HOW TO SAMPLE MARINE MICROAGGREGATES IN SHALLOW AND TURBID ENVIRONMENTS? - Problems and Solutions

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Introduction

Microaggregates (5-500 µm) are the main component of suspended particulate matter (SPM) in shallow and turbid aquatic environments. They play an important role in biological, transport and sedimentation processes of organic and inorganic particles (GROSSART & SIMON, 1998; SIMON *et al.*, 2002). They are also a highly dynamic microhabitat for bacteria and other microorganisms (ZIMMERMANN, 1997). In recent investigations these microhabitats have often been described from a physical and chemical point of view (EISMA *et al.*, 1991; MCCANDLISS *et al.*, 2002), - but what about biology? Investigations of the functional role of aggregate-associated bacteria for aggregation and disaggregation processes as well as the biochemical composition and alteration of particulate and dissolved organic matter (POM and DOM) on small scales are rare (MANNINO & HARVEY, 2000; SCHUMANN *et al.*, 2001; SCHWEITZER e*t al*., 2001). Most of the data available on the bacterial decomposition of aggregates are from pelagic marine systems (ALLDREDGE *et al.,* 1986; SIMON *et al*., 1990; SEMPERE *et al.*, 2000; SMITH *et al.*, 1992) or from estuaries (CRUMP & BARROSS, 2000; KERNER & EDELKRAUT, 1995; MURRELL & HOLLIBAUGH, 2000; MURRELL *et al.*, 1999).

This is partly due to methodological problems in highly turbid environments. For instance, concentrating water samples on glass fiber filters is a very common method for estimating basic parameters, e. g. SPM and POM (BEHRENDS & LIEBEZEIT, 1999; CANUEL & ZIMMERMAN, 1999; KOENIG & FROHSE, 1996). Information about the size structure and *in situ* abundance of fragile aggregates are lost by this treatment. Traditionally, marine samples concentrated on glass fiber filters are washed by *aqua dest.* to remove the salt, which also accumulates in the pore water of the filter and biases gravimetric results. Because rinsing the filter with distilled water causes significant losses of POM we developed an easy and accurate correction on the basis of the known salinity and the pore water volume of the used filter.

Further, to sample and separate the small and fragile aggregates, we designed and tested a new type of water sampler. It provides the opportunity to combine measurements of optical and biochemical together with biological parameters from the same sample. The analysis of different aggregate fractions separated by their gravity in addition to bulk samples provides new insights into their composition and potentially into their microbial colonization.

Methods

Sampling

Samples were collected (1) in the German Wadden Sea at the Otzumer Balje (53° 44.9´N, 07° 40.0´E) in the Spiek eroog back barrier tidal flat system at different seasons on board RV Senckenberg, and (2) in the German Bight in April 2003 on board RV Heincke.

Salt Correction

Dry weight (DW) was estimated by filtering 500 ml of water on preweighed and precombusted GF/F filters (Whatmann, d=47 mm) in duplicates. One filter was rinsed with 5 ml *aqua dest.* after filtration. The filters were kept frozen at –20°C until further analysis within one week. Then the filters were dried (1 h, 110°C), weighed, combusted (2 h, 500°C) and w eighed again. The dry weight of the rinsed (rin) and untreated (unt) filters was calculated as the weight difference of the dried filter after filtration (F_d) and its net weight (F_n):

$$DW_{rin/unt} = F_{d\ rin/unt} - F_n \quad [mg]$$

The ash weight (AW = inorganic content) was calculated by the difference of the filter weight after combustion (F_c) and its net weight:

$$AW_{rin/unt} = F_{c\ rin/unt} - F_n \quad [mg]$$

POM was calculated as:

$$POM_{rin/unt} = DW_{rin/unt} - AW_{rin/unt} \quad [mg]$$

Afterwards DW, AW and POM were normalized to 1 litre. To correct the values of the rinsed filters for POM loss they were compared with the values of the untreated filters and the difference was added before normalizing (DW_{rincor}):

