

Response to the review of Iversen and Ploug:

Title: "Temperature effects on carbon-specific respiration rate and sinking velocity of diatom aggregates – potential implications for deep ocean export processes"

We are grateful to both reviewers for their much appreciated and useful comments and suggestions to the manuscript.

Reviewer #1:

This is an interesting and highly relevant paper that provides insight into how remineralization of POC flux changes with depth in the oceans. The study is a nice addition to existing results that are in the literature. The methods are well explained, and the discussion is insightful. The graphs are well done and convey their information well.

1) **Reviewer:** One point that confused me somewhat is on page 380: Why should disaggregation of the large aggregates occur in the 15C treatment? Had the aggregates grown too large (Kolmogorov scale issues) or did something else happen? **Response:** The most obvious reason for the disaggregation observed in the 15 °C treatment seems due to degradation of the organic matter within the aggregates. We think this could cause the diatom frustules to fall apart and therefore cause disaggregation. This would also explain the less porous and more compact aggregates observed after the disaggregation event. However, this can only be speculated as we did not directly measure this. We do see both an increase in size-specific excess density and dry weight which can only be explained with more material packed in the same volume, which would be the case if smaller particles made up the aggregates (i.e. individual diatom frustules fell apart instead of whole diatoms). Therefore, we think that this disaggregation is more due to a biological mediated process and not only due to

increased hydrodynamic stresses on the falling particles. Of course the sinking of the aggregates causes fluid motion and thus hydrodynamic stresses, without those the aggregates would stay aggregated when their binding forces were weakened by biological degradation. Therefore, the biological degradation processes were the direct reason for the disaggregation, but the fluid motion causes the hydrodynamic stress which eventually caused the disaggregation. We have inserted a discussion paragraph to the disaggregation in the ms; page 386, line 9 - see under **a)** in the **general comments** from reviewer 2.

2) **Reviewer:** There are a few typos that should be corrected and places where a more unambiguous wording could be chosen. For example, page 378: line 5. Is Cd really defined by equation (1)? It is calculated using equation (2) **Response:** The reviewer is correct, Cd is defined by equation 2, it has been corrected in the text.

3) **Reviewer:** Under the section "Aggregate TEP concentration", this was a bit confusing: TEP increased from 0.3 to 8 μg but in which particles? 1 - 5 mm aggregates? **Response:** This has been clarified in the text and now reads: "The content of transparent exopolymer particles (TEP) within the aggregates in both treatments was low but increased with increasing aggregate size from $\sim 0.3 \mu\text{g}$ TEP (Xanthan equivalents) in 1 mm large aggregates and $\sim 0.8 \mu\text{g}$ TEP (Xanthan equivalents) in 5 mm large aggregates.".

Reviewer #2:

Iversen and Ploug present an interesting data set from a carefully conducted experiment on the sinking, size distribution, and organic carbon oxidation of a set of aggregates at 15°C and 4°C. Unsurprisingly, the aggregates at the lower temperatures are oxidized more slowly, exactly the comparison that can be made between food on countertops in kitchens and food kept in refrigerators.

General comments

a) **Reviewer:** What seemed to me the most interesting thing about the work was the apparent increase in excess density of the aggregates at 15°C during the last 6 days of the experiment. I would have liked the manuscript to have considered this in more detail especially as it is a derived parameter and there is no clear corresponding increase in the POC/DW, in the solid hydrated density, nor in the DW/ESD of the aggregates in question. It would be worthwhile for the manuscript to delve into this more quantitatively and with an expanded discussion compared to what is there now, which is mostly freestanding, unrelated statements here and there in the results and discussion that are left to a careful reader to patch together for themselves. **Response:** We did observe an increase in DW/ESD after the disaggregation event (see Fig. 2B). We have included a paragraph in the discussion speculating on the reasons for the disaggregation and the higher compactness of the aggregates after the disaggregation event. This has been inserted on page 386 line 9: “One may speculate on the reasons for an increase in the aggregate compactness. The lack of changes in POC:DW, TEP:ESD, and solid hydrated density throughout the study suggests that the increased compactness cannot be explained by remineralisation of relatively buoyant organic material. The size-specific dry weight and excess density of the aggregates increased after the disaggregation event, as observed on day 17 and 23 in the 15 °C treatment (Fig. 2b and c). Moriceau et al. (2009) suggested that box-shaped diatom frustules might open due to silica dissolution. It is therefore likely that the 3.5-fold higher carbon specific degradation rates at 15 °C compared to the 4 °C treatment caused the frustules of the diatoms within the aggregates to open up and fall apart, explaining the observed disaggregation in the 15 °C treatment. This also implies that the individual diatom cells in the roller tanks at 15 °C had a smaller cell size and possible shorter chain length after day 9. Therefore, the re-aggregation and scavenging of the shorter diatom cell chains and smaller diatom cell sizes would form denser aggregates as compared to the aggregates measured before the disaggregation, since

smaller particle sizes can be packed more compact within an aggregate. The absence of disaggregation in the 4 °C treatment, indicates that the low microbial activity did not lead to opening of the diatom frustules.”

