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Chemical and Biological Composition of Suspended Particles and Aggregates in the Baltic Sea in Summer (1999)

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Suspended particles and particle aggregates, which formed from concentrated field samples on the roller table, were characterized biologically and chemically along a transect through the Baltic Sea in summer 1999. Phytoplankton composition in field samples was dominated by cyanobacteria, including the filamentous diazotrophic cyanobacteria *Aphanizomenon ' baltica*', *Nodularia spumigena* and *Anabaena* spp. These species formed aggregates together with diatoms, mainly *Skeletonema costatum* and *Chaetoceros* spp. and with dinoflagellates, mainly with *Dinophysis norvegica*. Compared to the Redfield ratio, concentration ratios of particulate organic carbon, nitrogen and phosphorus, [POC]:[PON]:[POP], indicated an enrichment of carbon, especially in aggregates. However, regression analysis indicated a higher production rate of PON relative to POP and POC and significant background concentrations of POC. In field samples the concentration of transparent exopolymer particles (TEP) varied around 200 µg Xanthan Equiv. l^{-1} and comprised a volume fraction of 2–7 ppm and an abundance of about 10^5 TEP ml^{-1}. TEP were enriched in aggregates as inferred from volume ratios of TEP to conventional particles. It is suggested, that TEP contribute substantially to the background concentration of POC, while the high production rate of PON is attributed to nitrogen fixation of diazotrophic cyanobacteria.

Keywords: biogeochemical elements; organic particles; aggregates; filamentous cyanobacteria; Baltic Sea

Introduction

The formation of visible particle aggregates (marine snow) and their importance for the vertical transport of elements through the water-column have been acknowledged widely during the last decades (Fowler & Knauer, 1986; Gardner, 1997). Sinking of aggregates is responsible for pulsed sedimentation of phytoplankton (Alldredge & Gotschalk, 1989; Riebesell, 1991; Kiørboe *et al.*, 1994) and the occurrence of intense mass fluxes in the deep Ocean (Asper *et al.*, 1992), providing a tight coupling between the pelagic and benthic ecosystems. Through aggregation otherwise floating particles such as positive buoyant cyanobacteria and small picoplankton become entrained in settling aggregates and participate in the vertical particle flux (Grossart, 1996; Waite *et al.*, 2000).

Little is known about the composition of aggregates relative to the bulk fraction of suspended particles and their role for the sequestration of biochemical elements or for the selection of species. Aggregates often harbour high abundance of autotrophic and heterotrophic organisms (Alldredge & Silver, 1988) and can represent microhabitats. High biological activity in aggregates may therefore lead to chemical alteration of the aggregate (Smith *et al.*, 1992; Grossart & Simon, 1998). Due to differential aggregation, e.g. by different attachment probabilities or adhesion properties (Kiørboe *et al.*, 1990; Crocker & Passow, 1995), particles can be selectively enriched in aggregates. For example, transparent exopolymer particles (TEP), or mucus particles in general, frequently occur in high concentration in aggregates (Alldredge *et al.*, 1993; Passow & Alldredge, 1994). In comparison to most conventional particles like plankton and detritus, TEP have a higher C:N ratio (Engel & Passow, 2001). Thus, an enrichment of TEP in aggregates may be a pathway for the selective removal of organic carbon from surface waters. Since aggregation increases the sinking velocity of particles, differential aggregation may also influence the competition among species (Hansen *et al.*, 1995). However, only a few studies have addressed the enrichment of natural species or groups of organisms in aggregates (Caron *et al.*, 1986; Revelante & Gilmartin, 1991).

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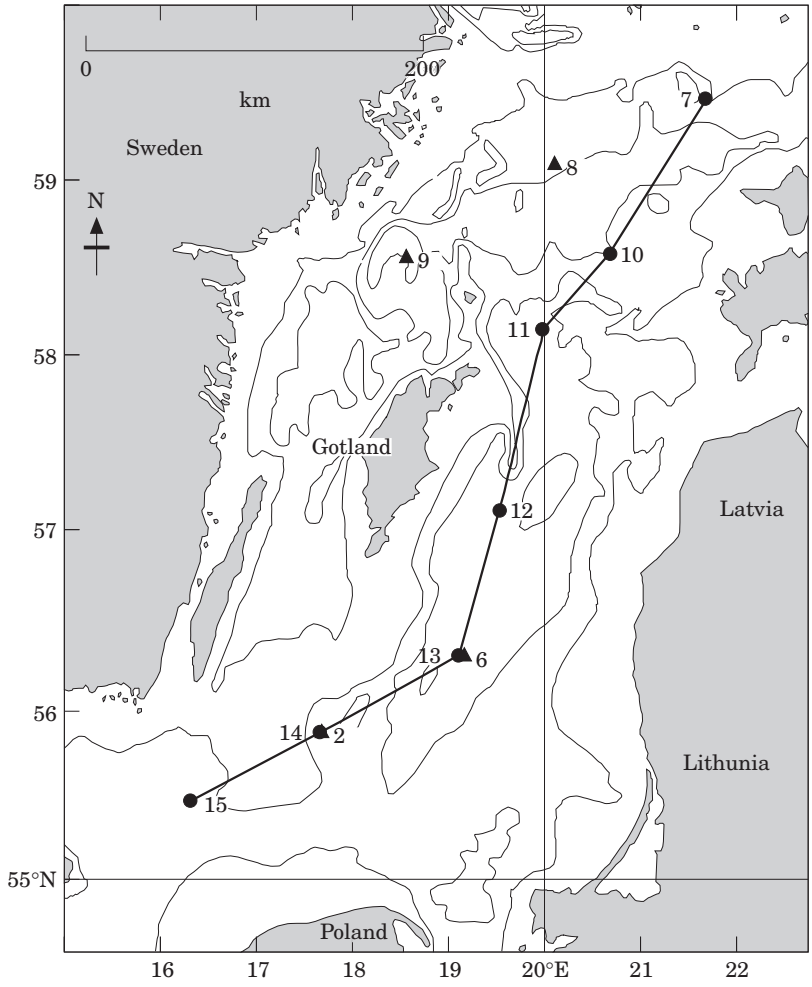

FIGURE 1. Map of the central Baltic Sea (Baltic Proper) with transect (●) and additional stations (▲) sampled during a cruise in June 1999.

