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Interactive comment on “Technical Note: Sampling and processing of mesocosm sediment trap material for quantitative biogeochemical analysis” by T. Boxhammer et al.

T. Boxhammer et al.

tboxhammer@geomar.de

Received and published: 31 March 2016

Response to Reviewer #1 (Anonymous Reviewer)

We thank the reviewer for the constructive comments on this technical note. Our responses to the reviewer's comments, including modifications to the manuscript, are detailed in the following:

Comment 1 by Reviewer #1: In my opinion, a proper evaluation of the potential bias in keeping zooplankton in the sediment trap samples must be presented. Questions to answer and/or discuss: what is the proportion of swimmers in samples collected

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since 2010? According to Niehoff et al. (2013), most organisms collected in Svalbard sediment traps were alive (referred to as swimmers in opposition to sinkers).

Author response: The described methods were primarily developed for biogeochemical analysis of the vertical flux of organic matter inside mesocosms to allow elemental budgeting of the enclosed systems. Due to the restricted mesocosm length, the vertical organic matter flux includes vertical migrating zooplankton and settling larvae, which is why all particles and organisms collected in the sediment traps were analysed as a whole. It is generally very difficult to distinguish between swimmers and sinkers reaching the mesocosm sediment traps, as the traps are very shallow compared to open ocean sediment traps so that even dead individuals do not experience much degradation until collection. As the sample volumes are too high to screen the entire samples (see Author response to comment 2 by Reviewer #1) the best chance to calculate general mesozooplankton contribution (swimmers and sinkers) is to analyse subsamples as done for example by Niehoff et al. (2013). To get a direct impression on the sample compositions we started to take high-resolution images of the collected material with a plankton scanner in 2013. The attached Fig. 1 shows image details, highlighting the usually very low ratio of copepods (potential swimmers) to phytoplankton detritus.

Comment 2 by Reviewer #1: How efficient and precise is this protocol to evaluate zooplankton contribution based on subsamples of at most 5% of the sampling volume, especially considering the occasional “patchy” distribution of particles mentioned by the authors?

Author response: The subsample volume of less than 5% for evaluation of zooplankton contribution was chosen to allow for time efficient estimation of zooplankton abundance in the sediment trap samples keeping the subsampling bias on the main sample as low as possible. Screening of the entire sample volumes for zooplankton organisms is usually impossible within limited time as the samples often consist of a very dense particle suspension easily reaching volumes of several litres. Just as an extreme example: We collected 540 samples during a single study in 2013 which were adding up to 907 kg of particle suspension in total. However, Niehoff et al. (2013) have shown that even this

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relatively small subsample volume can be used to generate reliable zooplankton data from mesocosm sediment trap samples.

Comment 3 by Reviewer #1: The authors further mention removing “Mesozooplankton actively swimming in the liquid phase, mostly copepods, . . . together with the supernatant from the settled material” If “some” swimmers are indeed manually removed, how do you precisely evaluate swimmers contribution for subsequent biogeochemical analyses? Are there some alternatives, for instance, solutions to "repel" swimmers from sediment traps or to avoid sampling for them?

Author response: The subsamples for zooplankton contribution analysis were taken after sample collection but prior to processing of the bulk samples (page 18697, line 13 – 14). Thus the removal of the supernatant including actively swimming copepods after settling of the particles just affected the biomass of the bulk sample. However, this was only to a negligible extent, as it was a very small number of copepods (in the range of less than a hundred individuals $\leq 70 \mu\text{mol C}$) compared to the overall biomass collected (1000 – 465000 $\mu\text{mol C sample}^{-1}$). Carbon data used for this comparison originates from a long-term study in 2013 covering very low and extremely high productive phases of the plankton communities. To avoid confusion, we will remove the sentence that Reviewer #1 refers to on page 18698, line 28 to page 18699, line 2. As the mesocosms are closed systems there is no possibility of adding toxins or other solutions to the collecting cylinders of the sediment traps without affecting the whole systems. Accumulated particles are known to be attractive to swimmers and so far we have not seen a possibility to exclude them from the samples.

Comment 4 by Reviewer #1: Sampling of the mesocosms: Although I do really see the advantage of a surface sampling (avoiding frequent diving in cold areas), I wonder whether the system, used for several years, has been occasionally blocked (a 1 cm inner diameter hose seems small to me) or prone to malfunctions.

