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Carbon content and C:N ratio of transparent exopolymeric particles (TEP) produced by bubbling exudates of diatoms

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ABSTRACT: The carbon content of transparent exopolymeric particles (TEP) was measured in the laboratory in particles produced by bubbling exudates of the diatom *Thalassiosira weissflogii*, grown under nitrogen non-limited conditions (N:P = 7). The carbon content of these particles (TEP-C) appears to vary as a function of their size according to $TEP-C = 0.25 \times 10^{-6} r^{2.55}$ ($\mu\text{g C TEP}^{-1}$), where r is the equivalent spherical radius of the TEP particle (μm). This relationship implies that TEP are fractal aggregates having a fractal dimension $D = 2.55$. When this value was applied to historical TEP size spectra from a coastal area (Kattegat, Denmark), TEP carbon concentration in the surface mixed layer was on the order of $230 \pm 150 \mu\text{g C l}^{-1}$. This is high relative to other sources of particulate organic carbon (e.g. phytoplankton) and depending on TEP turnover rates, suggests that TEP is an important pathway for dissolved organic carbon in coastal seas. The carbon to nitrogen ratio of TEP was measured from particles formed by bubbling exudates of the diatoms *T. weissflogii*, *Skeletonema costatum*, *Chaetoceros neogracile* and *C. affinis*. Each of these diatom species was grown under various N:P ratios, from N-non-limited to N-limited conditions. While the C:N ratio of the diatom cells grown under N-limited conditions was high (C:N ≥ 14), the TEP aggregates formed by coagulation of the extra-cellular release produced by these cells exhibited a C:N ratio relatively constant (C:N = 7.3 ± 2.6) and apparently independent from that of the cells.

KEY WORDS: TEP · Carbon · C:N ratio · Bubbling

INTRODUCTION

The recent discovery of a new class of particles, transparent exopolymeric particles (TEP) (Alldredge et al. 1993), has challenged our understanding of the physical and biological aspects of pelagic processes. TEP, ranging in size from 1 to 100 μm or more in diameter, have been found in high concentration ($>5 \times 10^3 \text{ ml}^{-1}$) in various regions of the ocean (Alldredge et al. 1993, Passow & Alldredge 1994, Mari & Kiørboe 1996, Kiørboe et al. 1998) and even in freshwater (Worm & Søndergaard 1998). The use of a recently improved enumeration technique (Mari & Burd 1998)

showed that these particles may occur at concentrations 1 order of magnitude higher than those reported previously (i.e. $>5 \times 10^4 \text{ ml}^{-1}$) and that their volume fraction exceeded that of phytoplankton by about 2 orders of magnitude (i.e. from 3 to 310 ppm).

The potential importance of TEP in carbon fluxes in the pelagic zone are 2-fold: (1) TEP may enhance the vertical transport of substances by coagulation-sedimentation mechanisms (Jackson 1995, Logan et al. 1995) and, thus, explain the discrepancy between observed and predicted mass flocculation and subsequent sedimentation from cell-cell collision models (McCave 1984, Hill 1992); (2) TEP are believed to be formed by coagulation of dissolved or colloidal organic matter (Alldredge et al. 1993, Kiørboe & Hansen 1993, Mari & Burd 1998) and may be consumed by microphageous protozoans (Shimeta 1993, Tranvik et al.

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1993), by larvaceans (Flood et al. 1992) and even by copepods (Carman 1990, Decho & Moriarty 1990). They may, therefore, represent a significant pathway for dissolved organic carbon (DOC) in the ocean and represent an alternative to the microbial loop, so far considered the most important for the transfer of energy from the dissolved fraction back to the trophic food web. However, the significance of TEP in carbon fluxes depends closely on their composition and estimates of their carbon content are needed to evaluate the importance of this pathway.

It has been shown that the volume concentration of TEP (Mari & Burd 1998) and the concentration of dissolved organic matter (DOM) (Copin-Montégut & Avril 1993, Carlson et al. 1994, Williams 1995, Zweifel et al. 1995) have similar seasonal patterns, i.e. increase from the spring bloom to the end of the summer period. Furthermore, the turnover rates of TEP and rates of pelagic primary production also have similar seasonal patterns (Mari & Burd 1998). Since a large fraction of DOM in the ocean is exopolymeric material produced by phytoplankton (Painter 1983, Hoagland et al. 1993), this further suggests that the organic material released by phytoplankton cells represents the main source of TEP. Thus, it is to be expected that TEP and exopolymers will have similar compositions. Exopolymers are composed primarily of carbohydrates and, thus, TEP are likely to present a high carbon content.

However, the composition of TEP may vary somewhat with the phytoplankton species involved in leaking DOM (Decho 1990), and with external factors such as the concentration of inorganic nutrients (Mykkestad & Haug 1972, Mykkestad 1974, 1977). Furthermore, because TEP possess a large surface area for exchange reactions (Sutherland 1972) and possess high binding affinities for many dissolved compounds (Logan & Hunt 1987), they may act as sorption sites for solutes. Thus, while TEP are expected to be composed mainly of carbohydrates, the composition of TEP may be further influenced by the adsorption of dissolved and colloidal compounds, such as amino acids (Schuster et al. 1998) or metals (Niven et al. 1997).

The scope of this study was, thus: (1) to estimate the carbon content of TEP produced in the laboratory from diatom exudates, (2) to evaluate TEP C:N ratio under different nutritional conditions of the phytoplankton and (3) to discuss the implications of TEP for carbon fluxes.

MATERIALS AND METHODS

TEP production by bubbling. The bubbling method, used in previous studies to produce aggregates from DOM derived from macrophytes (Kepkay & Johnson

1988, Alber & Valiada 1994) or phytoplankton (Mopper et al. 1995), has been adapted in order to produce 'pure' TEP from exudates of diatoms (Mari & Kiørboe 1996).

Four different diatom species (i.e. *Thalassiosira weissflogii*, *Skeletonema costatum*, *Chaetoceros neogracile* and *C. affinis*), chosen for their ability to produce large amounts of extracellular polymeric substances, were grown in batch cultures on *f/2* media with silica (Guillard 1975) at constant illumination (ca 120 $\mu\text{E m}^{-2} \text{s}^{-1}$) and under different N:P ratios. The initial nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations in the culture medium are given in Table 1. For each bubbling experiment, diatom culture in the stationary growth phase (10 d old culture inoculated at ~10 ppm) was filtered at a low and constant vacuum pressure (<150 mbar) through 125 mm diameter, Whatman GF/C filters (average pore size = 1.2 μm). The filtrate was subsequently diluted 4 times with 0.22 μm filtered seawater. This solution was added to a bubble adsorption column of borosilica glass (100 cm high, 10 cm diameter), and bubbled with Ultra Zero Grade Air ($\text{CO} + \text{CO}_2 < 1 \text{ ppm}$, $\text{H}_2\text{O} < 5 \text{ ppm}$). The gas flow rate was 100 ml min^{-1} (Kepkay 1991). The flow rate was constant during and between bubbling experiments. Bubbles were produced by a glass frit of 10 to 20 μm pore size (Johnson et al. 1986). Since the glass frit used was not calibrated, the size range of the bubbles produced was unknown. The glass frit was fitted on-line with a 0.1 μm air filter (Millipak 200, Millipore) and a trap containing molecular sieves (5 Å, 45/60 Mesh) for removal of organic impurities and moisture. Between each experiment, the glass frit was soaked overnight in 35% HCl and was rinsed 3 times with distilled water prior to the setup of the bubbling column. Blanks were prepared by bubbling 0.22 μm filtered seawater.