In the Baltic Sea nitrogen limits phytoplankton growth after the spring bloom. Filamentous cyanobacteria, which overcome nitrogen limitation by fixing atmospheric N_2 are therefore important and abundant primary producers during summer and episodically form extensive surface blooms. Although total export rates are usually small during summer, pulsed sedimentation of the filamentous cyanobacteria *Nodularia* spp., *Anabaena* spp. and *Aphanizomenon* spp. occurred, as measured with sediment traps below 100 m depth in the Baltic Sea (Gotland Sea) (Wasmund *et al.*, 1999). Aggregates are a major component of sinking material in the stratified Baltic Sea during summer (Lundsgaard *et al.*, 1999; Olli & Heiskanen, 1999) and may enable the vertical export of the N_2-based ' new production ' in this system.

The aim of this study was to investigate the potential role of aggregate formation for the sequestration of particulate organic matter in the central Baltic Sea (Baltic Proper) during summer. Since TEP are important agents in aggregate formation, but no data on TEP exist for the central Baltic Sea, special focus was laid on the distribution of TEP and their fraction in aggregates. The question whether aggregates and suspended particles differ with regard to their chemical and biological composition was addressed by comparing suspended particles with aggregates that were produced artificially on a roller table.

Material and methods

Hydrography and field sampling

Samples were collected in the Baltic Sea along a transect between 59°25′N, 20°10′E and 55°27′N, 16°20′E (Stations 7, 10–15) and at four additional

locations (Stations 2, 6, 8, 9,) in June 1999 (Figure 1). Water was sampled from the surface and at 4, 9, 14 and 20 m depths with 10 l Niskin bottles at 5:00 a.m. local time. Salinity and temperature were measured by CTD instruments. Irradiance above sea level was measured all day by a plane 2pi-LICOR quantum sensor.

Aggregation experiments

At six stations (2, 6, 7, 9, 11, 14) aggregates were produced on the roller table as described by Shanks and Edmondson (1989). At each station 20 l were sampled from the surface and also from 4 and 9 m depth. Because particle concentration was too low to generate a sufficient quantity of aggregates for the biological and chemical analysis, each of the field samples was enriched in particulate material: First, particles >0·16 µm were concentrated from 20 l to approximately 13 l by tangential flow filtration (TFF). TFF is a gentle method for the concentration of plankton without significant damage to the cells structure or viability (Petruševski *et al.*, 1995). One litre of filtrate (<0·16 µm) was stored at 5 °C for later resuspension of aggregates. The sample was further enriched with Plankton from 1–2 net catches, collected by Apstein net hauls (20 µm mesh, 16·5 cm diameter) from 20 m depth to the surface at a towing speed of 20 cm min^{-1}. Each of the enriched samples was split: ten litres were filled into an acrylic cylinder and subjected to rotation on the roller table at 0·5 rpm. Two litres were filled into Polycarbonate flasks and served as control for changes in the biological and chemical variables that occurred independent of aggregate formation. The roller table and the controls were kept in darkness for 24 h at 15 °C. Afterwards, visible aggregates (>1 mm) were isolated from the cylinders using a 25 ml syringe with a 2 mm diameter needle. The total volume of the aggregate slurry of each cylinder was measured to the nearest 0·5 ml within the syringe. Aggregate slurries were diluted with 200 ml of appropriate TFF-filtrate and aggregates disintegrated by gentle mixing.

The aggregate slurries consisted of a mixture of aggregates and of surrounding seawater (SSW) collected together with the aggregates, with the volume of SSW being typically much larger than the volume of aggregates. However, particle concentration in aggregates was generally 2–4 orders of magnitude higher than in the SSW. In order to obtain a conservative estimate of particulate components within the aggregates solely, all concentrations of particulate components in the SSW were subtracted from those in the slurry. The aggregate slurries, the SSW and the controls were analysed separately as described later.

For data presentation the mass content of aggregates was related to the solid aggregate volume (SV_{AG}). The SV_{AG} is the sum of the volumes of solid particles within an aggregate that can be detected by the Coulter Counter. In order to compare the results of this study with earlier findings, which relate the mass content of an aggregate to its visible dimensions, we derived the equivalent visible aggregate volume (VV_{AG}). The difference between SV_{AG} and VV_{AG} is due to the porosity (P), which is the volume fraction of an aggregates occupied by the fluid:

$$SV_{AG}/VV_{AG} = 1-P \quad (1)$$

Alldredge and Gotschalk (1988) determined the porosity of various natural aggregates and showed that (1–P) decreased with the visible diameter of the aggregates (d_{AG}, measured in millimetres) to the power of $-1{\cdot}6$:

$$1-P = 0{\cdot}008\ (d_{AG})^{-1{\cdot}6} \quad (2)$$

Assuming that the aggregate is spherical, equation (1) can be rewritten to:

$$SV_{AG}\ (mm^3) = (1/750)\pi(d_{AG},\ mm)^{1{\cdot}4} \quad (3)$$

where the exponent 1·4 is considered as the dimension of fractal scaling (D3). Thus, the visible diameter of an aggregate is related to the solid volume by:

$$d_{AG}\ (mm) = 750\pi(SV_{AG})^{(1/D3)} \quad (4)$$

Biological and chemical analysis

The particulate organic fractions of carbon (POC), nitrogen (PON) and phosphorus (POP) were determined from 250 to 500 ml of field samples, SSW and controls, and from 50 ml of aggregate slurries filtered onto pre-combusted GF/F filters and frozen until analysis at −18 °C. POC and PON were analysed with a CHN-O-rapid autoanalyser (*Hereaus*). POP was determined colourimetrically after persulfate oxidation (Koroleff & Grasshof, 1983). Filters were prepared in duplicates, except for the aggregate slurries. TEP were determined colourimetrically according to Passow and Alldredge (1995) from field samples (100 ml), SSW (20 ml), controls (20 ml) and aggregate slurries (5 ml), each filtered onto 0·4 µm Nuclepore filters. Semi-permanent TEP slides were prepared within 1 hour after sampling from field