Author response: The vacuum sampling method keeps the mesocosms entirely closed

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while sampling the sediment traps, but involves a small risk of malfunctions at depth. Current related bending of the silicon tube can easily block the system but we found solutions to almost exclude this risk. The tube itself has relatively thick walls of 3 mm and is only fixed at two points, the collecting cylinder and the flotation frame to avoid potential kinks (Fig. 1a). A wire helix hose coating the first 1.5 m of the tube where it is connected to the collecting cylinder of the trap to prevent bending at depth. To make clear why we have connected the tube at only two points of the mesocosm structure, we will change page 18696, line 21 – 22 in a revised manuscript to read: 'The silicon tube itself is only connected to the bottom of the mesocosm and fixed to the floating frame above sea surface to avoid any kinks (Fig. 1a).' The second reason for a system failure can be a blockage of the outlet of the collecting cylinder by lost parts of equipment. We have been using several methods to retrieve objects that fell into the mesocosms while keeping the mesocosms closed had highest priority. Only if retrieval with live view camera support by hooks, magnets or small nets failed we opened up the collecting cylinder. This is possible at the upper and the lower end, but involves the risk of losing collected material. However, over six years of annual KOSMOS experiments (each lasting for several weeks or months) we only once had to sample one of the sediment traps 24 hours later than planned after the schedule. To include this information in the revised manuscript we will add the following sentence on page 18705, line 4 to read: 'Only in case of a non-reversible blockage of the outlet of the collecting cylinder by artificial objects one can open up the cylinder at the top and the bottom.'

Comment 5 by Reviewer #1: Are you 100% sure of the efficiency of this sampling procedure (i.e. that all sinking material is collected)? A very informative evaluation would be to show average deviations in terms of collected mass between replicated mesocosms (control mesocosms for instance) during the various experiments.

Author response: The particles sinking down in the cylindrical mesocosm bags are concentrated by the sediment traps, which form the conical bottom end of the mesocosms

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and ensure quantitative collection. The critical steps to achieve quantitative sampling are the transfer of particles from the funnel surfaces into the collecting cylinders (please see also Authors response to comment 6 by Reviewer #2) and the emptying of the cylinders. We frequently lowered down a camera system inside to mesocosms to monitor the material accumulating in the sediment traps. A short sequence of one of these videos can be seen in a published video of the sampling strategy to empty sediment traps of the KOSMOS setup, cited in the manuscript on page 18696, line 26 (Boxhammer et al., 2015 (video)). We have followed the suggested evaluation by Reviewer #1 for a perfectly suitable KOSMOS study, which included two sets of five replicates, one set with manipulated pCO₂ while the other set served as control/ambient mesocosms. The experiment lasted for 107 days, covering the whole winter-to-summer plankton succession in a marine fjord system. The average cumulative mass flux in terms of dry-weight was 196 ± 20 g for one set while 193 ± 25 g for the other (author's unpublished data). First the result shows how similar the mass flux was between treatments but secondly also how high the variation was between replicates. This variation is attributed to the slightly different volumes enclosed in the mesocosms (50.74 ± 2.5 t) but also to the plankton communities developing separately from each other for several months after mesocosm closure. Following our observations and the mass flux evaluation we are convinced to collect all settling particles in our mesocosm systems.

Comment 6 by Reviewer #1: Separation of particles from bulk seawater: This is a very informative section based on a proper evaluation of the efficiency of each technique. Since it leads to one of the main conclusion of this manuscript, it should be clearly highlighted in the abstract that does not provide any recommendations so far.

Author response: We thank Reviewer #1 for highlighting this point. We will adjust the abstract so that it reads in a revised manuscript (page 18694, line 10 – 12): 'The particulate matter of these samples was subsequently separated from bulk sea water by passive settling, centrifugation or flocculation with ferric chloride and we discuss the advantages and efficiencies of each approach.'

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Comment 7 by Reviewer #1: I think that Figure 5 is not very informative and not easy to read as presented. What the reader wants to know and easily verify is: how many times did you observe an “unnatural” undersaturation in treated samples? A simple xy plot, Omega_ar before vs. after chemical treatment should be sufficient.

