Technical note: Sampling and processing of mesocosm sediment trap material for quantitative biogeochemical analysis

Tim Boxhammer[1], Lennart T. Bach[1], Jan Czerny[1], and Ulf Riebesell[1]

[1]{GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany}

Correspondence to: Tim Boxhammer (tboxhammer@geomar.de)

Abstract

Sediment traps are the most common tool to investigate vertical particle flux in the marine realm. However, the spatial decoupling between particle formation in the surface ocean and the collection in sediment traps often handicaps reconciliation even within the euphotic zone. Pelagic mesocosms have the advantage of being closed systems and are therefore ideally suited to study how processes in natural plankton communities influence particle formation and settling in the ocean's surface. We therefore developed a protocol for efficient sample recovery and processing of quantitatively collected pelagic mesocosm sediment trap samples for biogeochemical analysis. Sedimented material was recovered by pumping it under gentle vacuum through a silicon tube to the sea surface. The particulate matter of these samples was subsequently separated from bulk seawater by passive settling, centrifugation or flocculation with ferric chloride and we discuss the advantages and efficiencies of each approach. After concentration, samples were freeze-dried and ground with an easy to adapt procedure using standard lab equipment. Grain size of the finely ground samples ranged from fine to coarse silt (2 – 63 µm), which guarantees homogeneity for representative subsampling, a widespread problem in sediment trap research. Subsamples of the ground material were perfectly suitable for a variety of biogeochemical measurements and even at very low particle fluxes we were able to get a detailed insight on various parameters characterizing the sinking particles. The methods and recommendations described here are a key improvement for sediment trap

applications in mesocosms, as they facilitate processing of large amounts of samples and allow for high-quality biogeochemical flux data.

1 Introduction

Sediment traps of various designs are the most common tool to study vertical particle flux in the oceans since mid of the last century (Bloesch and Burns, 1980). During this period, the impact of anthropogenic pollution and climate change on marine biogeochemical cycles has grown steadily (Doney, 2010). Pelagic mesocosm systems enclose natural plankton communities in a controlled environment (Lalli, 1990; Riebesell et al., 2011) and allow us to investigate how changing environmental factors influence elemental cycling in the ocean's surface. The closed nature of these systems makes them particularly useful to investigate plankton community processes that quantitatively and qualitatively determine particle formation and settling. Cylindrical or funnel shaped particle traps were suspended inside various pelagic mesocosm designs (Schulz et al., 2008; Svensen et al., 2001; Vadstein et al., 2012; von Bröckel, 1982). Covering only a small share of the mesocosm's diameter they were prone to potential collection bias also well-known from oceanic particle traps in particular in the upper-ocean (Buesseler, 1991).

To study vertical particle flux in mesocosms it is essential to achieve collection of all particles settling to the bottom. This improves not only the measurement accuracy but also drains the material from the pelagic system, as it is the case in a naturally stratified water body. Different pelagic mesocosm designs like the "Controlled Ecosystem Enclosures" (CEE, (Menzel and Case, 1977), the "Large Clean Mesocosms" (Guieu et al., 2010) or the "Kiel Off-Shore Mesocosms for future Ocean Simulations" (KOSMOS, Riebesell et al., 2013) achieved quantitative collection of settling particles through a cone-shaped bottom of the columnar enclosures. Two different techniques were generally used to sample collected material of these sediment traps: (1) through replaceable collection cups or polyethylene bottles, regularly exchanged by divers (Gamble et al., 1977; Guieu et al., 2010), (2) by means of an extraction tube reaching down to the particle collector (Jinping et al., 1992; Menzel and Case, 1977; Riebesell et al., 2013).

The key difficulty of sediment trap applications in pelagic mesocosms is the sample processing after recovery. Depending on the setup (number of enclosures, trap design, sampling frequency, experiment duration), samples are high in number, relatively large in

volume (up to several liters) and can reach extremely high particle densities during aggregation events.

In the past the collected material was usually only partly characterized to answer specific questions (e.g. Harrison and Davies, 1977; Huasheng et al., 1992; Olsen et al., 2007) while the full potential of the samples remained unexplored and the methodology of sample processing was commonly described in little detail. To fill this gap and to facilitate a broader biogeochemical analysis of the collected material, we refined methods for efficient sampling, particle concentrating and processing of quantitatively collected mesocosm sediment trap samples. Our primary objective was the development of an efficient and easy to adopt protocol, which enables a comprehensive and accurate characterization of the vertical particle flux within pelagic mesocosms. The methods described in this paper were developed and applied during KOSMOS studies from 2010 until spring 2014 covering five different marine ecosystems at diverse stages in the succession of the enclosed plankton communities.

2 Protocol for sampling and processing

2.1 Sampling strategy

The sediment trap design of KOSMOS used since 2011 consists of a flexible thermoplastic polyurethane (TPU) funnel of 2 m in diameter, connected to the cylindrical mesocosm bag by a silicon-rubber-sealed glass fibre flange (Fig. 1A). A detailed description of the KOSMOS setup and maintenance requirements such as wall cleaning can be found in Riebesell et al. (2013). Settling particles are quantitatively collected on the 7 m^2 funnel surface, where they slide down in a 63° angle into the collecting cylinder of 3.1 L volume (Fig. 1B). A silicon tube of 1 cm inner diameter reaches down to the collecting cylinder outside of the mesocosm bag (Fig. 1A). A hose connector links the silicon tube to the conical bottom end of the collector while a wire helix hose coating the first 1.5 m prevents current related bending of the tube (Fig. 1B). 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). To empty the collecting cylinders, we connected 5 L Schott Duran® glass bottles via a Plexiglas® pipe to the silicon tubes attached at the floating mesocosm frames (Fig. 1B; Boxhammer et al., 2015 [video]). A slight vacuum of ~300 mbar was built up in the glass bottles by means of a manual kite surf pump, for gentle suction of the water inside the silicon tubes (step 1 in

1 Fig. 2). When first particles showed up in the Plexiglas® pipe the sampling process was
2 briefly interrupted, seawater in the bottles screened for particles and only discarded if clear.
3 The dense particle suspensions originating from the collecting cylinders were then vacuum-
4 pumped into the sampling flasks until no more particles were passing through the Plexiglas®
5 pipe in a sampled extra volume of about 0.5 L (Boxhammer et al., 2015 [video]).

6 Subsamples of sediment trap material for measurements such as zooplankton contribution
7 (Niehoff et al., 2013), particle sinking velocity (Bach et al., 2012) or respiration rates of
8 particle colonizing bacteria were taken with a pipette after sample collection but prior to
9 processing of the bulk sample for biogeochemical analysis. For this the particle suspension
10 (~1 – 4 L) was gently mixed and subsample volumes withdrawn immediately before re-
11 suspended particles were able to settle down. Total volume of all subsamples should be kept
12 low (ideally below 5 %) in order to limit the subsampling bias on the remaining sample that is
13 processed for quantitative biogeochemical analysis. We occasionally noticed a patchy
14 distribution of particles within the sampling bottles despite the mixing but we consider this
15 subsampling bias to be rather small because subsample volume was usually large enough to
16 tolerate a certain degree of sample heterogeneity. Quantities of the main sample and all
17 subsamples were gravimetrically determined with an accuracy of 0.1 g for individual share
18 calculations.

19 **2.2 Separating particles from bulk seawater**

20 Particulate material recovered from the mesocosm sediment traps and transferred into
21 sampling flasks needs to be separated from bulk seawater collected during the sampling
22 procedure. In this section we describe three different methods for separating particles from
23 bulk seawater, as this was the most critical and time-intense step in the sampling procedure.