Sampling. Samples for particulate organic carbon (POC) measurements were typically collected every 15 min during the first hour of bubbling and every hour thereafter until the end of the experiment. Samples for C:N ratio measurements were collected after 1, 3, 4 and 5 h of bubbling. All samples were collected in the middle of the column and were immediately filtered for TEP determination (see below), measurements of POC concentration and C:N ratio. Considering that the size distribution of TEP may vary as a function of the

Table 1 Initial inorganic nutrient concentrations used for the different diatom cultures

NO_3^- (μM)	PO_4^{3-} (μM)	N:P (atomic ratio)
85	170	0.5
170	170	1
340	170	2
1200	170	7

sampling depth in the column, different TEP size spectra could be observed for different sampling depths. However, this heterogeneity of distribution within the column does not cause any problem when estimating the TEP carbon content since it is calculated from the POC concentrations and the TEP size distribution measured at a given depth. A series of 6 aliquots of increasing volume, typically 2 to 20 ml for the POC measurements and 10 to 100 ml for the C:N ratio measurements, were immediately filtered onto 13 mm Whatman GF/F filters (average pore size = 0.7 μm) pre-combusted at 550°C for 2 h. Samples were filtered in a filtration unit consisting of a heavy steel funnel to hold the filter and a stainless steel support. After filtration the filters were placed in small petri dishes, dried at 60°C for 2 h and then frozen for later analysis. The effect of the nitrogen-limited conditions on the cell composition was studied by measuring the C:N ratio of diatom cells. We therefore filtered a series of aliquots of increasing volume (2, 3 and 5 ml) of each culture grown under N-limited conditions for C:N ratio determination. POC was determined on an InfraRed Gas Analyser (IRGA) and C:N ratio was determined on a Carbo-Erba CHN-analyser.

TEP determination. Slides of TEP were prepared largely following Passow et al. (1994) as follows. Several aliquots (2 to 10 ml) of each sample were filtered through 0.2 μm Nuclepore filters. TEP retained on the filter were stained with 500 μl of a solution of Alcian Blue. The TEP particles were then transferred to a microscope slide according to the Filter-Transfer-Freeze technique (Hewes & Holm-Hansen 1983). A minimum of 600 TEP were counted and sized on each slide at 3 successive magnifications (Mari & Burd 1998). The cross-sectional area of each TEP was measured by a semi-automatic image-analysis system, and its equivalent spherical diameter (ESD) was calculated. For each sample, counts from the 3 magnifications were combined and TEP were classified according to their ESD into 20 logarithmic size classes. TEP size distributions were described using a power relation of the type $dN/d(d_p) = kd_p^\delta$, where dN is the number of particles per unit volume in the size range d_p to $[d_p + d(d_p)]$ (e.g. McCave 1984). The constant k depends on the concentration of particles and the spectral slope, δ , describes the size distribution. Both constants were determined from regressions of $\log[dN/d(d_p)]$ versus $\log[d_p]$. Since the seawater passed through What-

man GF/C filter before bubbling, any particles that appeared during the bubbling period and after staining with Alcian Blue were operationally defined as TEP.

POC and PON measurements. Samples for POC measurements by IRGA were collected during 3 bubbling experiments conducted with the diatom *Thalassiosira weissflogii* grown under an N:P ratio of 7. TEP C:N ratio was determined from measurements of POC and particulate organic nitrogen (PON) by Carbo-Erba CHN-analyser, conducted with all diatom species using the different inorganic N:P ratios. The experimental protocol for the TEP carbon and nitrogen measurements is outlined in Fig. 1. The analytical principle for POC measurement by IRGA is a high temperature combustion at 600°C in a quartz combustion tube placed in a tube-furnace. The combustion was catalyzed by quartz wool with platinum powder and the CO_2 carried to an infrared gas analyser in an oxygen stream (Søndergaard & Middelboe 1993). Calibration was done with standard solutions of freshly prepared glucose added in small volumes (5 μl = 15 μg C) to a cold pre-combusted filter. The filter was shortly air dried before measurement. The concentrations of POC and PON

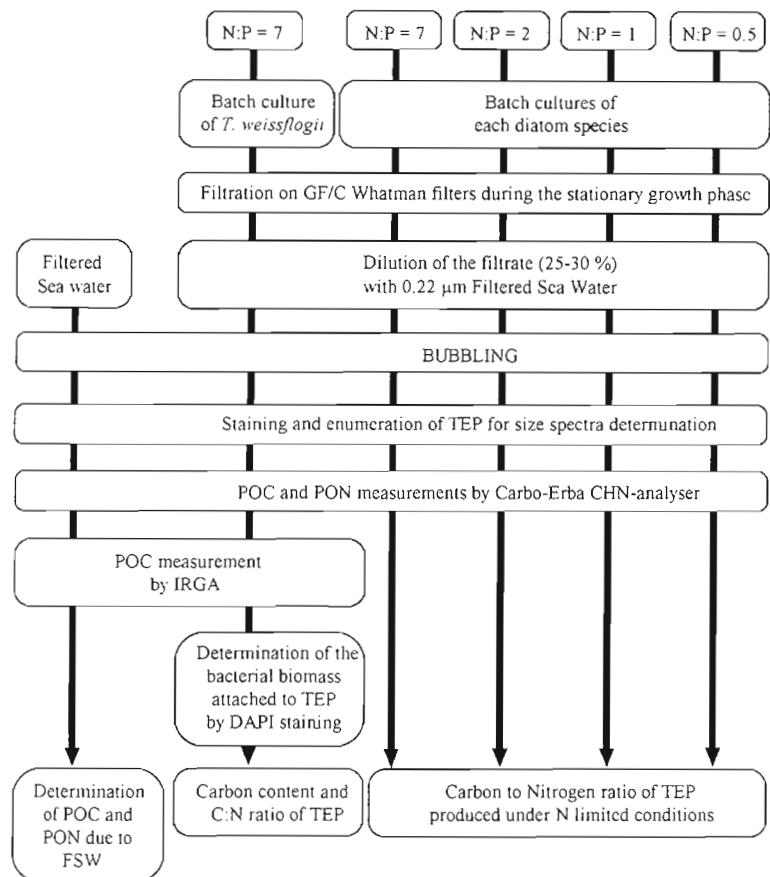


Fig. 1. Outline of experimental setup for determination of carbon content and C:N ratio of TEP produced from diatom exudates by surface coagulation

were calculated as the slopes of the regression lines of POC ($\mu\text{g C filter}^{-1}$) versus volume filtered (ml) and of PON ($\mu\text{g N filter}^{-1}$) versus volume filtered (ml).