samples (20 ml), SSW (10 ml), controls (10 ml) and aggregate slurries (3 ml), each filtered onto 0·4 μm Nuclepore filters (Passow & Alldredge, 1994). All TEP filters were prepared in duplicates. TEP slides were transferred to a compound light microscope and screened by a PANASONIC colour video camera on Digital Video with 400 × magnification. About 2 × 25 frames per slide were chosen in a cross section and digitized on a Macintosh PPC with an optical resolution of 0·17 μm^2 per Pixel. TEP were enumerated and sized semi-automatically by an image analysis program (NIH. Image 6.1 ppc., a public domain program developed at the US National Institute of Health). The equivalent spherical diameter (ESD) and the equivalent spherical volume (ESV) of individual TEP were calculated from area measurements assuming the symmetry of a sphere (Mari & Kiørboe, 1996). Only TEP were counted that did not touch the edge of the frame and contained at least five Pixel, yielding a minimum TEP size of 0·8 μm. Carbon content of TEP was calculated from colourimetrically determined TEP concentration using the empirical conversion factor of Engel and Passow (2001): C_{TEP} (μg) = 0·75 × TEP (μg Xanthan Equiv.). Concentration and size distribution of solid particles between 2 and 60 μm ESD were determined with the Coulter Counter (Coulter Multisizer II) from replicate 2 ml samples. A dilution with filtered seawater (<0·16 μm) in order of 1:2 for SSW and controls, and 1:10 to 1:20 for aggregate slurries was necessary to keep coincidence of particles at the aperture <5%. Prior to the Coulter Counter measurements aggregate slurries were again carefully mixed. This was necessary in order to avoid blocking of the orifice during measurement and to enable representative sub-sampling of the aggregate slurry, which is indicated by a small variability between replicate measurements. The content of pigments (chlorophyll and carotenoids) in field samples was determined by means of HPLC (High Performance Liquid Chromatography), using the method of Barlow *et al.* (1997). Identification of pigments was carried out by comparing their retention times and absorption spectra obtained with a diode array spectrophotometer (WATERS) with those of commercially available pigment standards. Chlorophyll *a* (chl *a*) was purchased from SIGMA, all other pigments from the International Agency for ^{14}C Determination, Denmark. The composition of the phytoplankton communities was calculated using the CHEMTAX program from Mackey *et al.* (1996), converting the concentrations of marker pigments to equivalents of chl *a* with suitable pigment to chl *a* ratios. These were obtained from isolations of filamentous cyanobacteria and size-fractionated filtration combined with multiple regression analysis. The abundance of filamentous cyanobacteria and of the dominant species of diatoms and dinoflagellates was also determined by microscopy from Lugol-fixed samples according to Utermöhl (1958). Samples were put under pressure (10 bar) prior to microscopy, which caused implosion of gas vesicles and enabled settlement of otherwise positive buoyant cyanobacteria to the bottom of the Utermöhl chamber. In order to minimize the counting error, at least 50 cells per species or 50 filaments in case of cyanobacteria were counted and at least 500 cells and filaments per sample. The dissolved inorganic compounds of nitrogen (DIN) and phosphorus (DIP) were determined in field samples immediately after sampling with an autoanalyser following the methods of Koroleff and Grasshof (1983).

Results

Hydrography and nutrient concentrations

The Baltic Sea is permanently stratified at ~60 m by a halocline (data not shown), separating the underlying more saline water of >10 psu from upper less saline water of <10 psu. The upper layer is well mixed during wintertime, whereas a sharp seasonal thermocline is formed during summer and was located between 10 and 18 m during the time of observation [Figure 2(a)]. Salinity in the upper layer increased steadily towards the south from 6·1 to 7·1 psu [Figure 2(b)]. Irradiance above sea level ranged between 500 $\mu E\, m^{-2}\, s^{-1}$ and 2000 $\mu E\, m^{-2}\, s^{-1}$, depending on the cloud coverage. Low but detectable concentrations of phosphate were observed along the transect with surface values between 0·05 $\mu mol\, l^{-1}$ and 0·1 $\mu mol\, l^{-1}$, increasing below 10 m to a maximum of 0·25 $\mu mol\, l^{-1}$. Nitrate was below detection limit (0·05 $\mu mol\, l^{-1}$) in almost every sample. Even the combination of the dissolved inorganic nitrogen (DIN) compounds NO_2^-, NO_3^- and NH_4^+ yielded low concentrations ranging between 0·1 $\mu mol\, l^{-1}$ and 0·2 $\mu mol\, l^{-1}$ throughout the study area.

Suspended particles along the transect

The phytoplankton communities along the transect were similar and consisted of small cyanobacteria (mainly *Synechococcus* spp.), filamentous diazotrophic cyanobacteria (*Aphanizomenon* '*baltica*', *Nodularia spumigena* and *Anabaena* spp.), crypto-, chloro-, and prymnesiophyceae, diatoms (mainly *Skeletonema costatum* and *Chaetoceros* spp.) and dinoflagellates

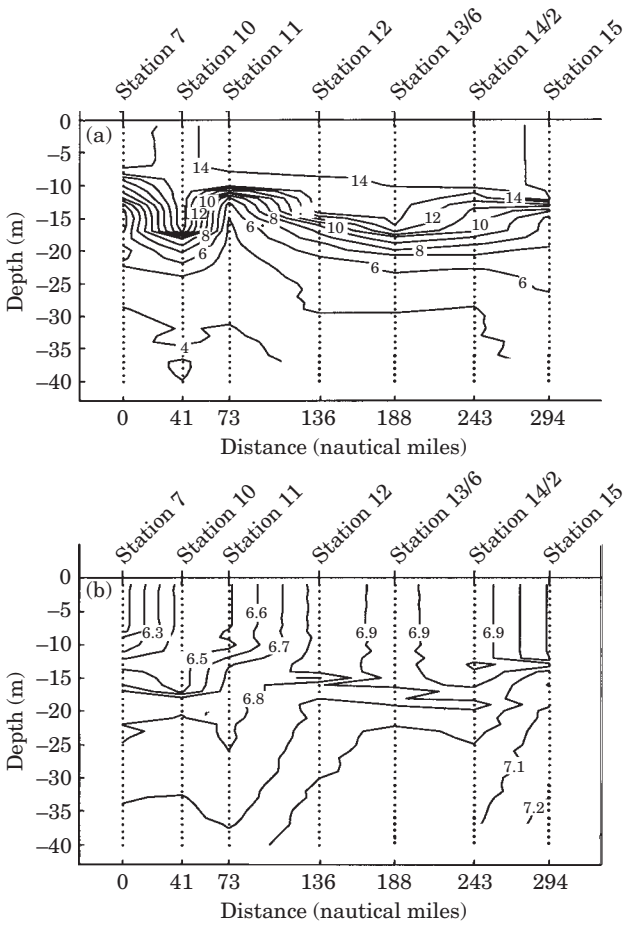

FIGURE 2. Hydrography of the sampling area. (a) Temperature (°C), (b) Salinity. Vertical lines indicate sampling locations along the transect. The distance indicated is referring to the most northern sampling station 7.

