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

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Abstract

Sediment traps are the most common tool to investigate vertical particle flux in the marine realm. However, the spatial decoupling between particle formation and collection often handicaps reconciliation of these two processes 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. 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 concentrated by passive settling, centrifugation or flocculation with ferric chloride and we discuss the advantages 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 ranges 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 silicone-rubber-sealed glass fiber flange (Riebesell et al., 2013; Fig. 1a). 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 tip of the collecting cylinder outside of the mesocosm bag (Fig. 1b). A hose connector links the silicon tube to 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 (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 Fig. 2). When first particles showed up in the Plexiglas® pipe the sampling process was briefly interrupted, seawater in the bottles screened for particles and only discarded if clear. The dense particle suspensions originating from the collecting cylinders were then vacuum-pumped into the sampling flasks until no more particles were visible in the Plexiglas® pipe (Boxhammer et al., 2015 (video)). The consistent inner diameter of the silicon tube and all connectors (1 cm) in combination with a low vacuum employed during the sampling process ensured to preserve the integrity of particles in the best possible way.

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 For this the particle suspension (∼ 1–4 L) was gently mixed and subsample volumes withdrawn immediately before re-suspended particles were able to settle down.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

2.2 Separating particles from bulk seawater

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

The particle concentration efficiency (%) of the three methods (Sects. 2.2.1–2.2.3) was determined as the percentage of total particulate carbon (TPC) concentrated in the processed samples in relation to the sum of concentrated and residual TPC in the remaining bulk water. Residual TPC in the bulk water was determined of subsamples that were filtered on combusted GF/F filters (Whatman, 0.7 µm pore size, 450 °C, 6 h) with gentle vacuum (< 200 mbar) and stored in combusted glass petri dishes (450 °C, 6 h) at −20 °C. Alive copepods, which could occasionally be found in the liquid, were carefully removed from the filters. 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. TPC 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 × 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 2 h 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). Mesozooplankton actively swimming in the liquid phase, mostly copepods, were removed together with the supernatant from the settled material. The dense particle suspension at the bottom of the glass bottles was concentrated in 110 mL tubes by centrifugation for 10 min 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 h was occasionally not long enough for small or low-density particles to settle.

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 min at 5236 × *g* using a 6–16 KS centrifuge (Sigma), followed by slow 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 min at 5039 × *g* in the small tubes (3K12 centrifuge, Sigma) samples were compressed into compact sediment pellets which can be frozen and stored in plastic screw cap jars as described in Sect. 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 ($CaCO_3$).

To quantify the $FeCl_3$ related pH reduction we added $FeCl_3$ to (1) a seawater sample originating from mesocosms deployed in Gullmar Fjord (Sweden 2013) and (2) to a seawater sample of the same origin in which we re-suspended sediment trap material. This test was carried out in 500 mL beakers at 25 °C using a stationary pH meter (NBS scale, 713, METROHM) to monitor changes of the seawater pH (Fig. 4). As expected, addition of 150 µL $FeCl_3$ (2.4 M) solution resulted in a distinct drop in seawater pH of about 3 units in the absence of particles (Fig. 4, blue, full boxes) and 1.3 units in the presence of re-suspended particles (Fig. 4, red, empty boxes). The pH decrease was compensated by stepwise titration with three molar NaOH reaching the initial seawater pH after addition of ~ 330 µL NaOH both in absence and presence of particles. In both cases the calculated aragonite saturation state, representing the more soluble form of biogenic $CaCO_3$, was well above $\Omega = 1$ (Fig. 4, grey dashed line), as calculated with CO2SYS MS Excel Macro (Pierrot et al., 2006) at 25 °C, 0 dbar, salinity = 29.62 and total alkalinity (TA) = 2206.1 (Bach et al., 2015) with constants of Mehrbach et al. (1973), refitted by Dickson and Millero (1987).