Author response: We agree with Reviewer #1 that Figure 5 is not the easiest to read but we think that this figure includes important information. With this figure we want to illustrate two different messages. First, using constant ratios of ferric chloride (FeCl₃) for particle precipitation and sodium hydroxide (NaOH) for pH compensation has lead to both positive and negative shifts in seawater pH of the samples. This is most likely the case due to different particle densities and characteristics of the sampled material as mentioned in the manuscript. The important information for the reader is that each sample has to be titrated with NaOH individually when using this method. Secondly, undersaturation with respect to aragonite was detected in samples that experienced a negative shift in pH as well as in samples where pH was increased after precipitation. We wanted to show this to illustrate that some of the samples were already undersaturated before any chemical treatment, while in fact the number of undersaturated samples was decreased after chemical treatment (page 18701, line 24 – 26). Using this method as recommended in Sect. 3.2. has the positive side effect of eliminating potential undersaturation of CaCO₃ in the samples as a consequence of CO₂ release by microbial degradation processes inside the collecting cylinders. To highlight this second point in the manuscript we will add the following sentence to page 18701, line 26 in a revised version: ‘This method can therefor also be used to eliminate undersaturation of CaCO₃ in the samples as a consequence of CO₂ released by microbial degradation of the collected organic matter.’

Comment 8 by Reviewer #1: Furthermore, did you check whether these pH decreases leading to stronger undersaturations resulted in significant carbonate dissolution during the flocculation process (how long did it last? maybe I missed it)?

Author response: We did not quantify calcium carbonate (CaCO₃) dissolution during

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the flocculation process, while calcifying organisms were not present in sufficient numbers inside the mesocosms to build up a considerable amount of CaCO_3 ending up in the sediment traps. After adding FeCl_3 and NaOH to the well-stirred samples we allowed the flocks to settle for one hour (page 18701, line 6 – 9). Depending on the amount of concentrated particles, centrifugation of the material took one to five hours before deep-freezing the material. To avoid Undersaturation of CaCO_3 when using FeCl_3 we highly recommend in the manuscript to adjust the pH with NaOH in each sample individually.

Comment 9 by Reviewer #1: Efficiency of grinding process: Table 2. Please provide results for N measurements as well, and if available for ^{13}C and ^{15}N . Showing CV% for C that represents 20-25% of the organic matter is ok. Providing similar estimates for N that is potentially 10-20 times lower in mass would be even better!

Author response: As suggested by Reviewer #1 we will add the results of nitrogen measurements to Table 2. The caption of Table 2, the description of the analysis of the ground material (Sect. 2.2) and the results of material homogeneity (Sect. 2.4) will be adjusted accordingly. In a revised manuscript these parts will than read:

Page 18714, line 1 – 2: ‘Results from replicated carbon and nitrogen measurements of ground sediment trap material in order to test its homogeneity.’

Page 18698, line 13 – 15: ‘Carbon and nitrogen content of the concentrated and subsequently dried and ground bulk material (processing procedure described in Sects. 2.3 and 2.4) was analyzed from subsamples of 2 ± 0.25 mg in tin capsules (5 x 9 mm, Hekatech).’

Page 18703, line 20 – 27 and page 18704, line 1 – 2: ‘We evaluated the homogeneity of finely ground sediment traps samples by five repetitive carbon and nitrogen measurements of samples collected during experiments in different ocean regions between 2010 and 2014 (Table 2). [...] The CV% estimates demonstrate that carbon (CV% = 0.15–0.99) and nitrogen (CV% = 0.28–1.86) measurements of the ground samples are

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at least equally reproducible as measurements of the two calibration standards acetanilide and soil standard with a CV% of 0.34 and 4.17 for carbon and 0.97 and 1.55 for nitrogen, respectively (Table 2).

Replicated measurements of samples including ¹³C and ¹⁵N have not been done.

Comment 10 by Reviewer #1: Figure 6 should be moved to the supplementary material.

Author response: In accordance with the suggestion by Reviewer #1, we will move Figure 6 to the supplementary material in a revised version of the manuscript.