24 The particle concentration efficiency (%) of the three methods (subsections 2.2.1 – 2.2.3) was
25 determined as the percentage of total particulate carbon (TPC) concentrated in the processed
26 samples in relation to the sum of concentrated and residual TPC in the remaining bulk water.
27 Residual TPC in the bulk water was determined of subsamples that were filtered on
28 combusted GF/F filters (Whatman, 0.7 µm pore size, 450°C, 6 h) with gentle vacuum
29 (< 200 mbar) and stored in combusted glass petri dishes (450°C, 6 h) at -20°C. Copepods,
30 which could occasionally be found in the liquid, were carefully removed from the filters right
31 after filtration. The filters were oven-dried at 60°C over night, packed into tin foil and stored

in a desiccator until analysis. Combusted GF/F filters without filtered supernatant were included as blanks and measured alongside with the sample filters. Carbon and nitrogen content of the concentrated and subsequently dried and ground bulk material (processing procedure described in sections 2.3 and 2.4) was analyzed from subsamples of 2 ± 0.25 mg in tin capsules (5*9 mm, Hekatech). For this subsamples were directly transferred into the tin capsules and weight determined on a microbalance (M2P, Satorius) with an accuracy of 0.001 mg. All samples were measured with an elemental analyzer (Euro EA–CN, Hekatech), which was calibrated with acetanilide (C_8H_9NO) and soil standard (Hekatech, Catalogue no. HE33860101) prior to each measurement run.

2.2.1 Separating particles from bulk seawater by passive settling

Particles were allowed to settle down for two hours in 5 L glass bottles in darkness at *in-situ* water temperature before separating the supernatant liquid. After this sedimentation period the supernatant was removed and transferred into separate vacuum bottles by means of a 10 mL pipette connected to a vacuum pump (Czerny et al., 2013; Gamble et al., 1977). We found removal of the supernatant to be most efficient when glass bottles were stored in a 60° angle so that particles could accumulate in the bottom edge of the bottles (step 2 in Fig. 2). The dense particle suspension at the bottom of the glass bottles was concentrated in 110 mL tubes by centrifugation for 10 minutes at 5039 * g (3K12 centrifuge, Sigma) to form compact sediment pellets (step 3 in Fig. 2). These pellets were then frozen at -30°C. A cable tie with its tip bent in a 90° angle was stuck into each sample before freezing in order to enable easy recovery of the material from the centrifugation tubes. The frozen samples were transferred to plastic screw cap jars (40 – 80 mL) for preservation and storage in the dark at -30°C before freeze-drying (sect. 2.3).

Separating particulate material from the liquid by passive gravitational settling resulted in a median concentration efficiency of 92.9%. The relatively wide range of scores (99.3 – 86.8%) reflects a non-ideal reproducibility of this particle concentration method (Fig. 3, green). The applied sedimentation period of 2 hours was occasionally not long enough for small or low-density particles to settle. To increase the concentration efficiency of passive settling, longer sedimentation periods of up to 48 hours e.g. for single plankton cells would be required. However, this is not practical at high sampling frequencies of a set of several mesocosms and would require poisoning of the samples to inhibit microbial degradation of organic matter.

2.2.2 Separating particles from bulk seawater by whole sample centrifugation

Centrifuging the entire sample volume, which is usually between 1 – 4 L, can considerably enhance gravitational separation of particles from bulk seawater. This procedure requires a large-volume centrifuge that is not necessarily standard lab equipment and difficult to take out into the field due to its high weight. For this approach we transferred particle suspensions originating from the sediment traps directly from the 5 L sampling flasks into 800 mL centrifuge beakers. Separation of particulate material was achieved within 10 minutes at 5236 * g using a 6-16KS centrifuge (Sigma), followed by slowly deceleration to avoid re-suspension of particles (step 3 in Fig. 2). The supernatant was then carefully decanted and collected for filtration, while the sample pellets were transferred into 110 mL centrifuge tubes. This procedure was repeated until the 5 L sampling flasks were emptied. In a second step of centrifugation for 10 minutes at 5039 * g in the small tubes (3K12, Sigma) samples were compressed into compact sediment pellets which can be frozen and stored in plastic screw cap jars as described in section 2.2.1.

Whole sample centrifugation resulted in a high concentration efficiency of particles with a median of 98.9% and a low variability (98.1 – 99.6%), indicating the high reproducibility of this method (Fig. 3, blue).

2.2.3 Concentrating samples by flocculation and coagulation of particles

Ferric chloride ($FeCl_3$) is well known as a flocculant and coagulant in sewage treatment (Amokrane et al., 1997; Renou et al., 2008), but can also be used for concentrating marine viruses (John et al., 2011) or microalgae (Knuckey et al., 2006; Sukenik et al., 1988). The iron ions form a series of metal hydrolysis species aggregating to tridimensional polymeric structures (sweeping flock formation) and enhance the adsorption characteristics of colloidal compounds by reducing or neutralizing their electrostatic charges (coagulation). Best precipitation results at salinity of 29.6 were obtained by addition of 300 µL of 2.4 molar $FeCl_3$ solution per liter of well-stirred particle suspension, resulting in a very clear supernatant. The disadvantage of particle precipitation with $FeCl_3$, however, is that $FeCl_3$ is a fairly strong Lewis acid and therefore reduces the pH upon addition to a seawater sample. A pH decline in sediment trap samples needs to be avoided in order to prevent dissolution of collected calcium carbonate ($CaCO_3$).

1 To quantify the $FeCl_3$ related pH reduction we added $FeCl_3$ to (1) a seawater sample
2 originating from mesocosms deployed in Gullmar Fjord (Sweden 2013) and (2) to a seawater
3 sample of the same origin in which we re-suspended sediment trap material. This test was
4 carried out in 500 mL beakers at 25°C using a stationary pH meter (NBS scale, 713,
5 METROHM) to monitor changes of the seawater pH (Fig. 4). As expected, addition of
6 150 µL $FeCl_3$ (2.4 M) solution resulted in a distinct drop in seawater pH of about 3 units in
7 the absence of particles (Fig. 4, blue, full boxes) and 1.3 units in the presence of re-suspended
8 particles (Fig. 4, red, empty boxes). The pH decrease was compensated by stepwise titration
9 with three molar NaOH reaching the initial seawater pH after addition of ~330 µL NaOH both
10 in absence and presence of particles. In both cases the calculated aragonite saturation state,
11 representing the more soluble form of biogenic $CaCO_3$, was well above $\Omega = 1$ (Fig. 4, grey
12 dashed line), as calculated with CO2SYS MS Excel Macro (Pierrot et al., 2006) at 25°C,
13 0 dbar, salinity = 29.62 and total alkalinity (TA) = 2206.1 (Bach et al. 2016) with constants of
14 Mehrbach et al., 1973, refitted by Dickson and Millero, 1987.