$$DW_{rincor} = DW_{rin} + (POM_{unt} - POM_{rin}) \quad [mg]$$

$$POM_{rincor} = POM_{rin} + (POM_{unt} - POM_{rin}) \quad [mg]$$

To calculate the true DW and AW the weight after filtration and drying was corrected for the salt contained in the pore volume (PV) of the filters (PV=0.51 ± 0.02 ml, n=5) by using the respective salinity (Sal) value, expressed as mg ml^{-1}:

$$Cor\ DW = DW_{unt} - (Sal\ [mg/ml]\ X\ PV\ [ml]) \quad [mg]$$

$$Cor\ AW = AW_{unt} - (Sal\ [mg/ml]\ X\ PV\ [ml]) \quad [mg]$$

The POM value is not affected by salt and thus calculated by the difference of the untreated DW and AW:

$$POM = DW_{unt} - AW_{unt}\ [mg]$$

Sampler

The features of the newly designed sampler (Fig. 1) are:
- soft door closing mechanism for careful sampling,
- quick and easy documentation of aggregate size and abundance by rectangular laser illumination and digital photography and subsequent image analysis,
- opportunity to fractionate different aggregate qualities by size and specific density,
- quantitative removal of the separated fractions.

The doors of the sampler are closed by remote control from shipboard while it is in horizontal position and oriented with the current. After retrieving the sampler on shipboard and bringing it into vertical position the aggregates were immediately illuminated by a red light laser (λ=658 nm, 50 mW, HB-Laser Components, Germany) in the lagged sampling chamber. The laser ray is extended by a semiplanar lens (Schott, Germany) and of defined width (1 mm). Aggregates of one sample were documented on three photos within 20 s using a digital camera (Sony Cybershot DSC-S70) and subjected to image analysis (analySIS, Soft Imaging System, Germany) for assessing aggregate size classes, area, equivalent circle diameter and abundance. Calibration shows that camera resolution is about 15 µm per pixel. To minimise counting errors, only particles ≥ 2 pixel (≥ 30 µm) were counted.

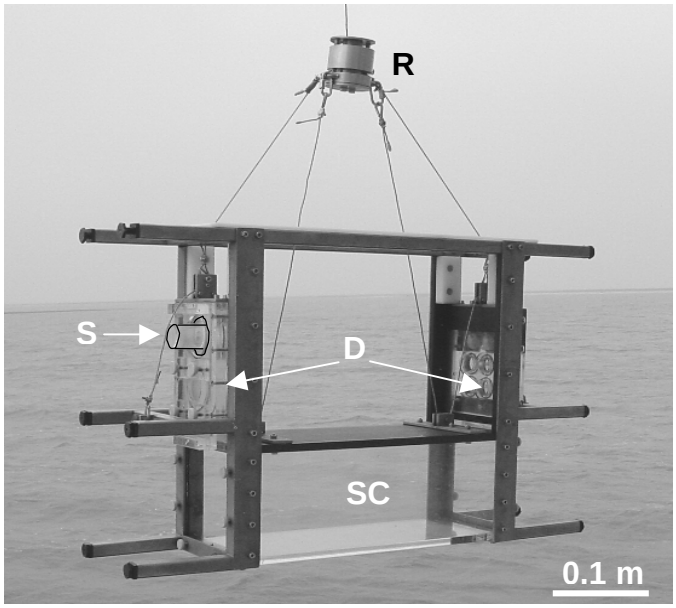

Fig. 1. Details of the newly designed water sampler for shallow and turbid environments: releaser (**R**), sliding doors (**D**), sampling chamber (**SC**), sedimentation chamber (**S**).

Results and discussion

Salt correction

DW of untreated filters (DW_{unt}) was systematically higher than that of rinsed filters (DW_{rincor} = corrected for loss of POM) and that corrected for calculation (Cor DW). DW of the rinsed filter and the corrected dry weight are nearly identical (linear regression, r^2=0.98, p<0.001).