Measurements of POC by CHN-analyser and IRGA were compared using filters coming from the same samples. As a result, for a given sample the POC measured by IRGA exceeded that measured by CHN-analyser according to the relationship $POC_{\text{IRGA}} = 1.377 POC_{\text{CHN}} - 0.003$ (Fig. 2), despite the same method of calibration for both types of measurement. This correction was applied only to the POC concentrations obtained from the CHN-analyser measurements used to estimate the TEP carbon content.

Bacterial biomass attached to TEP. The total bacterial abundance and the fraction of attached bacteria to TEP were calculated from the first bubbling experiment conducted with *Thalassiosira weissflogii* grown in a medium with N:P = 7. Total bacterial abundance was determined in 5 ml samples filtered onto 0.2 μm Nuclepore filters after staining with 0.1 $\mu\text{g ml}^{-1}$ 4',6'-diamidino-2-phenylindole (DAPI) (Porter & Feig 1980, King & Parker 1988). Bacteria were counted in 10 fields on each slide at 1000 \times magnification in an epifluorescence microscope. The number of bacteria attached to TEP was counted after double staining with DAPI and Alcian Blue (Passow & Alldredge 1994). All samples were prepared fresh. On each slide, 20 individual TEP were sized and their associated bacteria were enumerated by switching between UV and visible light. Since free bacteria can be retained above and beneath a TEP during the filtration, the number of free suspended bacteria likely to fall on or be retained by the filter beneath a TEP was calculated from: (1) the bacterial abundance, (2) the volume filtered and (3) the TEP size

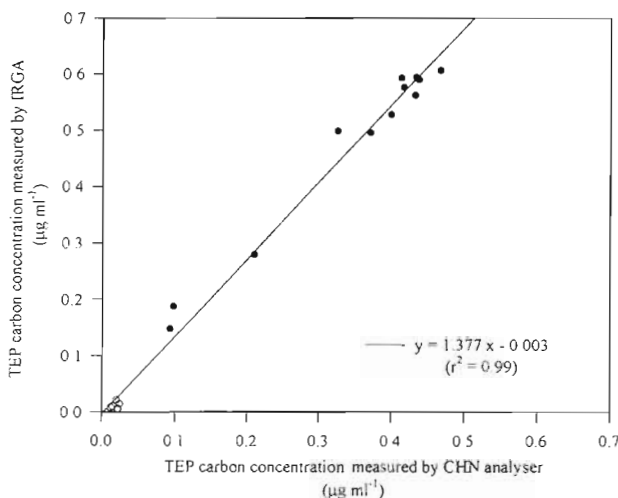


Fig. 2. Comparison between POC concentrations measured by IRGA and by CHN-analyser, for TEP produced from exudates of the diatom *Thalassiosira weissflogii* (●) and from filtered seawater (○)

spectra. This number was subtracted from the observed number of attached bacteria. The intrinsic TEP carbon content was obtained by subtracting the associated bacterial biomass, assuming a bacterial carbon content of 20 fg C cell⁻¹ (Lee & Fuhrman 1987). As a result, the bacterial biomass would add less than ~3% to the TEP carbon content, and was thus ignored.

TEP carbon content. Since TEP are fractal aggregates (Mari & Kjørboe 1996), the solid volume and consequently the carbon content of such particles are proportional to r^D , where r is the projected spherical encased radius of the TEP particle and D is the fractal dimension. Summed over all particle sizes, the total TEP carbon concentration (C) is given by the relationship:

$$C = a \sum_i n_i r_i^D$$

where n_i is the concentration of particles in size class i and a is a proportionality constant. a and D were estimated by least square method, i.e. by finding the values of a and D for which

$$SSD = \sum_j (C_j - a \sum_i n_{ij} r_i^D)^2$$

has minimum value. SSD is the sum of standard deviations, C_j is the TEP carbon concentration in sample j and n_{ij} is the concentration of size class i particles in sample j . The estimation of a and D allows the determination of the carbon content of a TEP particle as a function of its size.

RESULTS

Concentration and size distribution of TEP produced by bubbling

A few small TEP (<2 μm) were observed during the bubbling experiment conducted with only the 0.22 μm filtered seawater (blanks) and no large TEP were produced. This suggests that any TEP that occur during the bubbling experiments are the result of the coagulation of dissolved or colloidal organic material released by the cells.

During every bubbling experiments, TEP <10 μm ESD were recorded in relatively high concentration ($\sim 0.2 \times 10^5 \text{ ml}^{-1}$) but low volume concentration ($\sim 3 \text{ ppm}$), even before bubbling started (Fig. 3). This may be due to the flexible character of these particles which allows them to pass through the filter despite an ESD > pore size, and/or because coagulation occurred during handling and setup of the experiment. The power relation fitted the TEP size distributions very well at all sampling occasions and for all experiments, and as a result of the production of large TEP, the spectral slope, δ , of the TEP size distribution increased (Fig. 3).