According to the test, 660 µL NaOH (3 M) were simultaneously added with 300 µL $FeCl_3$ (2.4 M) to each liter of particle suspension to stabilize the sample pH and to achieve optimal particle precipitation (S1 (video) in the Supplement). The formation of dense and rapidly settling flocks allowed separation of the supernatant and concentration of the deposit as described in Sect. 2.2.1 after only one hour of sedimentation. Even though buffering the samples with NaOH, we still observed shifts in seawater pH. Delta pH (Δ pH) was calculated from 50 pH measurements before and after addition of $FeCl_3$ and NaOH to sediment trap samples (pH meter, 3310 WTW; InLab Routine Pt1000 electrode, Mettler Toledo). The resulting Δ pH (Fig. 5) differed between individual samples of the same day as well as between sampling days over the 107 days of experiment. A maximum spread of 0.46 pH units was observed on day 63 while the minimum difference of 0.15 units occurred on day 103. We did not detect a trend towards a positive or negative shift in pH as the variation in the data lead to an average Δ pH of −0.01. It is likely that differences in the amount and composition of particles in the samples led to the observed pattern. Aragonite and calcite saturation states of the samples after precipitation (Fig. 5) were calculated as described above using in situ storage temperature, pH measurements of the samples and TA values from mesocosm water column measurements (Bach et al., 2015). Undersaturation of both carbonate species already occurred in several samples prior to $FeCl_3$ addition as ocean acidification scenarios were established inside the mesocosm bags and CO_2 released by biomass degradation likely further reduced seawater pH. In fact the number of undersaturated samples after precipitation was reduced by 2 and 6 samples with respect to aragonite and calcite.

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

2.3 Freeze-drying samples

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

2.4 Grinding the desiccated material

The desiccated sediment pellets were cryogenically ground into a fine powder of homogeneous composition to guarantee representative subsampling. We therefore developed a ball-mill to grind sample sizes from 0.1 to 7.0 g dry-weight. Hollow spheres with volumes ranging from 11.5 to 65.5 mL were cut out of blocks of stainless steel (V4A/1.4571). Each hollow sphere is divided into two hemispheres of exactly the same shape only connected by two guide pins and sealed by a metal sealing (Fig. 6). The size of the grinding sphere was 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 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:

$$\text{CV\%} = \frac{\text{SD}}{\text{MEAN}} \times 100 \qquad (1)$$

The CV% estimates demonstrate that carbon measurements of the ground samples (CV% = 0.15–0.99) are at least equally reproducible as measurements of the two calibration standards acetanilide and soil standard with CV% of 0.34 and 4.17, 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. 7a and 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 are visible after the grinding procedure (Fig. 7c and d). Only at 2500–fold magnification, details such as pores of former diatom frustules become detectable in tiny fragments (Fig. 7e and 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. 7c and 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. 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.

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 column.
- 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 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.

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

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 × 10	5
1.5–2.5	24.4	1 × 15 + 2 × 10	5
2.5–5.0	47.7	2 × 15 + 2 × 10	5
5.0–7.0	65.5	1 × 20	5

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

Sample origin	Measured aliquots #	Aliquot weight [mg]	Grain size [µm]	Average carbon [$\mu mol\,mg^{-1}$]	SD	CV%
Soil STD *C = 3.429 %*	5	4 ± 0.25	ND	2.83	0.12	4.17
Acetanilide STD *C = 71.089 %*	5	1 ± 0.15	ND	58.81	0.20	0.34
Svalbard 2010 *# SV106*	5	2 ± 0.25	ND	22.74	0.12	0.51
Norway 2011 *# NO124*	5	2 ± 0.25	≤ 63	19.57	0.09	0.48
Finland 2012 *# FI114*	5	2 ± 0.25	≤ 63	22.53	0.03	0.15
Sweden 2013 *# SE502*	5	2 ± 0.25	≤ 63	29.03	0.23	0.80
Gran Canaria 2014 *# GC68*	5	2 ± 0.25	≤ 63	17.15	0.17	0.99

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

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. (2015)
Inorganic carbon	Calculated from total and org. carbon	Riebesell et al. (2015)
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)

