small particles than large ones, but there is a relatively higher proportion of large particles.

Figure 4: For the hour, is that local time or GMT? Can you say less small particles rather than more large

particles (we still have a negative slope).

Local time, as modified. We have modified the notation to “Proportionally more small particles” and “proportionally fewer small particles”. Since there may be more particles of all sizes when the PSD is flatter.

Also, it has to be binned a little bit?

We left these data intentionally unbinned to capture the variability in particle number and apparent particle size distribution slope.

L417: “that quantified how the change of flux…”

Corrected

Figure 5: For the hour, is that local time or GMT? Fix umol carbon

It was Local Mexican time. “μmol C” now always has a space in both panels.

l 509. Listing “disaggregation and

other processes” seems to be an oversimplification. You have active flux particles, disaggregation,

advection, and repackaging/aggregation. Also, why do we only have deviation from the model for the

small particles?

Modified to—

This value serves as a metric of processes that cannot be captured by a null model, which assumes that particles only sink and remineralize. Positive values suggest an excess of small particles, suggesting disaggregation or advection of small partices, while negative values suggest a dearth of small particles, suggesting repackaging and aggregation. DFM is only reported for small particles, because it is the inverse of the deviation from expected flux of large particles.

L565 HA2? Is this H2?

Good catch. There was a lot of re-naming of hypotheses in various drafts and this one snuck through.

L572: Wishner has demonstrated specialized lower oxycline communities. She suggests these

zooplankton are waiting for particles to fall out of the ODZ so this increased disaggregation could be the

result of a community that actively seeks out the lower oxycline and the particles that have escaped

remineralization.(Saltzman and Wishner 1997; Wishner et al. 1995)

Thank you.

“One possible source of disaggregation are zooplankton communities that have been found to specialize in feeding in the lower oxycline (Saltzman & Wishner, 1997; Wishner et al., 1995). This increased disaggregation could be the result of a community that actively seeks out the lower oxycline and the particles that have escaped remineralization. Such a community would likely be comprised primarily of small organisms which the EK60 is not able to measure at this depth.”

L579: The rate of change…

Thank you.

“This could indicate increased disaggregation in this region or horizontal transport of small particles through advection in this region. One possible source of disaggregation are zooplankton communities that have been found to specialize in feeding in the lower oxycline (Saltzman & Wishner, 1997; Wishner et al., 1995) (REFS). This increased disaggegation could be the result of a community that actively seeks out the lower oxycline and the particles that have escaped remineralization. Such a community would likely be comprised primarily of small organisms which the EK60 is not able to measure at this depth.”

.

L580: flux, is that…

Modified to

“The observation that the rate of change in flux changes with depth suggests some day-to-day variability in this transport.”

L592: Zooplankton are also known to …

Thanks.

L607: While, in principle, other…. this increasing in small particles, there is not reason…”

Thank you. Corrected.

L611: “could alternatively explain the increase”

Thank you.

L630: I would remove this sentence as I just found it confusing. You can put the citation in the prior

sentence and we can go there to see how the conclusion was made.

Removed as suggested.

L614: I really don’t think they are looking at temp or salinity – its light and o2 almost always. There is no

evidence that they care much about anything else. I would just remove this sentence.

We removed this sentence.

Saltzman, J., and K. F. Wishner. 1997. Zooplankton ecology in the eastern tropical Pacific oxygen

minimum zone above a seamount: 2. Vertical distribution of copepods. Deep Sea Research Part

I: Oceanographic Research Papers 44: 931-954.

Wishner, K. F. and others 1995. Pelagic and benthic ecology of the lower interface of the Eastern

Tropical Pacific oxygen minimum zone. Deep Sea Research Part I: Oceanographic Research

Papers 42: 93-115.