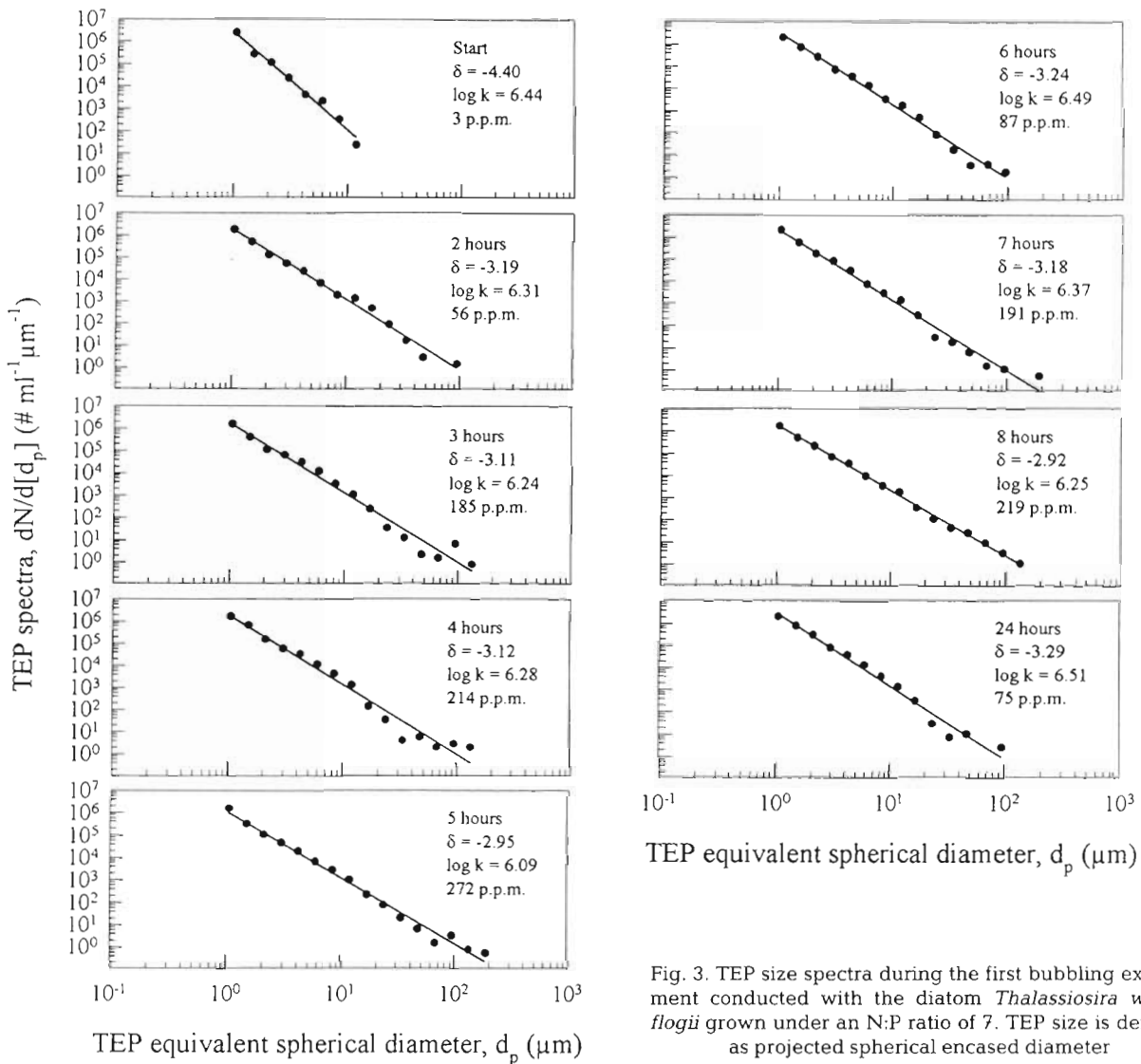


Fig. 3. TEP size spectra during the first bubbling experiment conducted with the diatom *Thalassiosira weissflogii* grown under an N:P ratio of 7. TEP size is defined as projected spherical encased diameter

The different bubbling experiments did not produce TEP with the same efficiency. As an example, the experiments conducted with the diatom *Thalassiosira weissflogii* grown under an N:P ratio of 7, produced from 10 to 60 ppm of TEP after 2 h of bubbling and from 15 to 270 ppm after 5 h (Fig. 4). For the first experiment with *T. weissflogii* (N:P = 7), TEP volume concentration increased from 3 to 270 ppm during the first 5 h and decreased thereafter to 75 ppm (24 h) (Fig. 5). While the observed increase was most likely due to formation of large TEP, the relative low volume fraction observed after 24 h of bubbling was either due to the settling of large TEP near the bottom or due to desegregation processes. Furthermore, the temporal pattern in POC concentration was closely related to the temporal pattern in TEP volume fraction (Fig. 5), suggesting that TEP contain a significant amount of

carbon and that they are the major component of the POC present in the column. The same correlation was observed for every bubbling experiment (data not shown).

Carbon content of TEP produced by bubbling

For the 4 bubbling experiments conducted with *Thalassiosira weissflogii* grown under an N:P ratio of 7, the TEP carbon content per volume of TEP (calculated as POC concentration/TEP volume fraction) decreased when δ increased, i.e. when large TEP were formed (Fig. 6). In other words, large TEP have a lower specific carbon content than smaller TEP. This pattern confirms that TEP are fractal aggregates. The estimate of a and D by the least square method gives $a = 0.25 \times 10^{-6}$

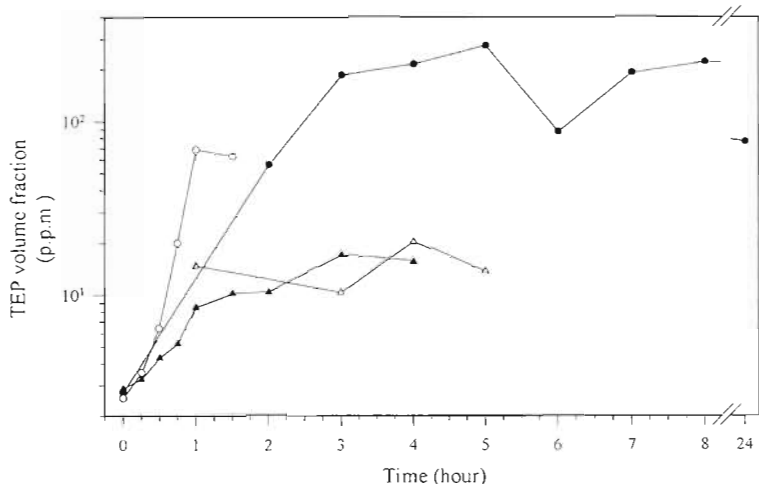


Fig. 4. Temporal variations of TEP volume concentrations observed during 4 bubbling experiments conducted with the diatom *Thalassiosira weissflogii* grown under an N:P ratio of 7. During (●), (▲) and (○), POC was measured by IRGA and during (▲) POC was measured by CHN-analyser

and $D = 2.55$. Thus, the carbon of a given TEP particle ($TEP-C$, $\mu\text{g C}$) with a radius r (μm) is given by:

$$TEP-C = 0.25 \times 10^{-6} r^{2.55}$$

which indicates a decrease in volume-specific carbon content for an increase in TEP size. As an example, a TEP with an ESD of $1 \mu\text{m}$ has a carbon content of $\sim 8 \times 10^{-8} \mu\text{g C } \mu\text{m}^{-3}$, while a TEP of $100 \mu\text{m}$ ESD has a carbon content of $\sim 10^{-8} \mu\text{g C } \mu\text{m}^{-3}$. Comparison between expected TEP carbon concentration from the

above relationship and observed POC concentration showed that the model explains about 80% of the observed carbon concentration for the experiments conducted with *T. weissflogii* (Fig. 7).

POC concentrations measured during the bubbling experiments conducted with the 3 other species of diatoms were slightly lower than, or similar to, those predicted from the size-carbon content relationship obtained for *Thalassiosira weissflogii*. The POC concentrations were between 45 and 135% of those predicted (Table 2). The low observed relative-to-predicted POC concentrations for the bubbling experiment conducted with *Chaetoceros neogracile* grown under an N:P ratio of 0.5 (~13%) is most likely an error due to a bad estimation of the POC concentration by the CHN-analyser, since the concentrations of POC during this experiment were very low (limit of sensitivity of the CHN-analyser). This experiment was thus ignored.

C:N ratio of TEP produced by bubbling

TEP produced from exudates of diatoms grown under N-limiting conditions had a relatively constant C:N ratio, between 5.9 and 9.2 (average = 7.3 ± 2.6) in accordance with the Redfield ratio (i.e. C:N = 106:16).