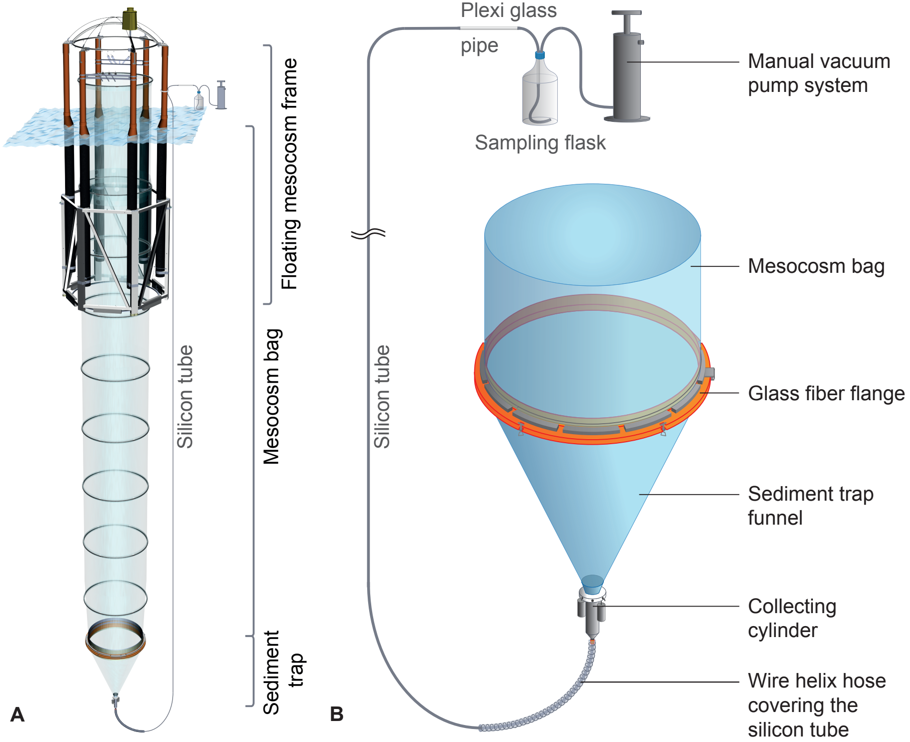

Figure 1. (a) Technical drawing of the KOSMOS flotation frame with unfolded TPU enclosure bag and attached funnel-shaped sediment trap. **(b)** A silicon tube connects the collecting cylinder at the tip of the sediment trap with a 5 L sampling flask. A wire-reinforced hose prevents current related bending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

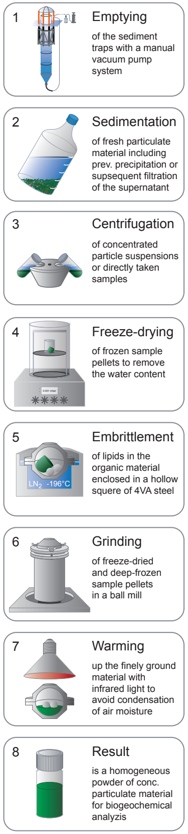

Figure 2. Protocol of mesocosm sediment trap sampling (1), particle concentration (2–3), freeze-drying (4) and grinding (5–8) to convert heterogeneous sediment trap samples into homogeneous powder for biogeochemical analysis.

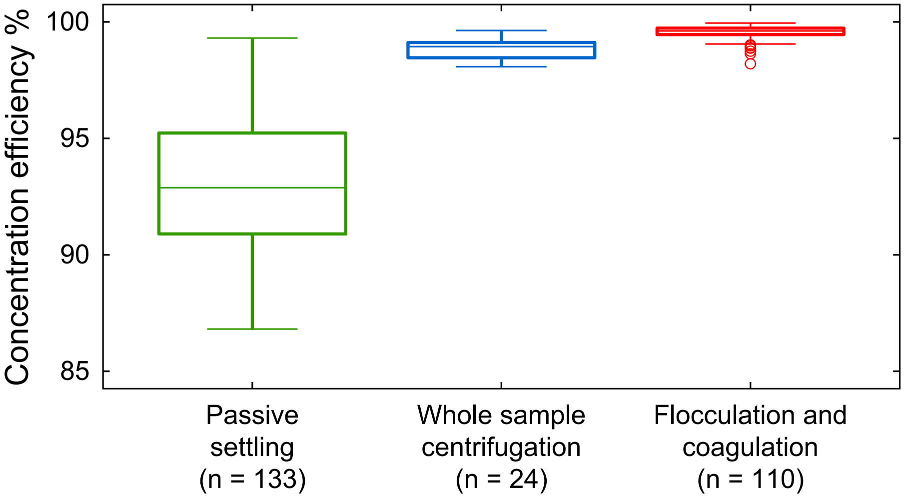

Figure 3. Boxplot of the concentration efficiency (%) of three different methods for particle concentration of mesocosm sediment trap samples. Concentration of particles by passive settling (green) is compared with gravitational deposition of particulates by whole sample centrifugation (blue). The third 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.