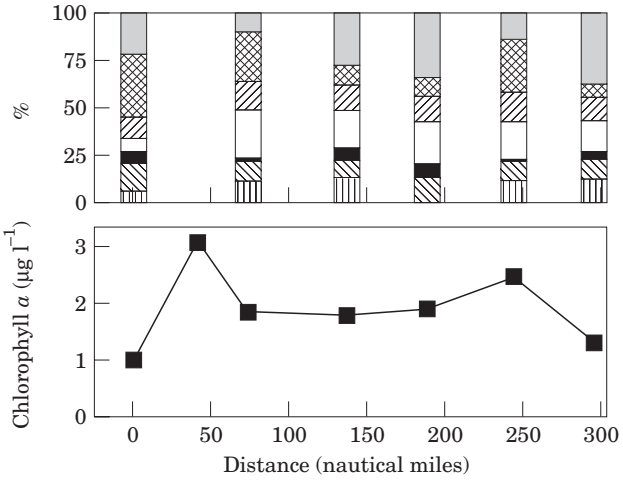

FIGURE 3. Concentration and partitioning of Chlorophyll *a* within the major phytoplankton groups as determined from accessory pigments by HPLC along the transect. Chlorophyll *a* concentration were averaged over the upper mixed layer (depth: 0–9 m). ▥ Dinoflagellates, ■ Prymnesiophyceae, ▨ Chlorophyceae, □ Other cyanobacteriae, mainly Synechococcus, ▧ Diatoms, □ Crytophyceae, ▩ Filamentous cyanobacteria.

(mainly *Dinophysis norvegica*). Chl *a* varied around a relatively low concentration of 2 μg l^{-1} and followed no spatial trend along the transect (Figure 3). In terms of relative chl *a* contribution, cyanobacteria dominated the phytoplankton by adding between 36 and 55% to total chl *a*, followed by cryptophyceae (up to 25%). The remaining chl *a* was shared between the other groups mentioned above.

Solid particles, detectable with the Coulter Counter (CCP) in the size range 2–60 μm ESD, occurred in numerical concentrations of $0{\cdot}9 \times 10^3$–$2{\cdot}0 \times 10^4$ ml^{-1} and comprised a volume fraction of 1–3 ppm. An increase in mean number and volume concentration of CCP was observed towards the southern Baltic Sea [Figure 4(a)]. Particle concentrations of TEP >0·8 μm ESD were one order of magnitude higher than CCP concentrations and varied between 6×10^4 and $1{\cdot}3 \times 10^5$ ml^{-1}. The total volume of TEP was about two to three times larger than the total volume of CCP [Figure 4(b)]. In terms of colourimetrically determined TEP, values ranged between 145 and 322 μg Xanthan Equiv. l^{-1}. Carbon contained in TEP (TEP-C) was on average 13 μmol l^{-1} and equivalent to roughly 40% of POC [Figure 4(c)]. Concentrations of particulate organic elements varied around average values of 32 ± 3 μmol l^{-1} POC, $4{\cdot}4 \pm 0{\cdot}4$ μmol l^{-1} PON and $0{\cdot}18 \pm 0{\cdot}03$ μmol l^{-1} POP and exhibited a similar spatial distribution [Figure 4(c, d)].

Covariations of POC, PON and POP above the thermocline (0–9 m) were detected from the slopes of linear regressions (Table 1). Slopes were concurrent with the expected Redfield ratio of C:N:P of 106:16:1 (Redfield *et al.*, 1963) in case of ΔPOC:ΔPOP (115 ± 18) but slightly lower for ΔPOC:ΔPON ($5{\cdot}6 \pm 0{\cdot}77$) and thus higher for ΔPON:ΔPOP ($19{\cdot}4 \pm 2{\cdot}2$). This indicates a relatively high production rate of PON with respect to POC and POP, and may be explained by the presence of N_2-fixing filamentous cyanobacteria, which have an exceptional high nitrogen content (Gabrielson & Hamel, 1985) and an uptake rate ratio of C:N of about 4·4 (Lindahl, 1987). Significant y-intercepts were observed for regressions of POC *vs*. PON or POP and indicate that a fraction of about 24–35% of POC was not affected by changes of PON and POP. Consequently, concentration ratios of POC:PON and POC:POP were