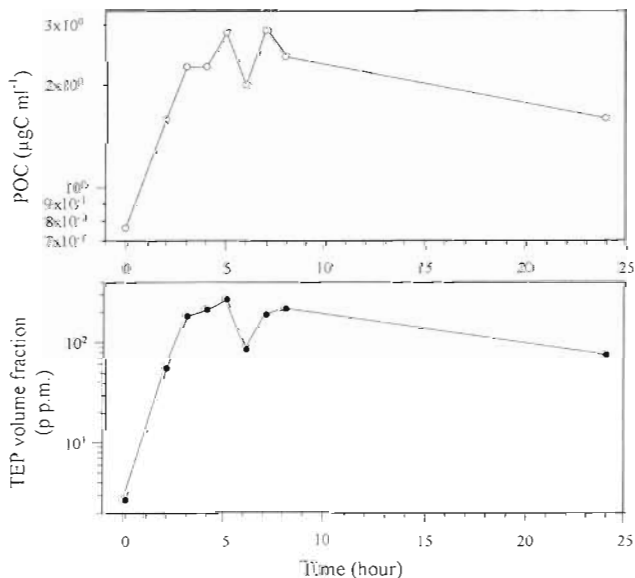


Fig. 5. Comparison between temporal variations of POC concentration (○) and TEP volume fraction (●) during the first bubbling experiment conducted with the diatom *Thalassiosira weissflogii* grown under an N:P ratio of 7

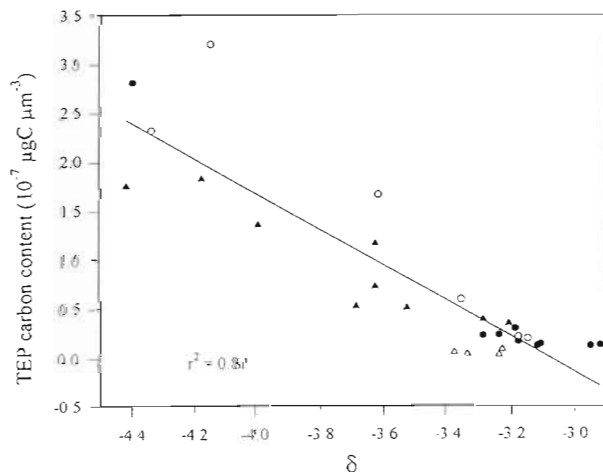


Fig. 6. Correlation between spectral slope of TEP size distribution, δ , and TEP carbon content per total volume of TEP, for 4 experiments conducted with the diatom *Thalassiosira weissflogii* grown under an N:P ratio of 7. Different experiments are distinguished by different symbols. In experiments shown by (●), (▲) and (○), POC was measured by IRGA and in the experiment shown as (▲) POC was measured by CHN-analyser and corrected with the relation $POC_{IRGA} = 1.377 POC_{CHN} - 0.003$

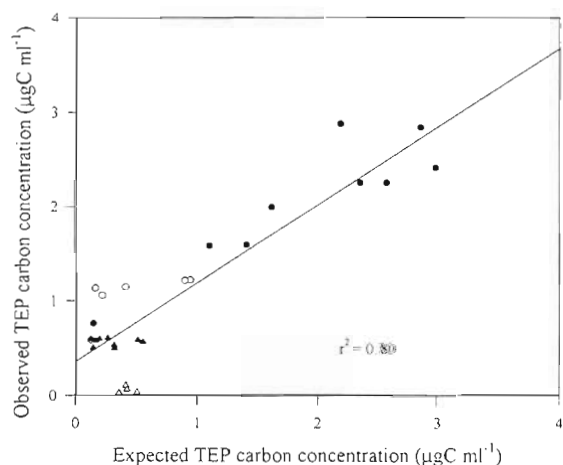


Fig. 7. Comparison between observed TEP carbon concentration and expected [from the relationship $TEP-C = 0.25 \times 10^{-6} r^{2.55}$ ($\mu\text{g C } \mu\text{m}^{-3}$) and the observed TEP size spectra] for the different experiments conducted with the diatom *Thalassiosira weissflogii* grown under an N:P ratio of 7. During (●), (▲) and (○), POC was measured by IRGA and during (△) POC was measured by CHN-analyser and corrected with the relation $POC_{IRGA} = 1.377 POC_{CHN} - 0.003$

In contrast, the diatom cells used to produce the TEP particles were characterized by a large deficit in nitrogen, i.e. the C:N ratio for cells increases with a decrease in inorganic nitrogen concentration of the growth medium ($C:N_{\text{average}} = 14.2 \pm 3.9$, 18.2 ± 4.5 and 21.9 ± 7.5 for N:P = 2, 1 and 0.5, respectively) (Fig. 8). This suggests either that the primary composition of TEP is not related to the inorganic nitrogen concentration of the medium and is independent of the C:N ratio of phytoplankton cells, or that TEP are capable of adsorbing and concentrating dissolved nitrogenous compounds present in the medium, after being formed.

DISCUSSION

Efficiency of the bubbling

The initial concentration in dissolved (DOM) or colloidal organic matter (COM) present in the column is supposed to control the efficiency of the TEP formation by bubbling, since it determines the frequency of collisions. Therefore, the bubbling experiments conducted utilizing the same diatom species and the same environmental conditions are expected to produce TEP with a similar efficiency, since the amount of DOM and COM released by the cells should be equivalent. However, it appears from the comparison of bubbling experiments conducted at similar conditions that the amounts of TEP produced differ significantly. Since the efficiency of the TEP formation by surface coagulation

Table 2. Comparison between POC concentrations observed during the bubbling experiments conducted with the diatom *Skeletonema costatum*, *Chaetoceros neogracile* and *C. affinis* with POC concentrations predicted from the relationship $TEP-C = 0.25 \times 10^{-6} r^{2.55}$

Initial N:P	POC observed/POC predicted		
	<i>S. costatum</i>	<i>C. neogracile</i>	<i>C. affinis</i>
7	0.49 ± 0.05	1.05 ± 0.25	0.45 ± 0.13
2	0.64 ± 0.08	0.86 ± 0.29	0.57 ± 0.17
1	1.35 ± 0.35	1.16 ± 0.27	0.63 ± 0.16
0.5	0.77 ± 0.48	0.13 ± 0.02	0.88 ± 0.20