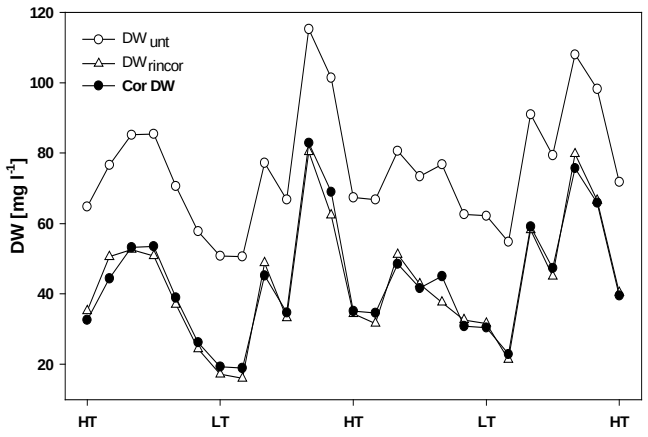

Fig. 2. DW over two tidal cycles (October 2002, Otzumer Balje) estimated with different calculations (see text); HT=high tide; LT=low tide.

In October 2002, the loss of POM (difference of POM_{unt} - POM_{rin}) was approximately 52.5 ± 10.4% of weight (Fig. 3). We found similar relationships for DW and loss of POM for tidal cycles in the Wadden Sea in August 2002 and February 2003 and for the German Bight in April 2003 at 20 stations (data not shown).

The results show that for estimating DW of SPM in marine samples by filtration it is necessary to correct the weight-based data for the amount of salt (Fig 2). To our best knowledge so far no clear-cut method is available for correcting this bias. Because rinsing with even very small amounts of distilled water results in substantial losses of POM (Fig. 3) we propose to use our method for correction. It is easy to apply and yields reliable data, as has been shown for various samples and sample volumes. Only when the sample volume becomes small such that the DW is in the range of the amount of salt in the filter, our method would not yield reliable data.

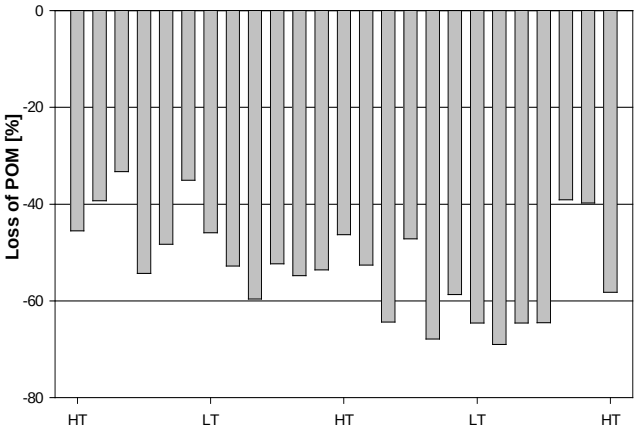

Fig. 3. Loss of POM [% of weight] by rinsing with 5 ml *aqua dest*. after filtration of 500 ml water (October 2002, Otzumer Balje).

Our calculated correction results in reduced DW (Cor DW) and unchanged POM values as compared to rinsing of samples and thus are enhancing the ratio POM/DW by 84.5 ± 34.35% (Fig. 4).

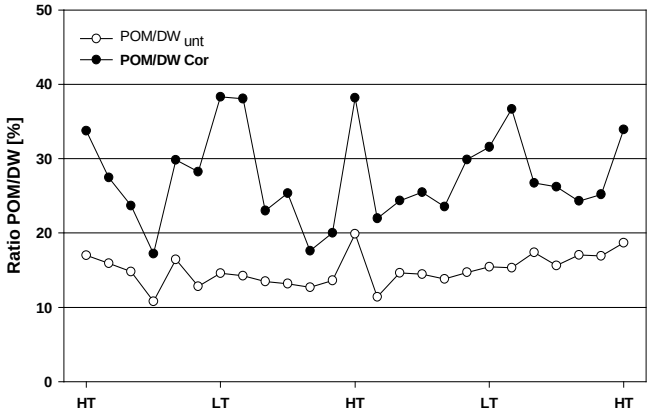

Fig. 4. Ratio of POM/DW in % estimated with untreated (unt) and corrected (Cor) DW in October 2002.