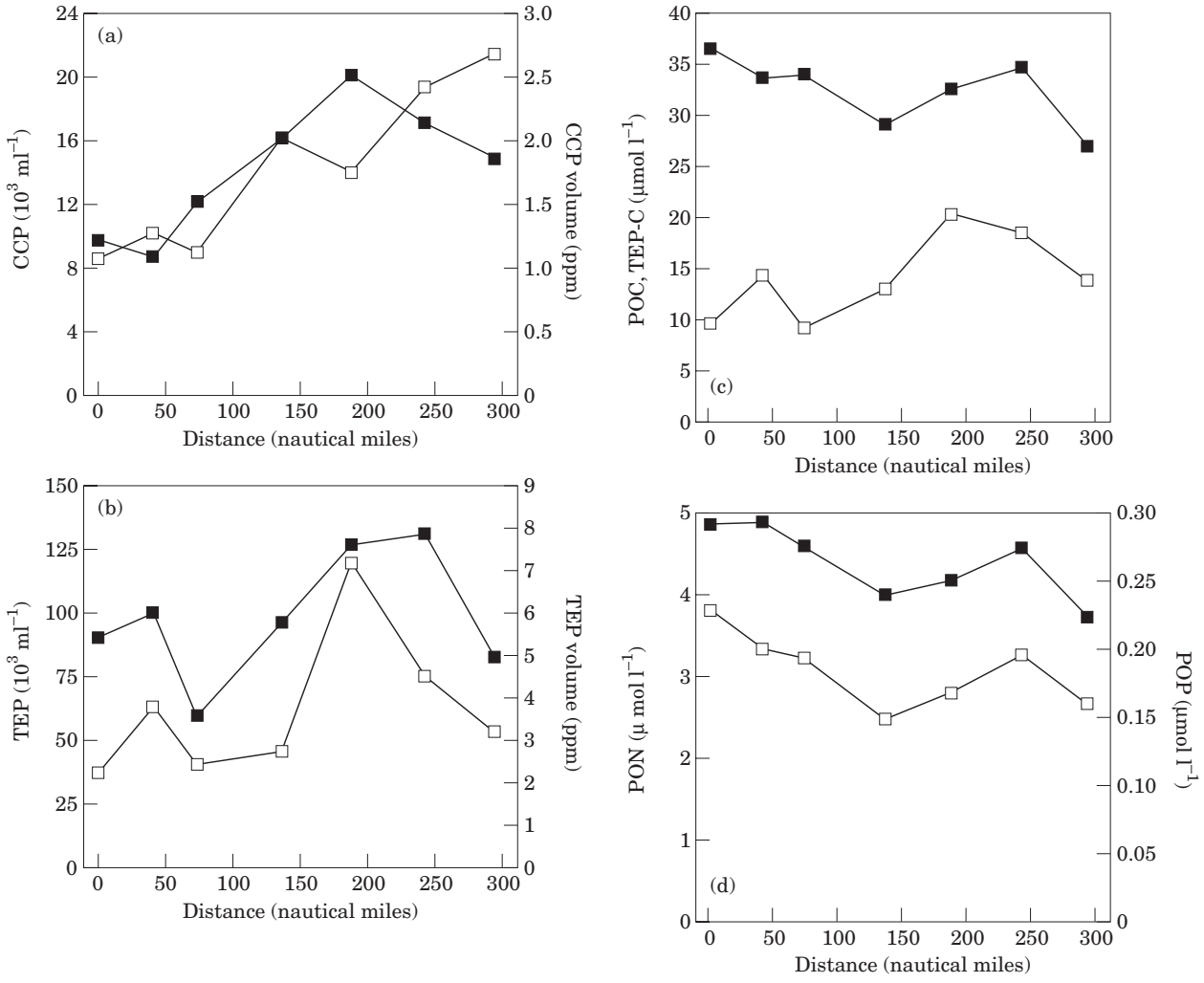

FIGURE 4. Particle composition within the field samples collected along the transect. Values are averages over the upper mixed layer (depth: 0–9 m). Shown are: (a) numerical concentration (■) and volume fraction (□) of CCP (Coulter Counter detectable Particles); (b) numerical concentration (■) and volume fraction (□) of TEP as determined by microscopy; (c) POC (■) and TEP-C as derived from colourimetrically determined TEP (□); (d) PON (■) and POP (□).

significantly higher than slopes of the respective regressions. Linear regression between total nitrogen (TN) defined as Σ[PON, NO_3^-, NO_2^-, NH_4^+] and total phosphorus (TP) defined as Σ[POP, PO_4^{3-}] yielded a slope not significantly different from the Redfield prediction of 16:1.

Aggregation experiments

In order to identify changes in the particle composition, due to enrichment by TFF and addition of net-plankton, control and field samples were compared. Particle enrichment was highly variable between the experiments. In the controls, concentrations of POC, PON and POP were about 2- to 7-fold higher and the volume fractions of TEP and CCP were 1·5- to 18-fold larger than in the field. No significant differences between field samples and controls were yet observed concerning the POC:PON:POP ratio and the TEP:CCP volume-ratio (Tables 1 and 4). Abundances of phytoplankton in the controls were 18- to 70-fold higher, but relative abundances of filamentous cyanobacteria, diatoms and dinoflagellates in the controls were within the range of the observed natural variability in the field (Figure 5).

Aggregates that formed during roller table incubations comprised only a minor fraction (<0·01%) of the total volume in the cylinders and ranged from 10^0 to 10^2 ml in terms of ‘ aggregate slurry ’ volume and from 10^{-3} to 10^{-2} ml in terms of total SV_{AG}. Solid

TABLE 1. Concentration ratios and regression analysis of particulate organic elements from samples of the upper mixed layer (0–9 m), collected along a transect at the Baltic Sea in 1999. TN*: combined data of DIN (NH_4^+, NO_2^-, NO_3^-) and PON; TP*: combined data of PO_4^{3-} and POP; (TEP:CCP): volume ratios of TEP and Coulter Counter detectable particles, ns: not significant

Variables (y, x)	Concentration ratio [y]/[x]		Regression analysis				
	median	range	Slopes Δ[y]/Δ[x]	R^2	*P*<	y-intersect	% of y
POC:PON	7·3	6·7–8·3	5·6 ± 0·77	0·78	0·001	8·2 ± 3·6	24 ± 11
POC:POP	179	155–195	115 ± 18	0·73	0·001	12 ± 3·5	35 ± 5
PON:POP	23·9	21·2–27	19·4 ± 2·2	0·84	0·001	ns	—
TN*:TP*	17·9	15·9–20	16·2 ± 2·4	0·78	0·001	ns	—
TEP:CCP	2·3	0·8–6·9	ns	—	—	—	—

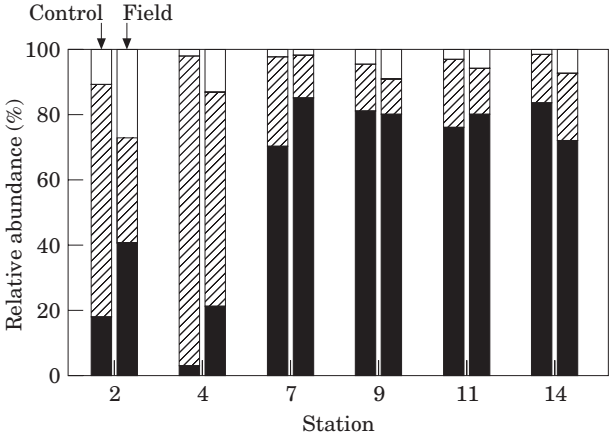

FIGURE 5. Relative abundance of the major phytoplankton groups (filamentous cyanobacteria, diatoms and dinoflagellates) in the enriched control samples and in the respective field samples. Values are averages over samples from the surface, 4 and 9 m depth for each station. □ Dinoflagellates, ▨ Diatoms, ■ Filamentous cyanobacteria.

particles comprised a volume fraction of 2×10^2–3×10^3 ppm in aggregates, which is 2–3 orders of magnitude higher than the CCP volume fraction in field samples. The total SV_{AG} of each cylinder increased exponentially with the CCP concentration in the control samples (P<0·001, data not shown). This indicates that aggregate formation was primarily a function of initial particle concentration.