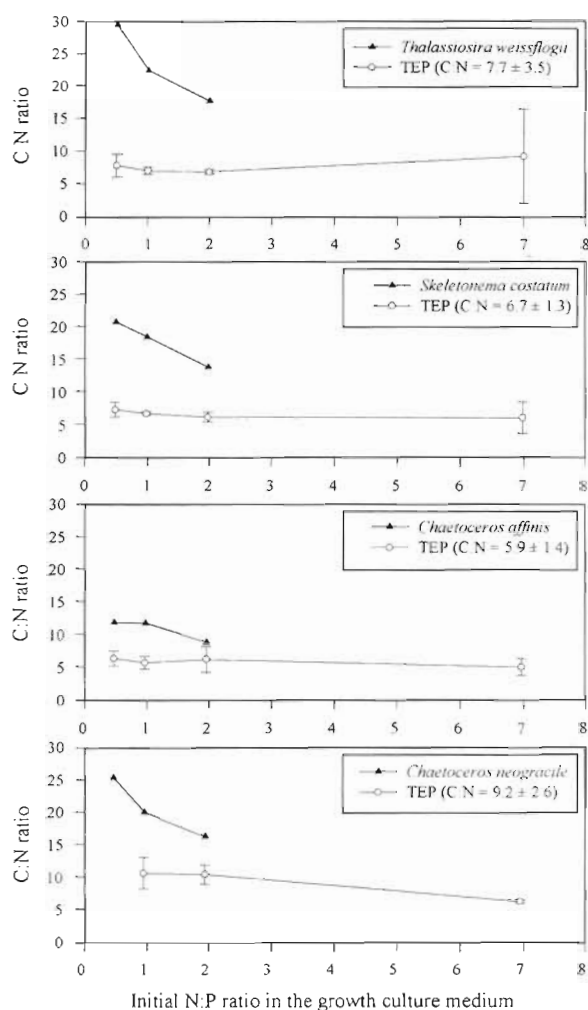


Fig. 8. Influence of inorganic nutrient concentrations in growth medium on C:N ratio of diatoms (▲) and TEP produced from the diatom exudates (○)

during the bubbling depends on collision and sticking mechanisms, the stickiness of the colloidal precursors of TEP may control TEP formation and integrity. The presence of EDTA (ethylenediamine tetraacetic acid)

in the growth medium may explain this variability in TEP formation efficiency. This strong chelating agent, commonly added in growth mediums to limit the flocculation of the culture, may decrease the coagulation efficiency by competing with sugars of adjacent polysaccharide polymers for divalent cations, such as Ca^{2+} or Mg^{2+} (Decho 1990). It has been demonstrated that the cation bridges giving rise to diatom aggregates and TEP were rapidly disrupted by adding EDTA to the solution (Alldredge et al. 1993). Therefore, the concentration of divalent cations in the bubbled solution might be too low to allow the formation of cation bridges and, thus, be responsible for the low efficiency of the bubbling.

Another factor that may control the efficiency of the bubbling is the size of the column. In the present study a 1 m bubbling column was used, which implies that colloids coagulated only on bubbles rising with mobile interfaces (Kepkay 1994). In contrast, bubbling experiments carried out with a 2 m column, as used in Kepkay & Johnson (1988), produced aggregates by coagulation on mobile bubble surfaces during the first meter and on immobile surfaces during the second meter. It appears that the size of the bubbling column may lead to the production of TEP with different structures and may control the efficiency of the bubbling. Although the relationship between the structure of TEP produced by bubbling and the size of the column should be investigated, the similarity of structure (same fractal dimension) and of size spectra observed for TEP produced by bubbling and for naturally occurring TEP suggests that the column used during the present study was efficient at producing TEP similar to naturally occurring TEP.

Finally, a potentially important factor that may control the efficiency of the bubbling is the alteration of the size of the bubbles. The mechanism that may modify the size of the bubbles is a reduction of the pore size of the glass frit caused by an accumulation of organic material in the frit. Despite having cleaned the glass frit with concentrated HCl, it is still possible that this method was not efficient enough to remove all the sorbed organics. Thus, considering the potentially major impact that an alteration of bubble size could have, this possibility should be acknowledged and considered for future work.

Sources and formation of TEP

It has been suggested that TEP are formed from DOM and that colloids coagulate to form larger and larger aggregates that eventually appear as TEP (Alldredge et al. 1993). Colloidal organic particles are the most abundant particles in the ocean (i.e. $>10^7 \text{ ml}^{-1}$;

Wells & Goldberg 1992). Thus, the formation of TEP in the ocean is a potentially important pathway for DOM and colloids. Since the first study of the DOM-to-POM conversion by Sutcliffe et al. (1963), this pathway has been intensively studied, and it is now acknowledged that non-living particles in the ocean can be formed from DOM and colloids (Batoosingh et al. 1969, Johnson et al. 1986, Kepkay 1991, 1994, Kepkay et al. 1993). While many studies have investigated the formation of 'conventional' particles from DOM and colloids, either due to shear coagulation (Kranck & Milligan 1988, Kepkay 1994, Passow et al. 1994) or to surface coagulation (Johnson et al. 1986, Kepkay et al. 1990, Kepkay 1991), only a few studies reported the formation of TEP from DOM coagulation.

Mopper et al. (1995) and Mari & Kjørboe (1996) demonstrated that TEP can be efficiently formed by bubbling solutions of filtered cultures of phytoplankton. In the present experiment, the bubbling method was inefficient at producing TEP from $0.22 \mu\text{m}$ filtered seawater, while TEP were produced copiously by bubbling solutions of diatom exudates filtered through $1.2 \mu\text{m}$ filters. Thus, it appears that the only possible source for TEP formation are the colloids released by phytoplankton cells. Although the formation of TEP by surface coagulation (aggregation of colloids at the air-sea interface, especially on rising bubbles; Johnson et al. 1986) is not supposed to be the major mechanism driving colloidal aggregation in the ocean and despite the other possible sources of DOM and colloids besides diatoms exudates, this experiment does demonstrate that TEP may be formed by coagulation of colloids and that diatoms may be one of the main sources of TEP precursors in the ocean.

TEP carbon content

The TEP carbon content estimated for TEP produced from exudates of the diatom *Thalassiosira weissflogii* varies as a function of the size of the TEP particle, as $\text{TEP-C} = 0.25 \times 10^{-6} r^{2.55} (\mu\text{g C TEP}^{-1})$. In comparison with marine phytoplankton (Fig. 9), TEP-specific carbon content is equivalent to about 10% of the phytoplankton-specific carbon content (Mullin et al. 1966, Strathmann 1967, Moal et al. 1987, Verity et al. 1992). Since phytoplankton cells contain on average ca 90% cellular water and about $10^{-7} \mu\text{g C } \mu\text{m}^{-3}$, and because TEP are supposed to contain 99% of 'bound' water (Sutherland 1972), a TEP carbon content on the order of $10^{-8} \mu\text{g C } \mu\text{m}^{-3}$ was expected. As for phytoplankton cells, the TEP carbon content decreases with an increase of TEP size (Fig. 9). For example, small procaryotic picoplankton cells contain between 1.5×10^{-7} and $5.5 \times 10^{-7} \mu\text{g C } \mu\text{m}^{-3}$ (Bratbak 1985, Lee & Fuhrman