15 According to the test, 660 µL NaOH (3 M) were simultaneously added with 300 µL $FeCl_3$
16 (2.4 M) to each liter of particle suspension to stabilize the sample pH and to achieve optimal
17 particle precipitation (S1 [video]). The formation of dense and rapidly settling flocks allowed
18 separation of the supernatant and concentration of the deposit as described in section 2.2.1
19 after only one hour of sedimentation. Even though buffering the samples with NaOH, we still
20 observed shifts in seawater pH. Delta pH (ΔpH) was calculated from 50 pH measurements
21 before and after addition of $FeCl_3$ and NaOH to sediment trap samples (pH meter, 3310
22 WTW; InLab Routine Pt1000 electrode, Mettler Toledo). The resulting ΔpH (Fig. 5) differed
23 between individual samples of the same day as well as between sampling days over the 107
24 days of experiment. A maximum spread of 0.46 pH units was observed on day 63 while the
25 minimum difference of 0.15 units occurred on day 103. We did not detect a trend towards a
26 positive or negative shift in pH as the variation in the data lead to an average ΔpH of -0.01. It
27 is likely that differences in the amount and composition of particles in the samples led to the
28 observed pattern. Aragonite and calcite saturation states of the samples after precipitation
29 (Fig. 5) were calculated as described above using in situ storage temperature, pH
30 measurements of the samples and TA values from mesocosm water column measurements
31 (Bach et al. 2016). Undersaturation of both carbonate species already occurred in several
32 samples prior to $FeCl_3$ addition as ocean acidification scenarios were established inside the
33 mesocosm bags and CO_2 released by biomass degradation likely further reduced seawater pH.

1 In fact the number of undersaturated samples after precipitation was reduced by 2 and 6
2 samples with respect to aragonite and calcite. This method can therefore also be used to
3 eliminate undersaturation of $CaCO_3$ in the samples as a consequence of CO_2 released by
4 microbial degradation of the collected organic matter.

5 The $FeCl_3$ approach yielded the highest concentration efficiency among the three methods
6 with a median of 99.6% and a narrow range of scores (98.2 – 99.9%), indicating a remarkable
7 reproducibility (Fig. 3, red). The outliers seen in the boxplot are likely caused by extremely
8 high amounts of transparent exopolymer particles (TEP) in specific samples. We observed
9 TEP in the supernatant of these samples in the form of strings (Alldredge et al., 1993) likely
10 promoting buoyancy of attached particles (Azetsu-Scott and Passow, 2004) and thereby
11 explaining the slightly decreased concentration efficiency in these samples.

12 **2.3 Freeze-drying samples**

13 The water content of the frozen samples was removed by freeze-drying for up to 72 hours
14 depending on pellet size (step 4 in Fig. 2). Lyophilization is preferable to drying the material
15 in the oven for better preservation of phytoplankton pigments (McClymont et al., 2007) and
16 significant improvement of pigment extraction (Buffan-Dubau and Carman, 2000; van
17 Leeuwe et al., 2006). Sedimentation rates within the mesocosms (expressed as collected dry-
18 weight per unit time) were gravimetrically determined and should be corrected for sea salt
19 content. Residual sea salt can be estimated with known loss of water during freeze-drying and
20 known salinity of water in the respective samples. The alternative of removing sea salt before
21 freeze-drying with ultra pure water has the downside of potential osmotic cell rupture and loss
22 of intracellular compounds and should therefore be avoided.

23 **2.4 Grinding the desiccated material**

24 The desiccated sediment pellets were cryogenically ground into a fine powder of
25 homogeneous composition to guarantee representative subsampling. We therefore developed
26 a ball-mill to grind sample sizes from 0.1 to 7.0 g dry-weight. Hollow spheres with volumes
27 ranging from 11.5 to 65.5 mL were cut out of blocks of stainless steel (V4A/1.4571). Each
28 hollow sphere is divided into two hemispheres of exactly the same shape only connected by
29 two guide pins and sealed by a metal sealing (Fig. S1). The size of the grinding sphere was
30 selected according to the dry-weight of the freeze-dried sediment pellets (Table 1). A set

number and size of grinding balls (stainless steal, 1.3541) ranging from 10 – 20 mm in diameter is transferred into the hemisphere containing the sample pellet (Table 1). The second hemisphere is then put on top of the other so that the two hemispheres form a hollow sphere with the sample and the grinding balls locked inside. Sediment pellets heavier than 7.0 g have to be split up into multiple spheres and require homogenization after grinding. After loading the grinding spheres we cooled them down in liquid nitrogen (step 5 in Fig. 2) until the liquid stopped boiling (-196°C). We observed that deep-freezing of the samples is essential for embrittlement of lipids in the organic matter and additionally protects phytoplankton pigments from frictional heating during the grinding process. The deep-frozen spheres (ca. -196°C) were clamped on a cell mill (Vibrogen VI 6, Edmund Bühler) shaking with 75 Hz for 5 min (step 6 in Fig. 2), thereby grinding the material by impact and friction. Before opening the grinding spheres they needed to be warmed up to room temperature to avoid condensation of air moisture on the ground sample material. This was done by means of infrared light bulbs (150 W) installed in about 5 cm distance (step 7 in Fig. 2). The very finely ground samples were then recovered from the opened spheres with a spoon and transferred into gas tight glass vials to protect the powder from air moisture (step 8 in Fig. 2). Samples were stored in the dark at -80°C to minimize pigment degradation. All handling of the samples during the grinding process was done over a mirror for complete recovery of the ground material.

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). Reproducibility of the measurements was expressed by the coefficient of variation in percent (CV%) reflecting the dispersion of measurements relative to the mean:

$$\mathrm{CV\%} = \frac{\mathrm{SD}}{\mathrm{MEAN}} * 100 \qquad (1)$$

The CV% estimates demonstrated that carbon (CV% = 0.15–0.99) and nitrogen (CV% = 0.28–1.86) measurements of the ground samples were 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).

Homogeneity of ground samples is mainly determined by the grain size, which is therefore crucial for representative subsampling. Scanning electron microscopy (SEM) photographs of fresh sediment trap samples (Fig. 6 A, B) show that the collected material consists of a

heterogeneous mixture of all kind of debris particles such as agglutinated diatom chains, fecal pellets and macroscopic aggregates. None of these macroscopic structures were visible after the grinding procedure (Fig. 6 C, D). Only at 2500–fold magnification, details such as pores of former diatom frustules became detectable in tiny fragments (Fig. 6 E, F). Grain size representing grinding quality was in the range of fine to coarse silt (2 – 63 µm, international scale) independent of the sample origin and primary composition (Fig. 6 C, D).

3 Conclusions and recommendations

3.1 Sediment trap design and sample recovery

The quantitative collection of settling particles, as realized in several pelagic mesocosm designs (e.g. CEE, KOSMOS, Large Clean Mesocosms), combines the advantage of sampling all settling particles produced by the enclosed plankton community with the removal of settled organic matter from the bottom of the enclosures. Collecting all settling particles avoids the potential sampling bias of suspended particle traps in mesocosm enclosures and leads to more accurate particle flux rates. Removing the accumulating material prevents re-suspension and non-quantified resupply of nutrients and other dissolved compounds released by degradation back into the water column.

We applied the vacuum sampling method to allow easy sample recovery in short time intervals and to keep the systems sealed for minimal disturbance of the enclosed water bodies. Opening of the sediment traps even for a very short time can lead to water exchange due to density gradients between enclosed and surrounding water. The vacuum sampling method is therefore ideal to keep the mesocosm enclosures completely sealed and thereby exclude introduction of plankton seed-populations and to allow for proper budgeting of elements. Furthermore the extraction of the collected material from the sea surface does not require diving activities. Only in case of a non-reversible blockage of the outlet of the collecting cylinder by artificial objects divers need to open up the collecting cylinder at the top or the bottom.

Sediment traps of mesocosms can obviously not be poisoned to prevent organic matter degradation, raising the importance of frequent sampling. Sampling intervals of the traps should be kept short - two days or less - to limit bacterial- and zooplankton-mediated

remineralisation of the settled material and to avoid or minimize the time of possible carbonate undersaturation or anoxic conditions.