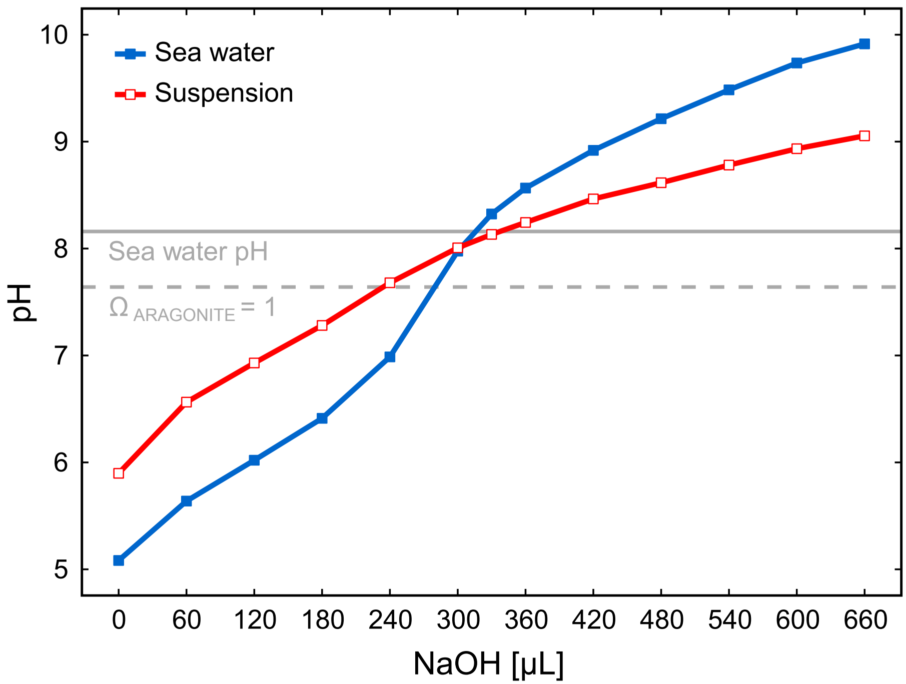

Figure 4. Titration of 500 mL sea water (blue, filled box and line) and 500 mL particle suspension (red, empty box and line) with 3 M NaOH after addition of 150 μL 2.4 M $FeCl_3$ solution. The grey solid line indicates the pH of seawater before any manipulation. pH (NBS scale) was measured at 25 °C with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

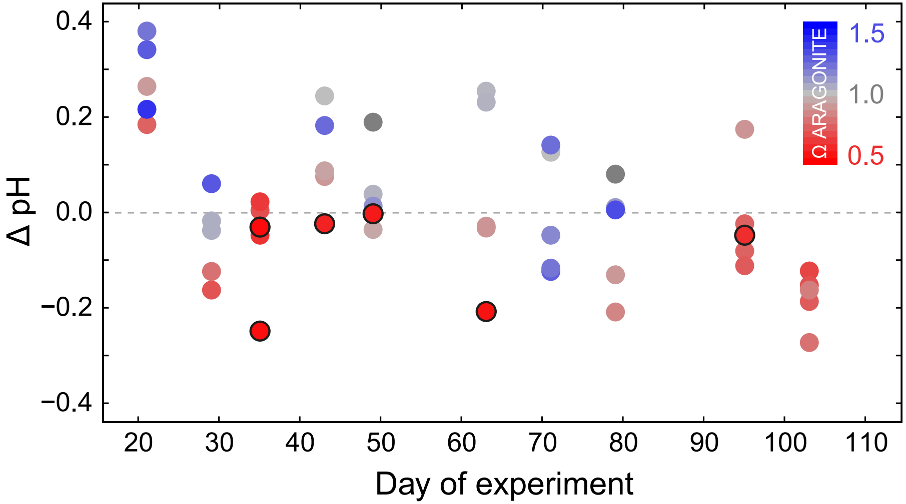

Figure 5. Delta pH of 50 sediment trap samples, calculated from pH measurements before and after addition of $FeCl_3$ (300 µL L^{-1}, 2.4 M) and NaOH (660 µL L^{-1}, 3 M) for precipitation of suspended particulate material. $\Omega_{ARAGONITE}$ after chemical treatment of the samples is indicated by 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.