b) **Reviewer:** It would also be good if this trend towards compactness in some of the aging aggregates could be at least briefly postulated on. Physically or chemically, what is happening? Do we have enough of an idea to even begin to make suggestions? Is it worth further study? Does it have a significant effect on carbon flux within the ocean? Is it the same thing that is happening with, for example, discarded larvacean houses as they sink? Is it the same effect that causes aggregates overloaded with inorganic particles (like clays or calcium carbonate) to fragment and lose porosity? The authors need not add a treatise, but some consideration of these things would be valuable, even if only to acknowledge any as yet unanswerable questions raised. **Response:** We have included a paragraph discussing the causes for the disaggregation and the increased compactness (see the reviewer comment above). However, since this is highly speculative and would need to be tested directly under controlled conditions we do not want to go too far with such discussions.

Major comments

1) **Reviewer:** p374, line 26; f/2 contains 105 μM Si and something like 880 μM N. Just to be picky, if Si and N were added in a 1:1 ratio, this is technically not f/2 but some sort of modified f/2 medium. Or more concern is exactly which concentration was used for Si and N, 105 μM or 880 μM ? It should be noted in the manuscript because this is a huge difference. I'd like to throw in some food for thought for the authors: What was their intention with the 1:1 ratio? Given what we know about this species (although not necessarily this strain) would a 1:1 ratio drive the culture into Si or N limitation? Looking at the two *S. cosatum* cultures tested in Brzezinski (1985) at less than continuous illumination would suggest that the

cultures Iversen and Ploug grew into stationary phase were Si limited, which has different implications for cell quotas of C and N, for mortality rates, and presumably, then, for remineralization (e.g., see De La Rocha et al., 2010 MEPS 412, 57-68). It might be worth including some brief words on this in the paper at some point. **Response:** The reviewer is correct we added Si at 106 μM and N at 880 μM , hence Si:N at a 1:8 molar ratio. This is corrected in the text. We observed growth to high diatom concentrations in the cultures, and therefore assume that we can exclude Si depletion from our study. We observed C:N ratios of ~5 for the whole study period which is comparable to the C:N ratios found during the non-starved conditions in De La Rocha et al. (2010). This suggests that we did not have Si or N limitations during our study, as De La Rocha et al. (2010) observed increased C:N ratios to levels above 5 during both nitrogen and silica starvation periods (see fig. 5 in De La Rocha et al. 2010).

2) **Reviewer:** p383, line 23-24; "Our measurements of respiration represent community respiration on aggregates, i.e. it includes respiration by diatoms and associated bacteria and protozoa." Are there really protozoa in GF/F filtered seawater? **Response:** There are no protozoa in the GF/F filtered seawater, however, the cultures were not kept sterile and though we did not observe any protozoa during the experiments, we have previously in other experiments observed that protozoa appeared in the cultures (e.g. ciliates). Those cultures were, however, discarded upon discoveries of protozoa. This statement is included to make the reader aware that measurements of oxygen consumption using microsensors includes respiration from the total living community associated with aggregates; bacteria, the diatoms forming the aggregates, and potential protozoa.

3) **Reviewer:** Discussion; To rephrase what was said in the general overview statement above: The observation of an increase in excess density that is related to (and calculated from) an increase in the sinking velocity vs. ESD relationship in some aggregates on day 17 at 15°C and in all aggregates measured on day 23 at 15°C is quite interesting. As the authors note, this

is not due to a change in the POC/DW of this material or to a change in the solid hydration density of this material. Thus it can only be due to a change in the porosity, or as they put it, "compactness" of the aggregates. However, I am confused as to how this could happen with no clear corresponding change in the DW/ESD of the aggregates. Is DW/ESD not sensitive enough or not determined with enough accurately (ESD is an approximation after all)? Or is there some circularity in the calculation of excess density from sinking velocity and ESD? It would be good to have a brief discussion of this in the paper. **Response:** We did observe an increase in the DW/ESD especially on day 23, which had much higher regression line than any other time point (see Fig. 2B). There is of course scatter in the DW measurements, since these are in the μg range, further, the ESD also have some uncertainties as the aggregates are not spherical but have different shapes, though often approaching ellipsoids. Considering these uncertainties in both dry weight and ESD measurements, we find that the tendency for increased DW/ESD on day 23 must be due to more compact aggregates. We have elaborated on this in the discussion on page 386, line 9 (see above).