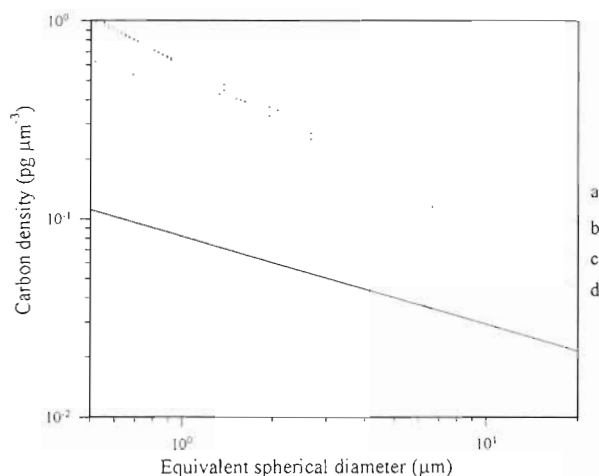


Fig. 9. Comparison between the carbon content of TEP (—) and of phytoplankton (.....) according to (a) Verity et al. (1992), (b) Moal et al. (1987), (c) Strathmann (1967) and (d) Mullin et al. (1966)

1987, Nagata & Watanabe 1990), while large ciliates contain between 0.9×10^{-7} and $1.9 \times 10^{-7} \mu\text{g C } \mu\text{m}^{-3}$ (Heinbokel 1978, Putt & Stoecker 1989). However, while the size-carbon relationship observed for TEP is due to the fractal nature of TEP aggregates, the carbon-size relationship observed for phytoplankton cells is due to differences in cellular water content and, thus, carbon density differences between taxa (Simon & Azam 1989, Verity et al. 1992).

The carbon-size relation implies that TEP have a fractal dimension of 2.55. During this study, the conservative characteristic of TEP aggregates used as a tracer of their solid volume was their carbon content, which one would expect to be directly proportional to the amount of polysaccharides they contain and, thus, represent the most reliable tracer of their solid volume used so far. In a previous study, Mari & Kjørboe (1996) estimated a TEP fractal dimension of ~ 1.5 , using the number of attached bacteria as a tracer of TEP solid volume. While attached bacteria may both multiply and degrade TEP material, bacteria cannot be considered conservative tracers. Thus, while the use of the number of attached bacteria to estimate TEP fractal dimension suggests that TEP are fractal aggregates, this approach does not allow a conclusion about their fractal dimension. In a previous study, Mari & Burd (1998) estimated TEP fractal dimension from field TEP size spectra and obtained a value similar to the one here. While TEP particles observed by Mari & Burd may not be formed by the same coagulation mechanisms as those occurring during the bubbling (surface coagulation), the similar fractal dimension obtained for 'natural' and 'artificial' TEP suggests that a fractal dimension of 2.55 is typical for TEP aggregates.

Carbon to nitrogen ratio of TEP

Since TEP produced during the bubbling experiments conducted for N-limiting conditions are formed from diatom exudates, the low C:N ratio of TEP (average = 7.3 ± 2.6) is inconsistent with the high C:N ratio of diatom cells (>14). Since it is most unlikely that severely N-limited cells leak exopolymers rich in nitrogen, the low C:N ratio of TEP measured may be due to an enrichment of TEP in nitrogenous compounds by adsorption mechanisms. The TEP formed from polysaccharides produced in large amount during N-limited conditions may act as efficient scavengers for charged molecules such as amino acids. This hypothesis is supported by the recent observation that polysaccharides, such as dextran, have high binding affinities for amino acids (Schuster et al. 1998). During this study, the authors showed that once amino acids are bound to dextran they are no longer easily accessible for bacteria. This suggests that, like dextran, TEP may efficiently scavenge nitrogenous compounds and, thus, explain the discrepancy between the C:N ratios of TEP and diatom cells. However, since there should not be much nitrogen left in the DOM fraction of the diatom culture in stationary growth phase, the most likely source of nitrogenous compounds for the proposed enrichment of TEP in nitrogen is the filtered seawater used to dilute the diatom culture filtrate prior to the bubbling.

One way to determine whether it is really the scavenging of nitrogenous compounds from the filtered seawater that causes the low C:N ratio is by repeating the experiment (TEP production by bubbling exudates of diatoms), but using artificial seawater (without any nitrate and amino acids) instead of filtered seawater. Thus, I attempted to examine the adsorption of nitrogenous compounds by TEP and their subsequent nitrogen enrichment, by comparing the C:N ratio of TEP produced from exudates coming from diatom species grown under N-limited conditions and diluted (1) with 0.22 μm filtered seawater and (2) with artificial seawater. Since the filtered seawater is likely to have a high concentration of dissolved amino acids and nitrate, the comparison with TEP produced by bubbling exudates solution diluted with artificial seawater may provide information on the potential role of TEP as scavengers for charged molecules such as amino acids.

Four batch cultures of the diatom *Thalassiosira weissflogii* were used to produce exudates under various N:P ratios, from N-non-limited to severely N-limited conditions (N:P = 7, 2, 1 and 0.5). For each experiment, the dense 10 d old culture was filtered (<150 mbar) through 125 mm diameter GF/C Whatman filters, and the filtrate split into 2 subsamples. The subsamples were diluted 4 times, one with 0.22 μm filtered sea-

water and one with artificial seawater. Both solutions were bubbled and sampled for TEP determination and C:N ratio measurements as above. As a result, the C:N ratio of TEP produced from exudates of *T. weissflogii* was relatively constant and was independent of the nature of the seawater used to dilute the culture filtrate (i.e. C:N = 6.7 ± 0.6 and C:N = 6.9 ± 1.2 for the filtrate diluted with artificial seawater and filtered seawater, respectively; data not shown). In contrast, the C:N ratio for the diatom cells used to produce the TEP particles increased with a decrease in inorganic nitrogen concentration of the growth medium (C:N = 9, 21, 30 and 35 for N:P = 7, 2, 1 and 0.5, respectively; data not shown). Since the filtered seawater used to dilute the diatom culture filtrate does not seem to be the source of the proposed enrichment of TEP in nitrogen, it is to be expected that this enrichment happens prior to the setup of the bubbling column.

One can construct a model in which TEP colloidal precursors may adsorb and concentrate dissolved nitrogenous compounds present in the growth medium shortly after being released from the cells. Thus, TEP formed by coagulation of these colloids rich in nitrogen may have a high nitrogen content despite the decrease in nitrogen in the DOM fraction of the growth medium.