Overall, aggregates contained a mixed community of filamentous cyanobacteria, dinoflagellates and diatoms, with *Aphanizomenon 'baltica'*, *Dinophysis norvegica*, *Chaetoceros* spp. and *Skeletonema costatum* being the dominant species [Figure 6(a)]. At two stations (2 and 6) aggregates were clearly dominated by dinoflagellates, mainly by *Dinophysis norvegica*, which comprised >60% of cell number. Selective enrichment of one species (E_i) was determined by comparing its relative abundances in aggregates and in the control:

$$E_i = \left(n_{i(AG)} / \sum_{i=1}^{N} n_{i(AG)} \right) \Big/ \left(n_{i(control)} / \sum_{i=1}^{N} n_{i(control)} \right) \quad (5)$$

where the total number of species considered (N) equalled 7. Selective enrichment was most pronounced for *Chaetoceros* spp., for thecate dinoflagellates (especially on station 2 and 6) and for *Nodularia spumigena* [Figure 6(b)]. Total phytoplankton abundances in aggregates and controls were not related. However, the abundance of *Anabaena* spp., *Nodularia spumigena* and *Skeletonema costatum* in aggregates increased significantly with the abundances of these species in the controls (Table 2).

Concentrations of particulate organic elements within aggregates ranged from 6·4 to 48 mmol l^{-1} POC, from 0·9 to 5·3 mmol l^{-1} PON and from 0·02 to 0·14 mmol l^{-1} POP. TEP concentrations ranged from 8·6 to 57 mg Xanthan Equiv. l^{-1}, equivalent to 0·5–3·6 mmol l^{-1} TEP-C. Linear relationships were calculated as a first order approximation for the increase in particulate elements and TEP with the solid volume of aggregates (SV_{AG}) [Figure 7(a–d)]. For easier comparison of aggregate composition with literature data, the visible volume (VV_{AG}) was calculated from the solid aggregate volume according to equation (4). The fractal scaling used for this calculation was set to D3=1·4, determined for *in situ* seized aggregates (Alldredge & Gotschalk, 1988) and to D3=1·9 for aggregates formed on the roller table (Engel & Schartau, 1999). Due to fractal scaling VV_{AG} increases nonlinearly with SV_{AG} [equation (2)] and exponential functions were assumed for the regressions of particulate organic elements and TEP *vs.* VV_{AG} (Table 3). The regression equations of POC and PON *vs.* VV_{AG} derived for D3=1·4 were similar

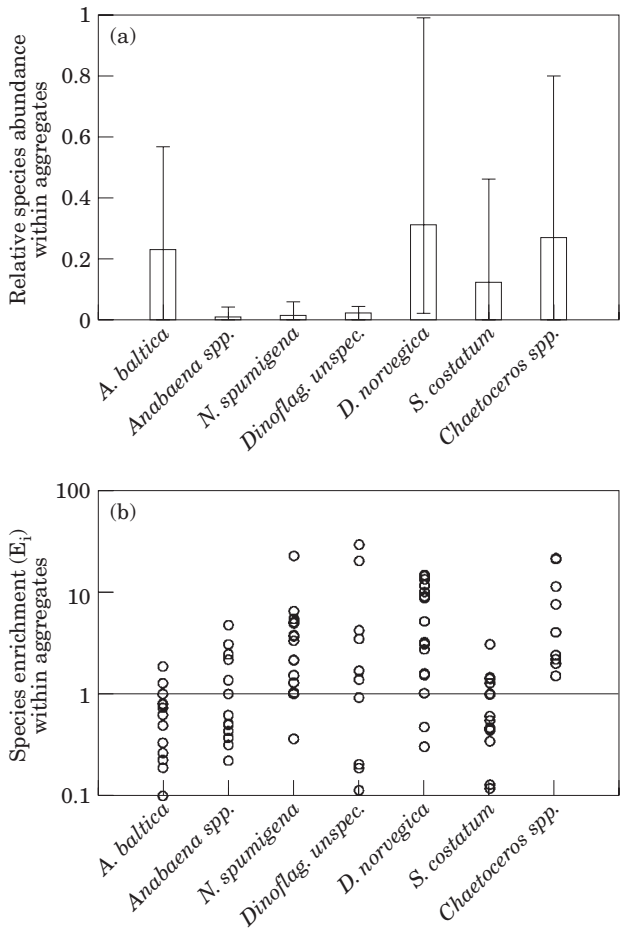

FIGURE 6. Abundance of major phytoplankton species within aggregates formed on the roller table (a) Given are the mean values (open bars) and the range of maximal and minimal abundances (error bars). Selective enrichment (E_i) of these species within aggregates (b) was calculated by comparing their relative abundance in the aggregates with their relative abundance in the controls and is shown for each experiment individually (○).

to the findings of Alldredge (1990) determined for marine snow isolated *in situ*, whereas for the higher fractal dimension the relationships were closer to the findings of Ploug and Grossart (2000), who used the roller table approach. This confirms that aggregates, which form on the roller table are more densely packed and have higher fractal dimensions than natural marine snow, as suggested earlier (Lick *et al.*, 1993). It also underlines that the knowledge of the scaling relationship between size and mass of an aggregate is crucial to derive its elemental content from visual measurements.

Linear relationships between POC and PON were determined for aggregates, SSW and controls (Table 4). The slopes (ΔPOC:ΔPON) were not significantly different from each other and from the slope that was determined for the field samples. (*t*-test: $P<0{\cdot}01$). Again, pronounced y-intersects were observed. The highest absolute and relative y-intersect was determined for aggregates, concomitant with highest concentration ratios of POC:PON. Covariations of POC and PON with POP were less tight and no significant y-intercepts were determined. Thus, no differences between slopes and concentration ratios were observed here. A pronounced deviation from Redfield's prediction was found for aggregates, where POC:POP and PON:POP as well as ΔPOC:ΔPOP and ΔPON:ΔPOP were about twice as high as expected and differed significantly from the suspended particles fractions (SSW, controls and field samples; $P<0{\cdot}001$). The volume fraction of TEP in aggregates was highly variable, but in general TEP comprised the major part of the particulate volume of aggregates. The volume ratio of TEP and CCP was highest in aggregates, which shows a significant enrichment of TEP in aggregates when compared to the suspended particles fraction (Tables 1 and 4).