3.2 Particle concentration

Centrifuging the entire sample volume (sect. 2.2.2) as well as precipitating particles with $FeCl_3$ (sect. 2.2.3) was shown to effectively concentrate sediment trap samples containing large amounts of bulk seawater without the need of separate analysis of the supernatant. In contrast, particle concentration by passive settling (sect. 2.2.1) should be complemented by additional measurements of material remaining in the supernatant as mean concentration efficiency is much lower and more depending on particle characteristics.

The simplest method to use in the field was centrifugation of the whole sample volume. We therefore recommend this method for sample volumes of up to three liters, as it avoids separate supernatant analysis or re-adjustment of the samples' pH and undesired enrichment with iron. Concentration of samples larger than three liters can be accelerated by precipitation of particles with $FeCl_3$ prior to centrifugation and is advisable during bloom and post-bloom events of high particle fluxes. If applied in the future, we strongly advise to adjust pH after $FeCl_3$ addition with NaOH in each sample individually to ensure $CaCO_3$ preservation. $FeCl_3$ is also known to precipitate dissolved inorganic phosphate (PO_4^{3-}) (Jenkins et al., 1971), but the relative contribution of precipitated PO_4^{3-} to particulate phosphorus in the samples is likely to be negligible. The potential of iron to interfere with the spectrophotometric analysis of biogenic silica or particulate phosphorus leading to increased absorption at very high iron concentrations (Hansen and Koroleff, 1999) can not be confirmed based on our observations (author's unpublished data).

3.3 Sample analyses

Processing of the sediment trap material to a finely ground and homogeneous powder proved to be ideally suited for reproducible elemental composition analysis. So far we successfully measured content of major bioactive elements such as total/organic/inorganic carbon, nitrogen, phosphorus and biogenic silica using standard methods for particulates in seawater (Table 3). Isotopic tracers such as ^{13}C and ^{15}N added to the mesocosms as well as natural isotope signals were additionally measured in settled organic matter (de Kluijver et al., 2013; Paul et al., 2015a). Furthermore phytoplankton pigments extracted from the ground samples

were analyzed revealing contribution of key phytoplankton groups to settling particle formation (Paul et al., 2015a). As only a few milligram of material are needed for these analyses, measurement of further parameters such as lithogenic material or amino acids should be tested in the future.

3.4 Recommendations

This section highlights the most important recommendations for improving particle collection in pelagic mesocosms along with sampling and processing of the collected material for biogeochemical analysis.

- Quantitative collection of settling particles with full-size funnel traps leads to accurate flux measurements and minimizes impact of organic matter degradation on the enclosed water columns.
- Vacuum sampling of the sediment traps via an extraction tube allows keeping the mesocosms sealed, excluding seawater and organism exchange.
- High sampling frequency limits organic matter degradation and potential carbonate undersaturation or anoxia in the traps.
- Separation of particles and bulk seawater in the samples is highly efficient when achieved by centrifugation or chemical precipitation with $FeCl_3$.
- Freeze-drying the collected material is preferable to drying the samples in the oven to better preserve phytoplankton pigments.
- Grinding of the entire samples guarantees representative subsampling for biogeochemical analysis.

Following our successfully applied protocol (Fig. 2, sect. 2) and the above recommendations will lead to accurate biogeochemical flux data of mesocosm sediment traps, irrespective of the magnitude of the particle flux.

Author contribution

U. Riebesell conceived the mesocosm experiments between 2010 and spring 2014. T. Boxhammer and J. Czerny developed the methods for sample acquisition and material processing. T. Boxhammer carried out the practical work, while the presented data were

1 analyzed by T. Boxhammer and L.T. Bach. T. Boxhammer prepared the manuscript with
2 contributions from all co-authors.
3

4 **Acknowledgments**

5 We thank the whole KOSMOS Team for deployment and maintenance of the KOSMOS
6 infrastructures during the five consecutive mesocosms studies between 2010 and spring 2014.
7 In particular, we thank Andrea Ludwig and Sebastian Krug for coordinating the logistics and
8 conducting CTD casts, Ylva Ericson and Leif Anderson for providing TA data, Allanah J.
9 Paul for support with RStudio, Jan Taucher for supervision, Sebastian Meier from the
10 Institute of Geology at the Christian-Albrechts University Kiel for support on SEM analyses,
11 Mario Deckelnick and Detlef Hoffmann for development of the ball mill as well as Michael
12 Sswat, Mathias Haunost, Hendrik Schultz, Saskia Audritz, Jana Meyer, Diana Gill, Kerstin
13 Nachtigall and Georgia Slatter for assistance during sampling, processing and measurements.
14 We are also grateful to the crews of MV *Esperanza*, RV *Alkor* (AL376, AL394, AL397,
15 AL406, AL420), RV *Håkan Mosby* (2011609), RV *Heincke* (HE360), RV *Poseidon*
16 (POS463) and RV *Hesperides* (29HE20140924) for transportation, deployment and recovery
17 of the mesocosms. This paper benefited from the constructive comments of two anonymous
18 reviewers. The mesocosm studies were funded by the Federal Ministry of Education and
19 Research (BMBF) in the framework of the coordinated projects BIOACID II (FKZ
20 03F06550) and SOPRAN II (FKZ 03F0611), as well as by the European Union in the
21 framework of the FP7 EU projects MESOAQUA (grant agreement no. 228224) and EPOCA
22 (grant agreement no. 211384).
23

1 **References**

2 Alldredge, A. L., Passow, U. and Logan, B. E.: The abundance and significance of a class of
3 large, transparent organic particles in the ocean, Deep-Sea Res. PT I, 40(6), 1131–1140,
4 doi:10.1016/0967-0637(93)90129-Q, 1993.

5 Amokrane, A., Comel, C. and Veron, J.: Landfill leachates pretreatment by coagulation-
6 flocculation, Water Res., 31(11), 2775–2782, doi:10.1016/S0043-1354(97)00147-4, 1997.

7 Azetsu-Scott, K. and Passow, U.: Ascending marine particles: Significance of transparent
8 exopolymer particles (TEP) in the upper ocean, Limnol. Oceangr., 49(3), 741–748,
9 doi:10.4319/lo.2004.49.3.0741, 2004.

10 Bach, L. T., Riebesell, U., Sett, S., Febiri, S., Rzepka, P. and Schulz, K. G.: An approach for
11 particle sinking velocity measurements in the 3–400 μm size range and considerations on the
12 effect of temperature on sinking rates, Mar. Biol., 159(8), 1853–1864, doi:10.1007/s00227-
13 012-1945-2, 2012.

14 Bach, L.T., Taucher, J., Boxhammer, T., Ludwig, A., The Kristineberg KOSMOS
15 Consortium, Achterberg, E.P., Algueró-Muñiz, M., Anderson, L.G., Bellworthy, J.,
16 Büdenbender, J., Czerny, J., Ericson, Y., Esposito, M., Fischer, M., Haunost, M., Hellemann,
17 D., Horn, H.G., Hornick, T., Meyer, J., Sswat, M., Zark, M., and Riebesell, U.: Influence of
18 ocean acidification on a natural winter-to-summer plankton succession: First insights from a
19 long-term mesocosm study draw attention on periods of low nutrient concentrations, in
20 review, 2016.

21 Bisutti, I., Hilke, I. and Raessler, M.: Determination of total organic carbon – an overview of
22 current methods, Trac-Trend. Anal. Chem., 23(10–11), 716–726,
23 doi:10.1016/j.trac.2004.09.003, 2004.

24 Bloesch, J. and Burns, N. M.: A critical review of sedimentation trap technique, Schweiz. Z.
25 Hydrol., 42(1), 15–55, doi:10.1007/BF02502505, 1980.