Minor Comments:

1) **Reviewer:** p374, line 24; The species name *Skeletonema costatum* should be either italicized or underlined. And if I 'm not mistaken, the name has been changed to *Skeletonema marinoi*. **Response:** The reviewer is correct and the text now read: "Cultures of the diatom *Skeletonema marinoi* (formerly known as *S. costatum*, Sarno et al. 2005) from the North Sea were grown at 15 °C in 0.2 μm filtered...". [Sarno et al. 2005; J. Phycol. 41:151-176]. Accordingly, *costatum* has been changes to *marinoi* in the ms.

2) Reviewer: section 2.8; Just a comment about the centrifugation to determine the solid hydrated density of the "aggregated particles": what does this mean? The term is ambiguous. Does "aggregated particles" mean "aggregates" or "particles making up the aggregates". There

is a significant difference between those two things. Did the aggregates remain whole during the centrifugation? Does it matter whether or not the aggregates remained unbroken during the centrifugation? If they did break, did material of different densities (e.g. organic matter versus silica) end up in different density layers (presumably not from the text). What is the significance of the two endmember densities chosen (1.05 and 1.43 g/cc)? Also, just curious, one only 1 ml of each 2 ml particle-containing density layer was removed for weighing? Were the particles evenly distributed throughout the layer? Some clarification of the above issues is needed in this section. Also "constitutes" in the header should be "constituents". **Response:** This has been clarified in the text. "aggregated particles" has been changed to "aggregates". The aggregates remain whole during the centrifugation due to the aggregates being formed from a mono-culture and therefore all the particle making up the aggregates had similar density, i.e. ended in the same density layer. The endmembers were chosen to ensure that the diatom aggregates would be collected somewhere in the seven layers made from mixing of the least and most dense layer. We sampled 1 ml to make sure that we did not mix to different density layers when sampling (each layer was 2 ml, e.i. 2 cm tall in the centrifugation tube).

3) **Reviewer:** section 2.9 header; Is it exopolymer or exopolymeric? Traditionally it has been the former (e.g. Passow 2002 Prog Oceanogr 55, 287-333). **Response:** The header has been changed to "Transparent exopolymer particles (TEP) measurements".

4) **Reviewer:** p379, line 21; It should be "onto each filter". **Response:** This had been corrected in the text.

5) **Reviewer:** p380, line 10-11; "...showed an increase in the abundance.....and a decrease in the abundance....". **Response:** This has been corrected in the text.

6) **Reviewer:** p380, line 14-16; "Lowering the temperature to 4°C for half of the roller tanks after 3 days did not have any effect on the aggregate size distribution (Fig. 1b)". What the authors are trying to say is correct, but how they say it is not accurate. Compared to leaving the temperature at 15°C, the lowering of the temperature DID have an effect on aggregate size

distribution in that the aggregates at the lower temperature did not disaggregate between days 9 and 12 as they seemingly would have done if the temperature had remained unchanged. It would thus be better to simply remove the sentence quoted above from section 3.1. **Response:** The sentence has been removed from the paragraph.

6) **Reviewer:** p380, line 24; It sounds better as "The amount of TEP within...." The "content of TEP" implies what TEP contains, not the quantity of it. **Response:** This has been corrected in the text.

7) **Reviewer:** Are the figures referenced in order? Fig 4 seems to be invoked before Figs 2 and 3, or have I just missed something? **Response:** Section 3.2 "Aggregate TEP content" has been moved to the end of the result section (below section 3.6) and the section numbers have been corrected accordingly. Hereby, Fig. 4 will not be invoked before Fig. 2 and 3.

8) **Reviewer:** The symbols used for the same day and treatment in the different figures is different (e.g. sometimes day 23 at 15°C is a white square, and sometimes it is a black square). This needs to be made the same from figure to figure (for the relevant figures) to facilitate comparison. **Response:** Each figure has been changed to have similar symbols for the same time points and treatments.

9) Reviewer: p378, line 5; "...where CD is the dimensionless drag forced defined in Eq. (1).."
That should say Eq. (2). **Response:** This has been corrected to Eq. (2) in the text.