Discussion

Preconditions for aggregation in the field

Particle aggregation depends on the rates of particle collision and particle adhesion. The former is a function of the concentration, size and velocity of particles (McCave, 1984), the latter depends on the physicochemical properties of the particles surface. TEP are of major importance for aggregation processes as they increase bulk particle concentration and bulk stickiness (Alldredge *et al.*, 1993; Logan *et al.*, 1995; Engel, 2000). The formation of aggregates and high production of TEP *in situ* has mostly been connected with diatom bloom (Alldredge & Gotschalk, 1989; Riebesell, 1991; Kiørboe *et al.*, 1994), and aggregation in stratified systems with low biomass is expected to be low. During this field study mean TEP concentrations of about 200 µg Xanthan Equiv. l^{-1} were measured. Expressed in particle abundance they comprised a volume fraction of 2 to 7 ppm with at a mean number of about 10^5 ml^{-1}. These are the first TEP data reported for the central Baltic Sea. In comparison, Kraus (pers. commun., 1997) determined TEP colourimetrically during the diatom spring bloom at a coastal Baltic Sea location (Kiel Bight), and yielded mean values of about 120 µg Xanthan Equiv. l^{-1} while the volume concentration of conventional particles (CCP) ranged between 2 to 6 ppm (Engel, 1998). The ratio of TEP and CCP during the diatom bloom was roughly 2- to 4-fold lower than during this study and the spring system

TABLE 2. Correlation between the abundance of one species in aggregates and in controls (a) and between the abundance of one species in aggregates and total phytoplankton abundance in controls (b). ns: not significant. Dinoflagellates other than *D. norvegica* were not specified

Species	(a)		(b)	
	R^2 species specific	$P<$	R^2 total phytoplankton	$P<$
Aphanizomenon ' baltica '	0·08	ns	0·13	ns
Anabaena spp.	0·75	0·005	0·05	ns
Nodularia spumigena	0·52	0·07	0·00	ns
Dinoflagellates unspec.	0·01	ns	0·17	ns
Dinophysis norvegica	0·08	ns	0·04	ns
Skeletonema costatum	0·60	0·05	0·20	ns
Chaetoceros spp.	0·18	ns	0·02	ns

was clearly dominated by phytoplankton. Thus, the relative importance of TEP in the Baltic Sea seems to be more pronounced during summer than during the diatom spring bloom. This is also supported by the findings of Mari and Burd (1998), who determined the seasonal distribution of TEP in the Kattegat (northwestern Baltic Sea) and observed that TEP concentration during summer was relatively high. There may be several reasons why TEP can be relatively important during summer. First, the removal rate of TEP from the upper water column at times of high CCP, like during diatom blooms, may be higher due to ongoing coagulation and settling of aggregates. Obernosterer and Herndl (1995) showed that exopolymers released by phytoplankton under phosphate limitation are more resistant to bacterial decomposition. In a similar way TEP produced under DIN deficiency in the Baltic Sea during summer may resist bacterial degradation also. On the other hand, TEP production rate may be higher during summer as nutrient limitation enhances TEP production (Kraus, 1997; Corzo *et al.*, 2000).

Particle sequestration during the aggregation experiments

In this study the potential of aggregate formation in the Baltic Sea was studied by producing artificial aggregates of natural phytoplankton on the roller table. The composition of aggregates that are formed from natural water samples on the roller table is quite similar to those formed *in situ* (Shanks & Edmondson, 1989). The roller table has therefore been appreciated as an alternative tool to study aggregates, their formation, composition and chemistry (Crocker & Passow, 1995; Engel & Schartau, 1999; Ploug & Grossart, 2000). Because the samples exposed to rotation on the roller table were enriched in net-plankton, the fraction of phytoplankton cells in the artificial aggregates may have been higher than in field aggregates. We therefore compared the phytoplankton composition of aggregates with enriched but not rotated control samples that were incubated under the same temperature and light conditions as the samples on the roller table. Although phytoplankton abundances in the controls were much higher than in the field samples the relative proportions of the major phytoplankton species, the ratios of the particulate organic elements C, N and P, and the ratio of TEP to CCP were quite similar. We therefore assume that the relative compositional changes that occurred during aggregate formation reflect natural processes even though the total amount of aggregates yielded during the roller table incubation was higher than *in situ*.

Aggregation of filamentous cyanobacteria has rarely been studied (Sellner *et al.*, 1988; Grossart, 1996), although floating aggregates have been reported as a final stage of filamentous cyanobacteria blooms. This study showed that the diazotrophic cyanobacteria *Aphanizomenon ' baltica '*, *Nodularia spumigena* and *Anabaena* spp. indeed coagulate and form aggregates with pelagic diatoms, such as *Chaetoceros* spp. and *Skeletonema costatum*. Different coagulation patterns for individual phytoplankton species were observed. The abundance of *Skeletonema costatum*, *Anabaena* spp. and *Nodularia spumigena* in aggregates depended on their initial concentration, but not on the bulk phytoplankton abundance, which indicates that these species coagulate specifically with themselves. While the relative abundance of *Aphanizomenon ' baltica '* in aggregates was lower than in the controls, yielding E_i-factors <1, *Nodularia spumigena* was highly enriched in aggregates. The cells of the latter species are covered by a mucus surface coating, which may render them stickier than the filaments of *Aphanizomenon ' baltica '*, which lack a mucus envelope. Coagulation of cyanobacteria with gravitationally