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

30 Buesseler, K. O.: Do upper-ocean sediment traps provide an accurate record of particle flux?

1 Nature, 353(6343), 420–423, doi:10.1038/353420a0, 1991.

2 Buffan-Dubau, E. and Carman, K. R.: Extraction of benthic microalgal pigments for HPLC
3 analyses, Mar. Ecol.-Prog. Ser., 204, 293–297, doi:10.3354/meps204293, 2000.

4 Czerny, J., Schulz, K. G., Boxhammer, T., Bellerby, R. G. J., Büdenbender, J., Engel, A.,
5 Krug, S. A., Ludwig, A., Nachtigall, K., Nondal, G., Niehoff, B., Silyakova, A. and Riebesell,
6 U.: Implications of elevated CO2 on pelagic carbon fluxes in an Arctic mesocosm study – an
7 elemental mass balance approach, Biogeosciences, 10(5), 3109–3125, doi:10.5194/bg-10-
8 3109-2013, 2013.

9 de Kluijver, A., Soetaert, K., Czerny, J., Schulz, K. G., Boxhammer, T., Riebesell, U. and
10 Middelburg, J. J.: A ^{13}C labelling study on carbon fluxes in Arctic plankton communities
11 under elevated CO2 levels, Biogeosciences, 10(3), 1425–1440, doi:10.5194/bg-10-1425-
12 2013, 2013.

13 Dickson, A. G. and Millero, F. J.: A comparison of the equilibrium constants for the
14 dissociation of carbonic acid in seawater media, Deep-Sea Res., 34(10), 1733–1743,
15 doi:10.1016/0198-0149(87)90021-5, 1987.

16 Doney, S. C.: The growing human footprint on coastal and open-ocean biogeochemistry,
17 Science, 328(5985), 1512–1516, doi:10.1126/science.1185198, 2010.

18 Gamble, J. C., Davies, J. M. and Steele, J. H.: Loch Ewe bag experiment, 1974, B. Mar. Sci.,
19 27(1), 146–175, 1977.

20 Guieu, C., Dulac, F., Desboeufs, K., Wagener, T., Pulido-Villena, E., Grisoni, J. M., Louis,
21 F., Ridame, C., Blain, S., Brunet, C., Bon Nguyen, E., Tran, S., Labiadh, M. and Dominici, J.
22 M.: Large clean mesocosms and simulated dust deposition: a new methodology to investigate
23 responses of marine oligotrophic ecosystems to atmospheric inputs, Biogeosciences, 7(9),
24 2765–2784, doi:10.5194/bg-7-2765-2010, 2010.

25 Hansen, H. P. and Koroleff, F.: Determination of nutrients, in: Methods of seawater analysis,
26 edited by K. Grasshoff, K. Kremling, and M. Ehrhardt, Wiley-VCH Verlag GmbH,
27 Weinheim, Germany, 159–228, 2007.

28 Harrison, W. G. and Davies, J. M.: Nitrogen cycling in a marine planktonic food chain:
29 Nitrogen fluxes through principal components and effects of adding copper, Mar. Biol., 43(4),
30 299–306, doi:10.1007/BF00396924, 1977.

1 Huasheng, H., Laodong, G. and Jingqian, C.: Relationships between particle characteristics
2 and biological activities in controlled ecosystems, in: Proceedings of a symposium held in
3 Beijing: Marine ecosystem enclosure experiments, Beijing, People's Republic of China, 9–14
4 May 1987, 230-243, 1992.

5 Jenkins, D., Ferguson, J. F. and Menar, A. B.: Chemical processes for phosphate removal,
6 Water Res., 5(7), 369–389, doi:10.1016/0043-1354(71)90001-7, 1971.

7 Jinping, W., Whitney, F. A., Shumin, H., Xiaolin, C., Dongfa, Z. and Shengsan, W.:
8 Introduction to the Xiamen Marine Ecosystem Enclosed Experiments, in: Proceedings of a
9 symposium held in Beijing: Marine ecosystem enclosure experiments, Beijing, People's
10 Republic of China, 9–14 May 1987, 158-173, 1992.

11 John, S. G., Mendez, C. B., Deng, L., Poulos, B., Kauffman, A. K. M., Kern, S., Brum, J.,
12 Polz, M. F., Boyle, E. A. and Sullivan, M. B.: A simple and efficient method for
13 concentration of ocean viruses by chemical flocculation, Environmental Microbiology
14 Reports, 3(2), 195–202, doi:10.1111/j.1758-2229.2010.00208.x, 2011.

15 Knuckey, R. M., Brown, M. R., Robert, R. and Frampton, D. M. F.: Production of microalgal
16 concentrates by flocculation and their assessment as aquaculture feeds, Aquacult. Eng., 35(3),
17 300–313, doi:10.1016/j.aquaeng.2006.04.001, 2006.

18 Lalli, C. M.: Introduction, in: Enclosed experimental marine ecosystems: A review and
19 recommendations: A contribution of the scientific committee on oceanic research working
20 group 85, edited by C. M. Lalli, Springer US, New York, NY, 1-6, 1990.

21 McClymont, E. L., Martínez-Garcia, A. and Rosell-Melé, A.: Benefits of freeze-drying
22 sediments for the analysis of total chlorins and alkenone concentrations in marine sediments,
23 Org. Geochem., 38(6), 1002–1007, doi:10.1016/j.orggeochem.2007.01.006, 2007.

24 Mehrbach, C., Culberson, C. H., Hawley, J. E. and Pytkowicx, R. M.: Measurement of the
25 apparent dissociation constants of carbonic acid in seawater at atmospheric pressure, Limnol.
26 Oceangr., 18(6), 897–907, doi:10.4319/lo.1973.18.6.0897, 1973.

27 Menzel, D. W. and Case, J.: Concept and design: Controlled ecosystem pollution experiment,
28 B. Mar. Sci., 27(1), 1–7, 1977.

29 Niehoff, B., Schmithüsen, T., Knüppel, N., Daase, M., Czerny, J. and Boxhammer, T.:
30 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.

Olsen, Y., Andersen, T., Gismervik, I. and Vadstein, O.: Protozoan and metazoan zooplankton-mediated carbon flows in nutrient-enriched coastal planktonic communities, Mar. Ecol.-Prog. Ser., 331, 67–83, doi:10.3354/meps331067, 2007.

Paul, A. J., Achterberg, E. P., Bach, L. T., Boxhammer, T., Czerny, J., Haunost, M., Schulz, K.-G., Stuhr, A., and Riebesell, U.: No observed effect of ocean acidification on nitrogen biogeochemistry in a summer Baltic Sea plankton community, Biogeosciences Discuss., 12, 17507-17541, doi:10.5194/bgd-12-17507-2015, 2015a.

Paul, A. J., Bach, L. T., Schulz, K. G., Boxhammer, T., Czerny, J., Achterberg, E. P., Hellemann, D., Trense, Y., Nausch, M., Sswat, M. and Riebesell, U.: Effect of elevated CO_2 on organic matter pools and fluxes in a summer Baltic Sea plankton community, Biogeosciences, 12(20), 6181–6203, doi:10.5194/bg-12-6181-2015, 2015b.

Pierrot, D., Lewis, E. and Wallace, D.: MS Excel program developed for CO_2 system calculations, ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee, doi:10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a, 2006.

Renou, S., Givaudan, J. G., Poulain, S., Dirassouyan, F. and Moulin, P.: Landfill leachate treatment: Review and opportunity, J. Hazard. Mater., 150(3), 468–493, doi:10.1016/j.jhazmat.2007.09.077, 2008.

Riebesell, U., Bach, L. T., Bellerby, R. G. J., Bermudez Monsalve, R. J., Boxhammer, T., Czerny, J., Larsen, A., Ludwig, A. and Schulz, K. G.: Ocean acidification impairs competitive fitness of a predominant pelagic calcifier, under review, 2016.

Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R. and Schulz, K. G.: Technical Note: A mobile sea-going mesocosm system – new opportunities for ocean change research, Biogeosciences, 10(3), 1835–1847, doi:10.5194/bg-10-1835-2013, 2013.

Riebesell, U., Lee, K. and Nejstgaard, J. C.: Pelagic mesocosms, in: Guide to best practices in ocean acidification research and data reporting, edited by U. Riebesell, V. J. Fabry, L. Hansson, and J.-P. Gattuso, Office for Official Publications of the European Communities, Luxembourg, 95-112, 2011.

1 Schulz, K. G., Riebesell, U., Bellerby, R. G. J., Biswas, H., Meyerhöfer, M., Müller, M. N.,
2 Egge, J. K., Nejstgaard, J. C., Neill, C., Wohlers, J. and Zöllner, E.: Build-up and decline of
3 organic matter during PeECE III, Biogeosciences, 5(3), 707–718, doi:10.5194/bg-5-707-
4 2008, 2008.

5 Sukenik, A., Bilanovic, D. and Shelef, G.: Flocculation of microalgae in brackish and sea
6 waters, Biomass, 15(3), 187–199, doi:10.1016/0144-4565(88)90084-4, 1988.

7 Svensen, C., Egge, J. K. and Stiansen, J. E.: Can silicate and turbulence regulate the vertical
8 flux of biogenic matter? A mesocosm study, Mar. Ecol.-Prog. Ser., 217, 67–80,
9 doi:10.3354/meps217067, 2001.

10 Vadstein, O., Andersen, T., Reinertsen, H. R. and Olsen, Y.: Carbon, nitrogen and phosphorus
11 resource supply and utilisation for coastal planktonic heterotrophic bacteria in a gradient of
12 nutrient loading, Mar. Ecol.-Prog. Ser., 447, 55–75, doi:10.3354/meps09473, 2012.

13 van Leeuwe, M. A., Villerius, L. A., Roggeveld, J., Visser, R. J. W. and Stefels, J.: An
14 optimized method for automated analysis of algal pigments by HPLC, Mar. Chem., 102(3–4),
15 267–275, doi:10.1016/j.marchem.2006.05.003, 2006.

16 von Bröckel, K.: Sedimentation of phytoplankton cells within controlled experimental
17 ecosystems following launching, and implications for further enclosure studies, in: Marine
18 mesocosms, edited by G. D. Grice and M. R. Reeve, Springer US, New York, NY, 251-259,
19 1982.

20

1 **Table 1.** Depending on the dry-weight of the freeze-dried sediment trap samples, different
2 grinding sphere volumes and numbers of grinding balls (10 – 20 mm) are recommended to
3 achieve optimal grinding results at a set run time of the ball mill (5 minutes). The optimal
4 combination of the different factors was determined empirically to achieve a grain size
5 smaller than 63 µm and to minimize frictional heating of the samples.

6

Sample dry-weight [g]	**Hollow sphere volume** [mL]	**# of grinding balls and size** [mm]	**Run time of the ball mill** [min]
< 1.5	11.5	1 x 10	5
1.5 – 2.5	24.4	1 x 15 + 2 x 10	5
2.5 – 5.0	47.7	2 x 15 + 2 x 10	5
5.0 – 7.0	65.5	1 x 20	5

1 **Table 2.** Results from replicate carbon and nitrogen measurements of ground sediment trap material in order to test its homogeneity.
2 Powdered samples originating from different pelagic mesocosm experiments were tested and compared with commercially available standards
3 commonly used for calibration of elemental analyzers (Soil Standard [STD], Acetanilide Standard [STD]). Homogeneity is expressed by the
4 coefficient of variation in percent (CV%). As well presented are the number of measured aliquots, the amount of material analyzed, average
5 carbon content, calculated standard deviation (SD) and grain size derived from scanning electron microscopy. ND = grain size not
6 determined.

7

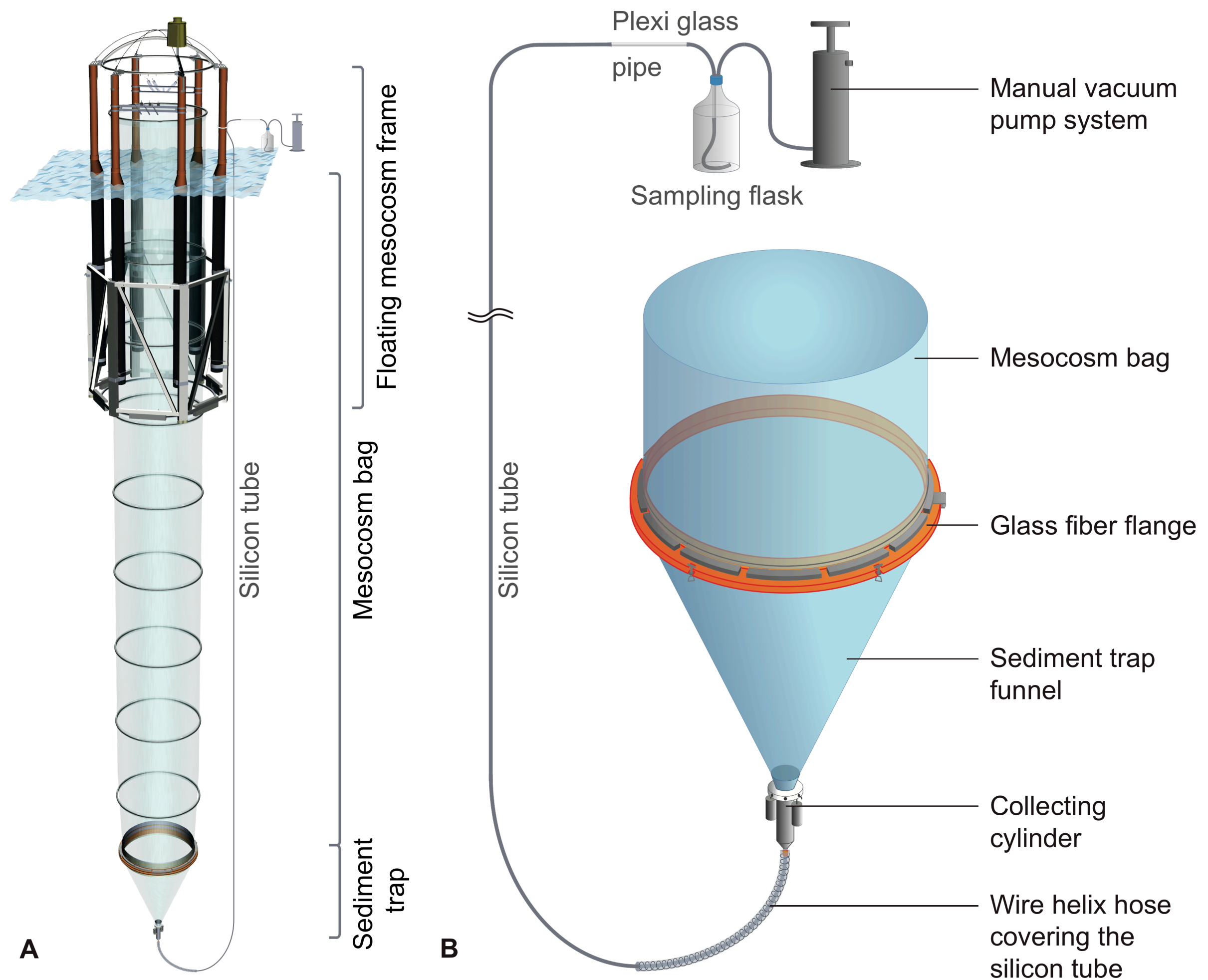
Sample origin	**Measured aliquots** #	**Aliquot weight** [mg]	**Grain size** [µm]	**Average carbon** [µmol mg^{-1}]	**SD (carbon)**	**CV% (carbon)**	**Average nitrogen** [µmol mg^{-1}]	**SD (nitrogen)**	**CV% (nitrogen)**
Soil STD *C = 3.429%*	5	4 ± 0.25	ND	2.83	0.12	**4.17**	0.16	0.00	**1.55**
Acetanilide STD *C = 71.089%*	5	1 ± 0.15	ND	58.81	0.20	**0.34**	7.34	0.07	**0.97**
Svalbard 2010 *# SV106*	5	2 ± 0.25	ND	22.74	0.12	**0.51**	3.77	0.01	**0.39**
Norway 2011 *# NO124*	5	2 ± 0.25	≤ 63	19.57	0.09	**0.48**	2.53	0.01	**0.54**
Finland 2012 *# FI114*	5	2 ± 0.25	≤ 63	22.53	0.03	**0.15**	3.58	0.01	**0.28**
Sweden 2013 *# SE502*	5	2 ± 0.25	≤ 63	29.03	0.23	**0.80**	1.65	0.03	**1.86**
Gran Canaria 2014 *# GC68*	5	2 ± 0.25	≤ 63	17.15	0.17	**0.99**	0.94	0.00	**0.28**