Because calculation does not affect POM concentration, our results imply that the SPM in the Wadden Sea exhibits a substantially higher POM fraction than assumed previously and possibly in other marine environments, in which DW has been assessed by similar methods.

Sampler

For fractionation we obtain a bulk sample at time 0 and allow the aggregates in the sample to settle for 45 minutes in the sampler covered by a black PE-jacket. During this time the larger and heavier aggregates sink out and accumulate in the connected sedimentation chamber (for details see Fig. 1). Smaller and lighter aggregates remain suspended or even move upwards in the supernatant. The latter fraction is carefully withdrawn by a tube from the top. The photos in Fig. 5 are corrected for brightness and contrast and show the different aggregate structures of the bulk sample at t=0 and the supernatant after 45 min:

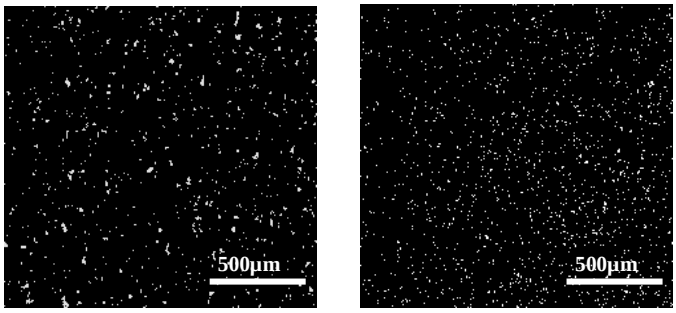

Fig. 5. Digital photos of a fractionated water sample (May 2002, high tide, Otzumer Balje). (A) bulk sample at time 0 and (B) supernatant after 45 min. Camera cut-out and scale is identical in both pictures.

Image analysis of the photos provides valuable parameters such as aggregate abundance and maximum diameter as in February 2002 (Fig. 6). All data available show similar patterns with close covariations of these parameters to DW and tidal dynamics (SIMON *et al*., this volume).

The separation of the various fractions by density and size provides valuable information on biological parameters such as chlorophyll and POM and how they vary over space and time, such as over tidal cycles in the Wadden Sea and in the German Bight at stations of different states of the phytoplankton spring bloom. (Figs. 7 and 8).

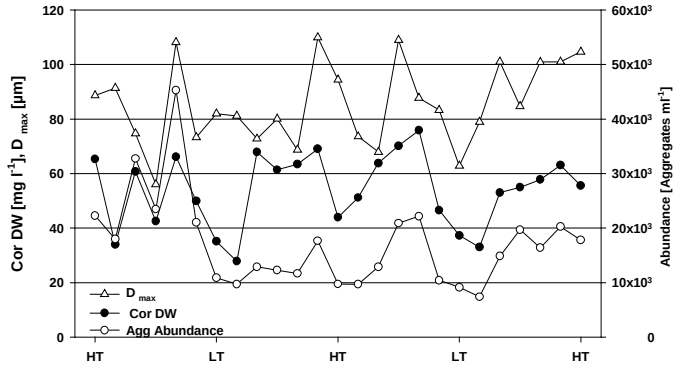

Fig. 6. Dynamics of aggregates >30 µm (February 2003, Otzumer Balje) in terms of dry weight (Cor DW), abundance (Agg Abundance) and maximum diameter (D_{max}).

The newly designed sampler is a useful tool to document the size structure and abundance of aggregates. The technique is relatively low-priced and easy to handle in comparison with elaborated optical *in situ* systems (EISMA *et al.*, 1996; KNOWLES & WELLS, 1996; SYVITSKI & HUTTON, 1996). A first comparison with **L**aser-**I**n-**S**itu-**S**cattering-and-**T**ransmissiometry data (LISST) (JOERDEL *et al*., this volume) and our data (see Fig. 6; SIMON *et al*., this Volume) gave promising results. The possibility to obtain optical measurements as described for lab experiments (CURRAN *et al.*, 2003; ZANEVELD *et al.*, 1982) in combination with biochemical and biological parameters from a single field sample is a promising step forward by our method.