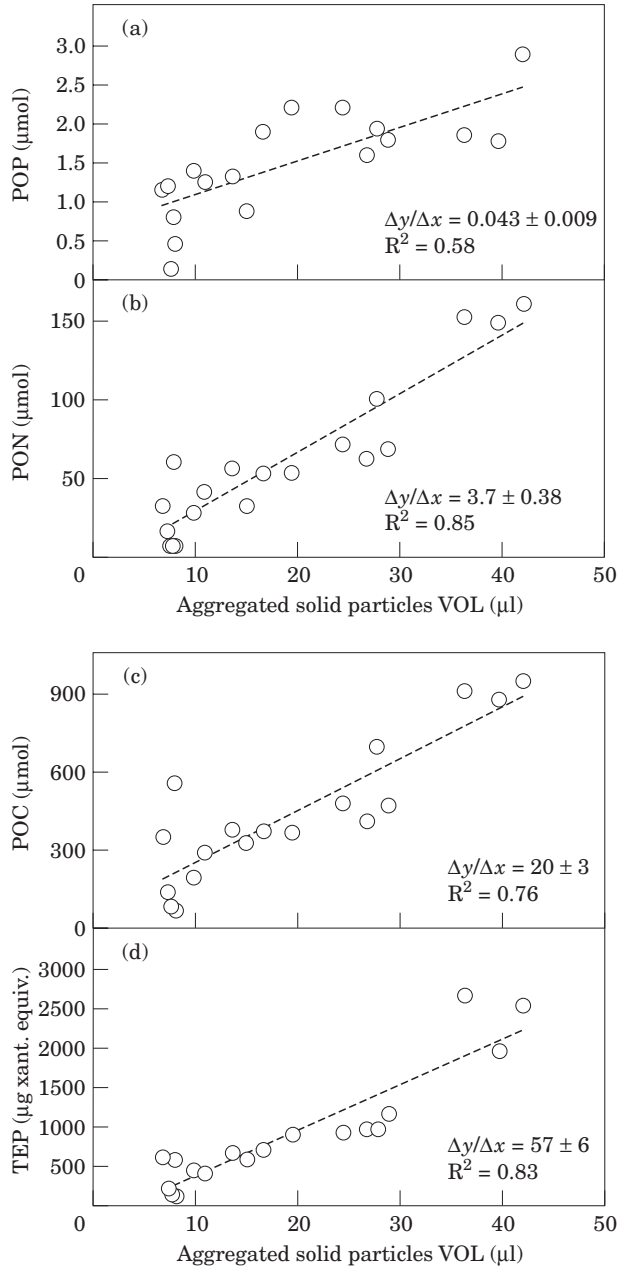

FIGURE 7 (a–d) Increase of particulate organic elements (POC, PON, POP) and TEP with the total volume of solid particles in aggregates.

settling algae will increase the sedimentation rate of cyanobacteria and may be responsible for pulsed sedimentation of filamentous cyanobacteria as observed by Wasmund *et al.* (1999).

The elemental composition of aggregates differed from the suspended particle fractions in the experiments and in the field. Relatively high background concentrations of carbon in aggregates (~32% of total POC) were inferred from regression analysis. Since this residual POC was independent of PON, it may represent carbon contained in refractory POM and TEP rather than carbon of living biota. TEP form abiotically from dissolved and colloidal exopolymeric carbohydrates (Mopper *et al.* 1995; Mari, 1999; Passow, 2000) and overlap to a certain, but unknown, degree with the POC pool. The C:N ratio of TEP is significantly higher than the Redfield ratio (Engel & Passow, 2001). The high ratio of TEP to conventional particles in aggregates may therefore explain the observed carbon enrichment.

During a filamentous cyanobacteria bloom in the Baltic Sea, Sörensen and Sahlsen (1987) observed an increase in the particulate C:N ratio below the thermocline. Calculating remineralization ratios Thomas *et al.* (1999) argued, that material sinking below the thermocline in the Baltic Sea must be enriched in carbon. In both publications the increase in C:N ratios with depth was attributed to the preferential degradation of nitrogen. This study indicates that sedimentation of carbon-enriched aggregates may be another factor responsible for the observed higher C:N ratios at depth. A preferential degradation of nitrogen within a short period (24 h) cannot be supported, neither for aggregated nor for suspended particles, because preferential degradation of PON would lead to an increase in the slope ΔPOC:ΔPON, which was not observed. This is consistent with the findings of Grossart and Ploug (2001), who observed that preferential degradation of PON and increase in POC:PON ratios within detrital diatom aggregates, formed during a similar experiments, did not occur until day five of the incubation. Concentration ratios of POC:POP in the field samples were higher than expected from Redfield's prediction whereas the slope of ΔPOC:ΔPOP was close by. This indicates that in fresh material the higher POC:POP ratios were due to enrichment of carbon and not to preferential degradation of the particulate phosphorus. In the aggregation experiments, where the samples were collected after 24 h of dark incubation, the slopes and the respective concentration ratios of POC:POP were more variable and higher than in the field samples. This was most pronounced for aggregates where a dramatic decrease of phosphorus as inferred from steep slopes (ΔPOC:ΔPOP) occurred. Degradation of POP is processed by the ectoenzyme alkaline phosphatase, which is released by several bacteria and phytoplankton species. Activities of alkaline phosphatase are higher in aggregates than in the suspended particles fraction (Smith *et al.* 1992, Grossart & Simon, 1998) and support a rapid remineralization of POP in aggregates. During this study the scaling relationship between visible volume and mass content

TABLE 3. Relationships between aggregate mass composition and volume (n=18). SV_{AG}: solid aggregate volume (µl), VV_{AG}: visible aggregate volume (µl), calculated with fractal dimensions (D3) of 1·4 and 1·9, respectively. ns: not significant

	POP (µg µl^{-1})		PON (µg µl^{-1})		POC (µg µl^{-1})		TEP (µg µl^{-1})	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
$y=a\ (SV_{AG})+b$	1·33	21	51·8	ns	240	ns	57	ns
$y=a\ (VV_{AG})^b$								
D3=1·4	0·13	0·33	0·07	0·54	3·3	0·42	5·3	0·40
D3=1·9	0·15	0·47	0·08	0·76	3·7	0·59	5·9	0·56
P<	0·05	—	0·001	—	0·005	—	0·005	—

TABLE 4. Concentration ratios and regression analysis of particulate organic elements in aggregates, surrounding seawater (SSW) and controls. (TEP:CCP): volume ratios of TEP and Coulter Counter detectable particles. Number of experiments was 18. ns: not significant

		Concentration ratio [y]/[x]		Regression analysis				
Fraction	Variables (y, x)	median	range	Slopes Δ[y]/Δ[x]	R^2	*P*<	y-intersect	% of y
SSW	POC:PON	6·2	5·6–7·0	5·4 ± 0·23	0·97	0·001	16 ± 5·0	13 ± 4
	POC:POP	135	63–173	129 ± 45	0·37	0·1	ns	—
	PON:POP	21·6	9·3–28	25 ± 7·7	0·41	0·05	ns	—
	TEP:CCP	2·3	0·9–11	ns	—	—	—	—
Control	POC:PON	6·1	5·6–8·1	5·1 ± 0·18	0·98	0·001	25 ± 4·5	19 ± 3
	POC:POP	143	63–169	139 ± 31	0·57	0·05	ns	—
	PON:POP	21·6	8·4–29	27 ± 5·8	0·59	0·05	ns	—
	TEP:CCP	2·5	0·6–11	ns	—	—	—	—
Aggregates	POC:PON	6·9	5·8–10·8	5·5 ± 0·3	0·96	0·001	85 ± 22	32 ± 8
	POC:POP	270	117–679	273 ± 72	0·47	0·05	ns	—
	PON:POP	37·7	14·2–84	52 ± 12	0·52	0·01	ns	—
	TEP:CCP	18	6·5–946	ns	—	—	—	—

indicated that aggregates, which formed on the roller table, were more densely packed than natural ones. Consequently activities of alkaline phosphatase may have been higher also and the degradation of POP faster than in natural aggregates. However, from an ecological point of view rapid solubilization of POP in aggregates would be advantageous in an N_2-fixing system because it retains phosphorus in the upper layer and sustains primary production.

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