1 **Table 3.** List of parameters measured from ground sediment trap samples originating from
2 KOSMOS experiments. The methods / instruments applied and the corresponding references
3 with data sets and detailed descriptions of the methods are furthermore provided.

4

Parameter	Method / Instrument	Corresponding publications
Total carbon	Elemental analyzer	Czerny et al., 2013; Paul et al., 2015b
Organic carbon	Removal of inorganic carbon by direct addition of hydrochloric acid (Bisutti et al., 2004); Elemental analyzer	Riebesell et al., 2016
Inorganic carbon	Calculated from total and org. carbon	Riebesell et al., 2016
Total nitrogen	Elemental analyzer	Czerny et al., 2013; Paul et al., 2015b
Phosphorus	Spectrophotometry (Hansen and Koroleff, 1999)	Czerny et al., 2013; Paul et al., 2015b
Biogenic silica	Spectrophotometry (Hansen and Koroleff, 1999)	Czerny et al., 2013; Paul et al., 2015b
Isotopic tracers (^{13}C, ^{15}N)	Mass spectrometry, Elemental analyzer	de Kluijver et al., 2013; Paul et al., 2015a
Phytoplankton pigments	High pressure liquid chromatography	Paul et al., 2015a

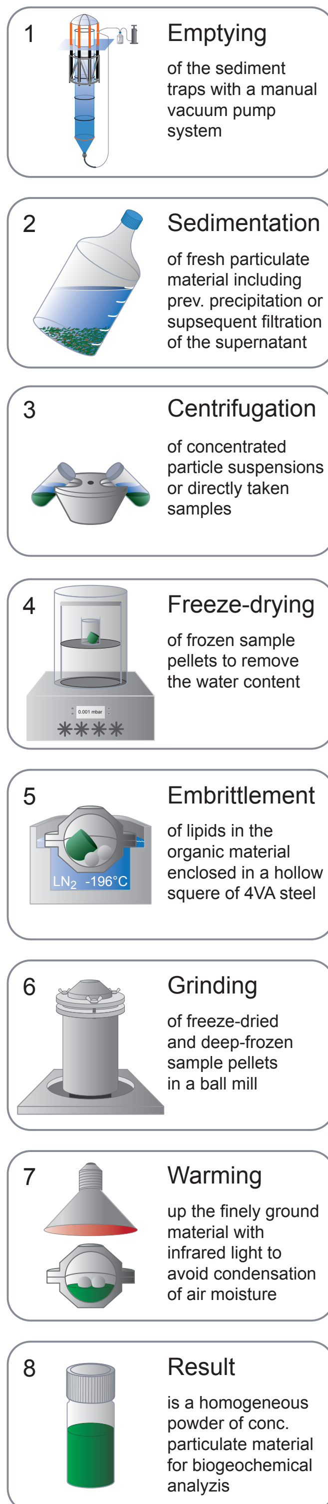
5



1

2

3 **Figure 1. (A)** Technical drawing of the KOSMOS flotation frame with unfolded TPU
4 enclosure bag and attached funnel-shaped sediment trap. **(B)** A silicon tube connects the
5 collecting cylinder at the tip of the sediment trap with a 5 L sampling flask. A wire-reinforced
6 hose prevents current related bending of the first 1.5 meters. Particles can be easily detected
7 in the Plexiglass® pipe linking up the silicon tube with the sampling flask.



1

2

3 **Figure 2.** Protocol of mesocosm sediment trap sampling (1), particle concentration (2 – 3),
4 freeze-drying (4) and grinding (5 – 8) to convert heterogeneous sediment trap samples into
5 homogeneous powder for biogeochemical analysis.

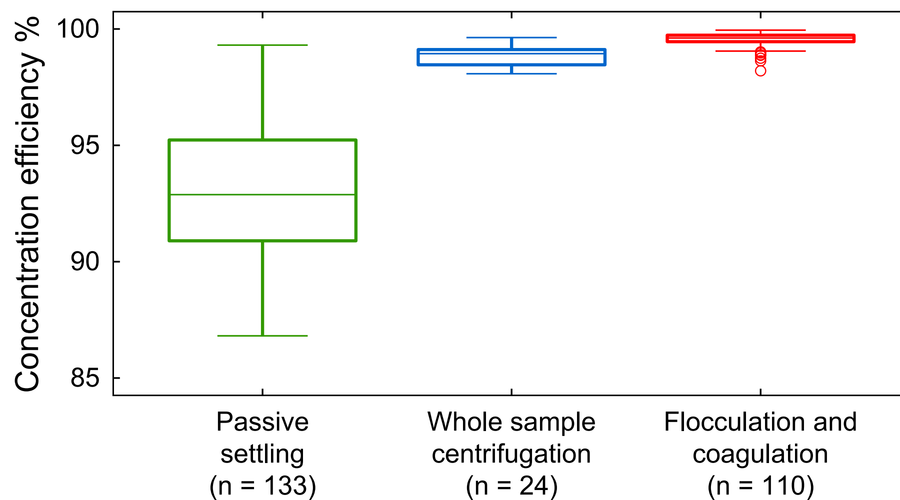
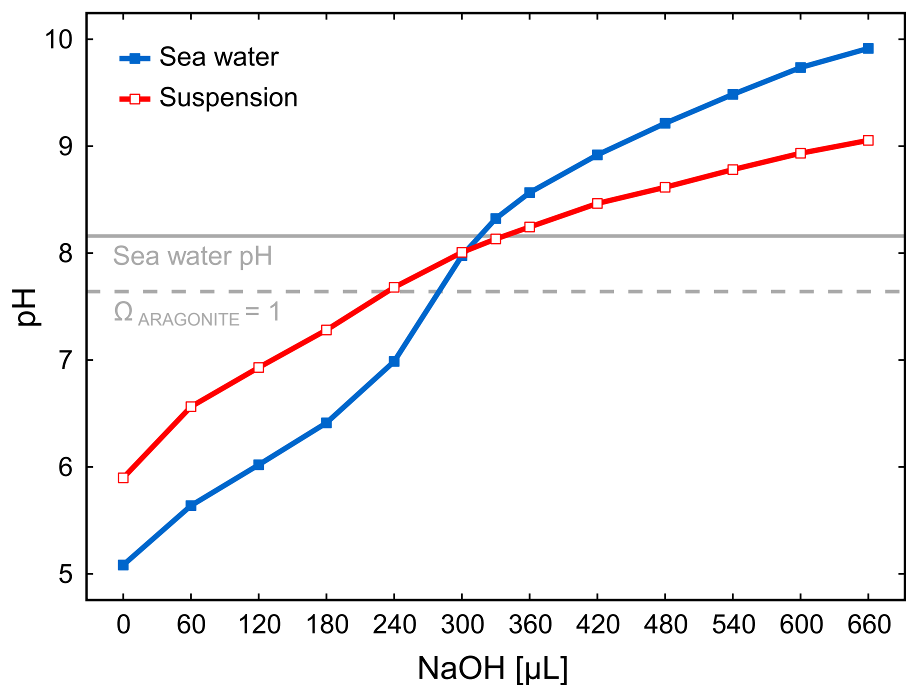