Comment 11 by Reviewer #1: Several cryogenic grinding systems are commercially available, providing (according to the technical specs) a powder of ~5 microns. One could ask what is the originality of your system. Is there a step forward compared to these commercial units (e.g. Cryomill from Retsch or others) that I do not see?

Author response: Our custom made ball-mill for cryogenic grinding of samples operates on the same principles of impact and friction at temperatures close to -196°C as commercial units, e.g. the CryoMill from Retsch. We developed our own system based on two reasons. First we needed larger volumes of the grinding jars/spheres, than commercially available. Even with our maximum volume of 65.5 mL we sometimes have to split up the samples into multiple spheres to homogenise them after the grinding process (page 18703, line 4 – 5). As mesocosm studies produce several hundred of sediment trap samples it is critical to minimize the number of grinding operations. Secondly we wanted to develop an easy to adapt procedure using standard lab equipment for the sample processing to keep the costs relatively low. While a commercial cryogenic ball-mill costs about 20000€ including accessories our system was comparably cheap with less than 1000€ to equip a standard cell-mill. However, commercial systems as the CryoMill of Retch will also be perfectly suitable to grind freeze-dried sediment trap samples.

References:

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Boxhammer, T., Bach, L. T., Czerny, J., Nicolai, M., Posman, K., Sswat, M. and Riebesell, U.: Video of the sampling strategy to empty sediment traps of the “Kiel Off-Shore Mesocosms for future Ocean Simulations” (KOSMOS), doi:10.3289/KOSMOS_SEDIMENT_TRAP_SAMPLING, 2015.

Niehoff, B., Schmithüsen, T., Knüppel, N., Daase, M., Czerny, J. and Boxhammer, T.: Mesozooplankton community development at elevated CO₂ concentrations: results from a mesocosm experiment in an Arctic fjord, Biogeosciences, 10(3), 1391–1406, doi:10.5194/bg-10-1391-2013, 2013.

Figures:

Fig. 1 High resolutions images of collected sediment trap material during a mesocosm experiment in Gullmar Fjord, 2013. While image A is dominated by aggregates of various detrital phytoplankton, image B shows mostly cells of *Coscinodiscus* spp. (diameter of about 200 μm). Red circles highlight adult copepods.

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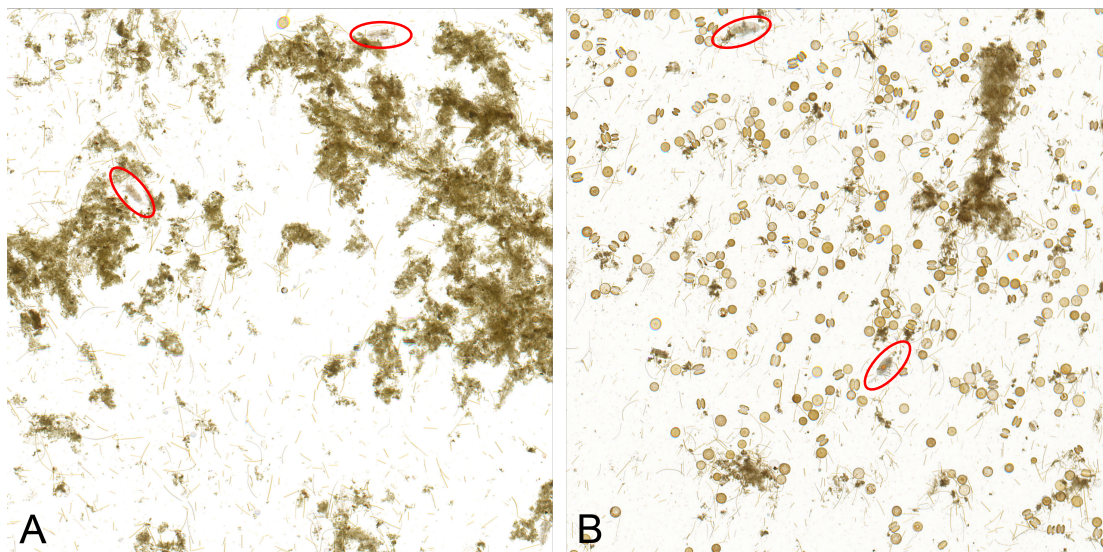


Fig. 1.

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