The potential drawback of structural modifications of the fragile aggregates during sample handling is tried to be minimized and compensated by the described improvement.

MCCANDLISS *et al.* (2002) showed that fractionation of the bulk sample offers the assessment of organic-inorganic interactions in sedimentation processes and show that comparing these data with hydrodynamic conditions makes this a good tool for validating models.

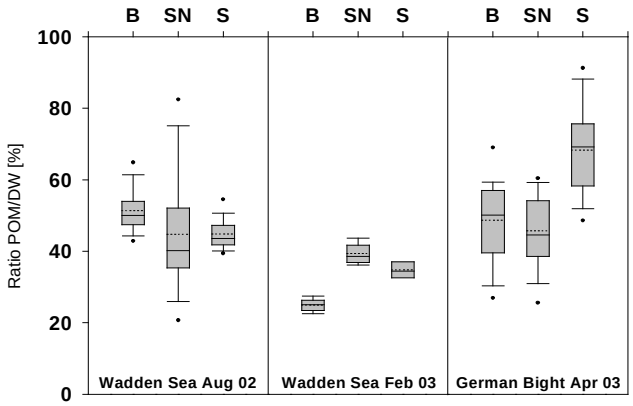

Fig. 7. Ratio of POM/DW from aggregate fractions separated in the sampler (B=bulk sample, t=0 min; SN=supernatant, t=45 min; S=sediment, t=45 min). Wadden Sea: one station (Otzumer Balje) sampled for two tidal cycles, German Bight: 20 different stations sampled within 72 hours; mean: dotted line; median: solid line.

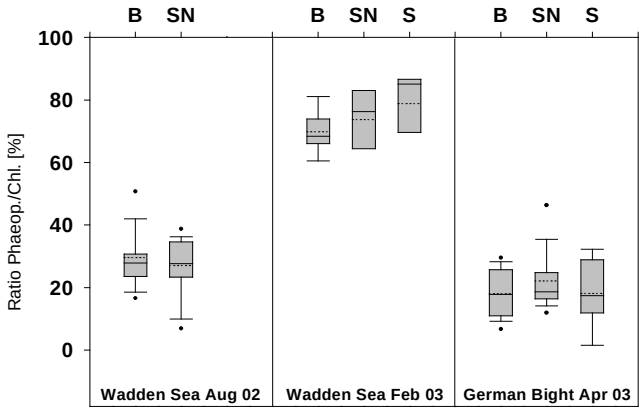

Fig. 8. Ratio of phaeopigments/chlorophyll from aggregate fractions separated in the sampler (B=bulk sample, t=0 min; SN=supernatant, t=45 min; S=sediment, t=45 min). Wadden Sea: one station (Otzumer Balje) sampled for two tidal cycles, German Bight: 20 different stations sampled within 72 hours; mean: dotted line; median: solid line.

Outlook

In order to obtain completely reliable results of the POM data in combination with our approach to correct for salt we are testing whether the combustion only affects loss of organic matter or if other components, e. g. clay-bounded water, are smoked out by this treatment. Further, we are going to determine directly POC and PON of rinsed as compared to untreated filters by elemental analysis.

With respect to assessing the abundance and size structure of aggregates by our sampling device and image analysis we will adjust our optical system with a LISST system (JOERDEL *et al.,* this volume) and will further improve the fractionation of the bulk sample.

After solving these methodological problems we shall become ready to focus on our main points of interest, i. e. which bacteria are dominant, where do they live and what is their impact on producing, metabolizing and decomposing POM (SIMON *et al*., this volume). We want to know if and how rapid changes in a physically driven system are reflected by bacterial metabolic activities. Together with experimental approaches, e. g. couette-chambers (DRAPEAU & DAM, 1993), we are going to intensify our investigations of aggregate-bacteria-POM-DOM interactions.

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