Figure 3. Boxplot of the concentration efficiency (%) of three different methods for particle concentration of mesocosm sediment trap samples. Concentration of particles by passive settling (green) is compared with gravitational deposition of particulates by whole sample centrifugation (blue). The third option of flocculation and coagulation with $FeCl_3$ for enhanced particle settling is presented in red. Concentration efficiency is defined as the percentage of TPC concentrated in the processed sediment trap samples in relation to the particulate carbon in the originally sampled suspensions (sum of concentrated and residual TPC in the bulk water). Outliers (circles) are defined as any data points below 1.5 * IQR (interquartile range) of the first quartile hinge or above 1.5 * IQR of the third quartile hinge.



1

2

3 **Figure 4.** Titration of 500 mL sea water (blue, filled box and line) and 500 mL particle
4 suspension (red, empty box and line) with 3 M NaOH after addition of 150 µL 2.4 M $FeCl_3$
5 solution. The grey solid line indicates the pH of seawater before any manipulation. pH (NBS
6 scale) was measured at 25°C with a stationary pH meter (713, METROHM). Calculated
7 aragonite saturation state of $\Omega = 1$ is represented by the grey dashed line.

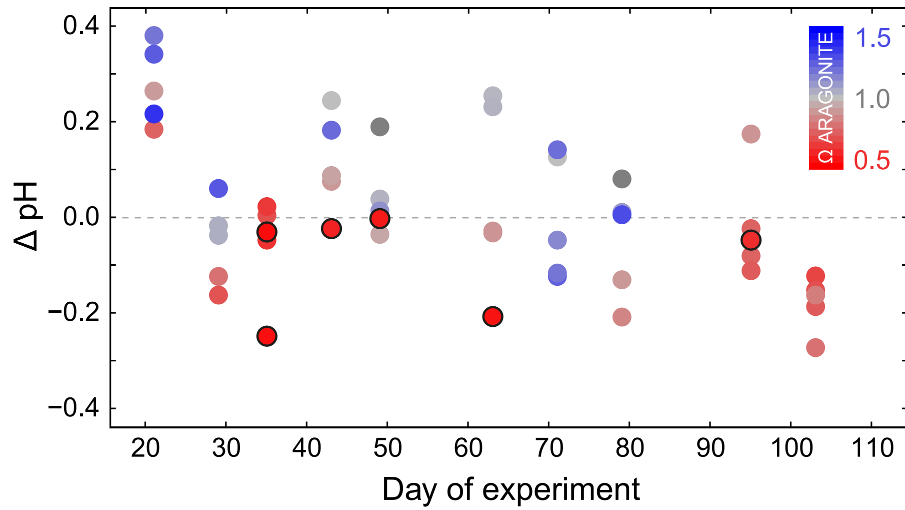
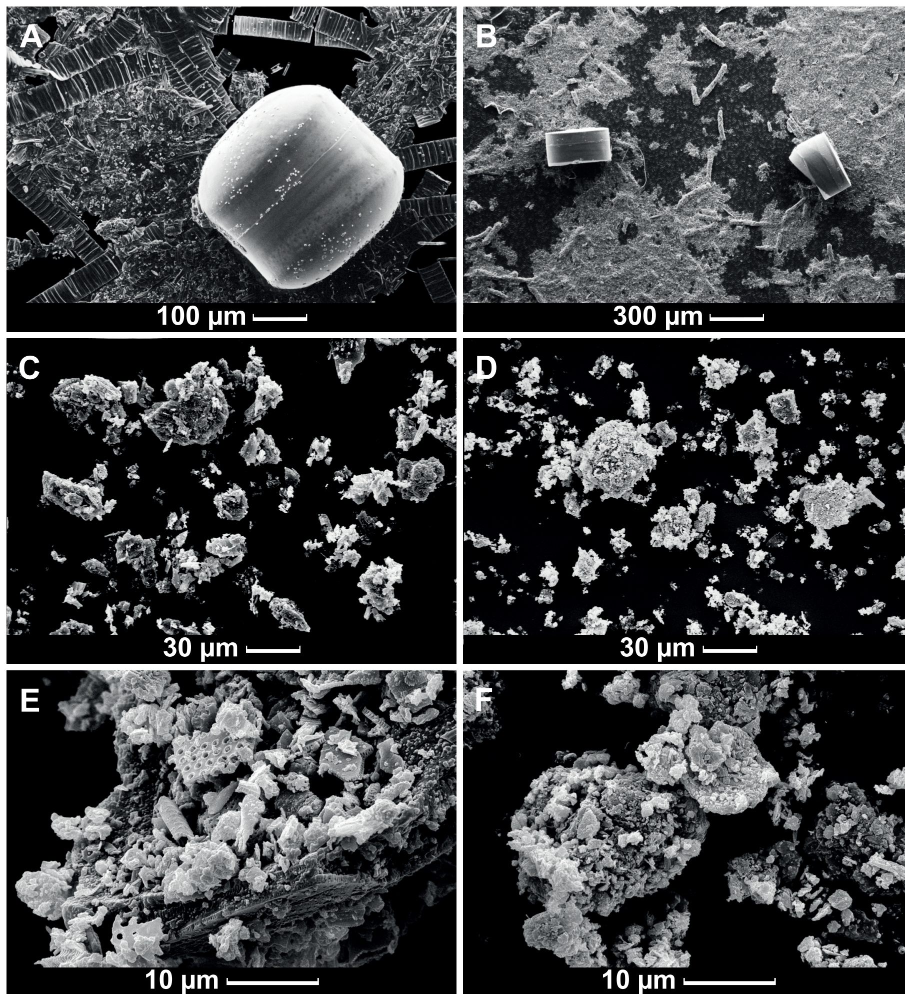


Figure 5. Delta pH of 50 sediment trap samples, calculated from pH measurements before and after addition of $FeCl_3$ (300 µL L^{-1}, 2.4 M) and NaOH (660 µL L^{-1}, 3 M) for precipitation of suspended particulate material. $\Omega_{ARAGONITE}$ after chemical treatment of the samples is indicated by a color gradient from red over grey to blue, representing undersaturated, saturated and oversaturated samples, respectively. $\Omega_{CALCITE} < 1$ is tagged by black edging of the colored data points.

1 

2

3 **Figure 6.** Scanning electron microscopy (SEM) photographs of two sediment trap samples
4 before **(A, B)** and after grinding **(C – F)**. **(C)** and **(D)** represent the average grain size of the
5 ground samples, while **(E)** and **(F)** reveal details visible at 2500 fold magnification.