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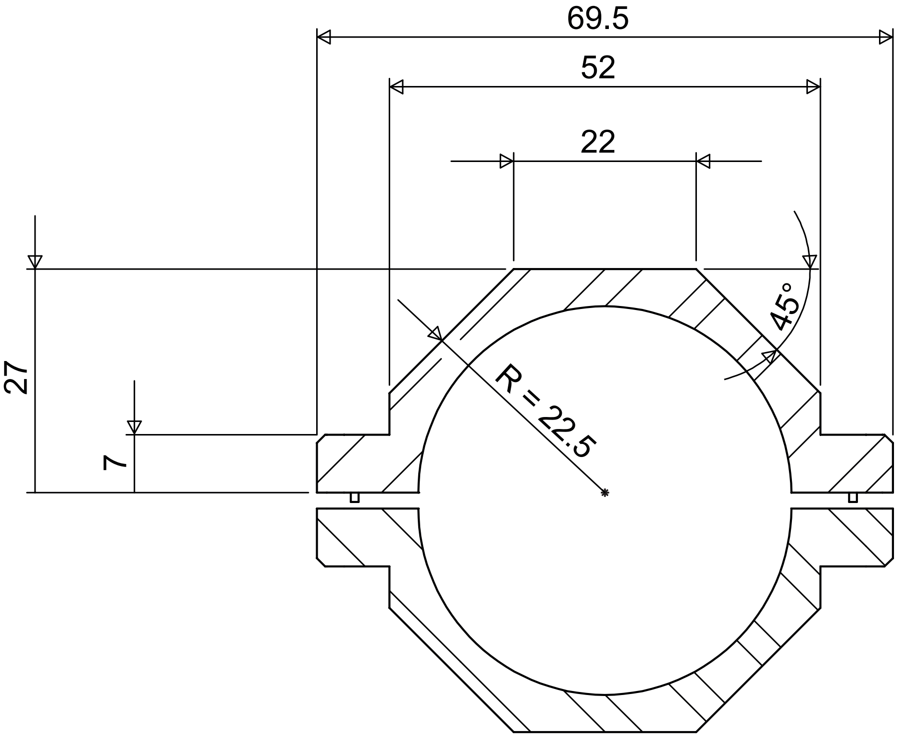

Figure 6. Technical drawing (lateral view) of a dividable hollow sphere cut out of stainless steel (V4A/1.4571) for grinding of concentrated and freeze-dried sediment trap samples. The sphere consists of two hollow hemispheres, which are only connected by two guide pins and sealed by a metal sealing. All physical dimensions are given in millimeters. In this case, the inner radius was 22.5 mm corresponding to a volume of about 47.7 mL.

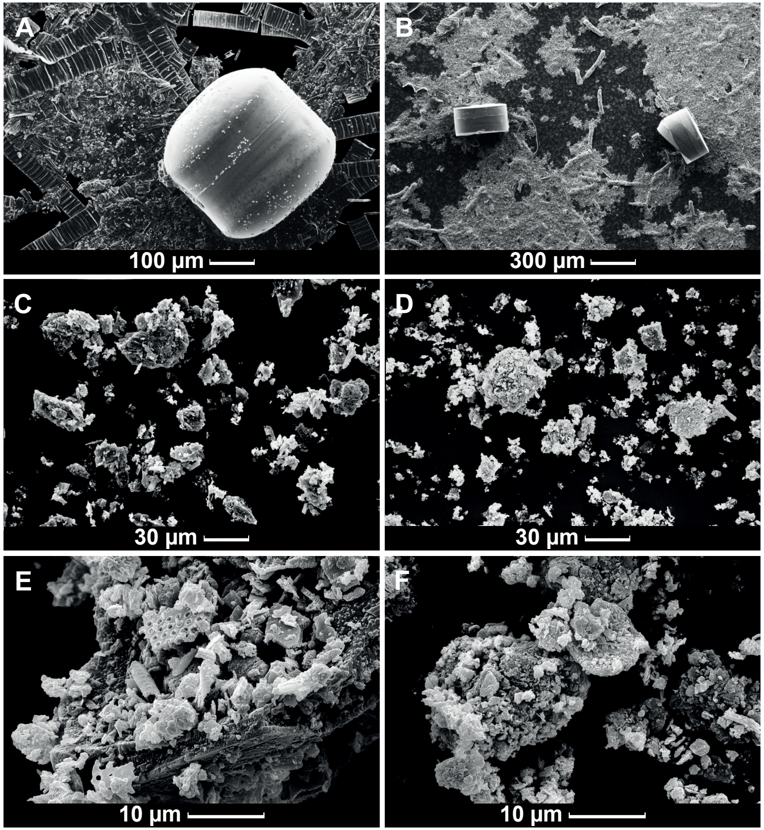

Figure 7. Scanning electron microscopy (SEM) photographs of two sediment trap samples before **(a, b)** and after grinding **(c–f)**. **(c)** and **(d)** represent the average grain size of the ground samples, while **(e)** and **(f)** reveal details visible at 2500 fold magnification.