TEP carbon concentration in the ocean

The use of the carbon-size relationship obtained for TEP produced in the laboratory by bubbling *Thalassiosira weissflogii* exudates to predict the carbon content of naturally occurring TEP presents 2 problems. First, TEP produced by bubbling do not necessarily have the same structure as that of natural TEP, due to the formation by different means. During the bubbling experiment, TEP are supposed to be formed by surface coagulation, while 'natural' TEP are supposed to be formed by coagulation processes controlled by various collision mechanisms (e.g. Brownian motion, differential sedimentation and shear). The mode of formation determines TEP structure and fractal dimension (Jiang & Logan 1991, Logan & Kilps 1995) and, thus, carbon content. Therefore, carbon content of field TEP and laboratory produced TEP may differ. However, the fractal dimension of field TEP, calculated from the field TEP size spectra (Mari & Burd 1998), and that estimated for laboratory produced TEP in the present study are similar. This suggests that 'natural'

and 'artificial' TEP have similar structures. Second, while TEP produced during the different bubbling experiments had a single source of dissolved and colloidal organic matter (i.e. polymers exuded by diatoms), the DOM and COM producing TEP in natural environment is likely to have multiple sources. Even though diatoms are one of the main components of marine phytoplankton and, thus, are supposed to produce a large amount of exopolymeric material (Painter 1983, Decho 1990, Hoagland et al. 1993), the colloidal precursors of TEP may have other origins. For example, it has been demonstrated that some flagellates can produce exopolymeric material which is able to coagulate and form TEP (Kjørboe & Hansen 1993), and it is to be expected that mucilaginous substances secreted by fish (Daniel 1981), corals (Ducklow & Mitchell 1979), appendicularians (Alldredge & Silver 1988), bacteria (Myklestad 1974) and macrophytes (Wilson et al. 1986) may also be sources of TEP in the ocean. The origin of TEP processors may constitute an important factor controlling the specific sugar composition of TEP and,

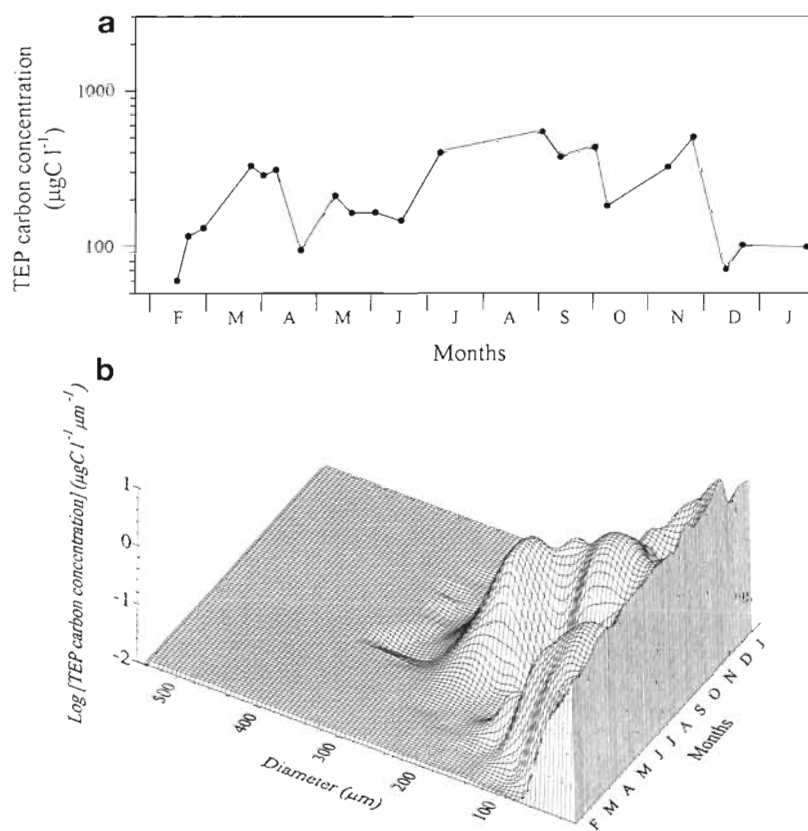


Fig. 10. Seasonal variations in the surface mixed layer of (a) TEP carbon concentration and of (b) TEP carbon concentration per size class. TEP carbon concentrations were calculated from the carbon-size relationship and field TEP size spectra. TEP size spectra were obtained from a field study in the Kattegat in 1995 (Mari & Burd 1998)

thus, it is possible that 'natural' TEP carbon content differs from that obtained for 'artificial' TEP.

However, since this study constitutes the first attempt to estimate TEP carbon content, it is worthwhile to apply the carbon-size relation obtained for TEP produced from *Thalassiosira weissflogii* exudates to field TEP size spectra in order to estimate the TEP carbon concentration in the ocean. During a study in a coastal sea (Kattegat, Denmark), Mari & Burd (1998) collected seasonal data on TEP size spectra and abundance during a complete annual cycle. During this field study, TEP concentration was high ($>0.5 \times 10^5 \text{ ml}^{-1}$) on all sampling occasions and at all sampling depths, increased subsequent to the spring bloom and remained high ($>2 \times 10^5 \text{ ml}^{-1}$) during the summer period until the end of the autumn bloom. The TEP volume fraction varied between 3 and 310 ppm, and increased on 2 occasions: during the spring bloom and during the summer period. The TEP carbon concentrations in the surface mixed layer estimated from the size-carbon content relation were typically $>100 \mu\text{g C l}^{-1}$, and around $230 \pm 150 \mu\text{g C l}^{-1}$ (Fig. 10a). An increase in TEP carbon concentration was observed during the summer period. This seasonal pattern was confirmed by the 3-dimensional representation of the seasonal variations of the spectral TEP carbon concentration (Fig. 10b).

During a study in an adjacent area in 1989, Olesen & Lundsgaard (1995) reported average concentrations of total POC of 680 and $330 \mu\text{g C l}^{-1}$, during the spring bloom and the summer period, respectively. They also estimated that the non-algal POC concentration was on the order of $250 \mu\text{g C l}^{-1}$ during the spring bloom, and on the order of $180 \mu\text{g C l}^{-1}$ during summer. Thus, the TEP carbon concentration estimated in the present study is similar to the non-algal POC reported by Olesen & Lundsgaard (1995) and is similar to the total POC recorded during summer. Despite uncertainties related to the application of the size-carbon content relation (obtained for TEP produced from *Thalassiosira weissflogii* exudates) to field data, this study suggests that TEP may represent a significant fraction of POC. Furthermore, since TEP turn over rapidly, between 0.1 to 1.0 d^{-1} (Mari & Burd 1998), the formation of TEP from dissolved or colloidal organic matter may represent an important pathway for DOC in the ocean.

While this study suggests that TEP produced in the laboratory from diatom exudates contain a significant amount of carbon, the application of the results obtained during this experiment to estimate field TEP carbon concentration presents several reservations. One possible way to obtain more realistic estimates of field TEP carbon content would be to measure the carbon content of TEP produced by bubbling 'natural' filtered seawater.

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LITERATURE CITED

- Alber M, Valiada I (1994) Production of microbial organic aggregates from macrophyte-derived dissolved organic material. *Limnol Oceanogr* 39:37–50
- Allan GG, Lewin J, Johnson PG (1972) Marine polymers. IV. Diatom polysaccharides. *Bot Mar* 15:102–108
- Allredge AL, Silver MW (1988) Characteristics, dynamics and significance of marine snow. *Prog Oceanogr* 20:41–82
- Allredge AL, Passow U, Logan BE (1993) The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Res* 40:1131–1140
- Amon RMW, Benner R (1994) Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature* 369:549–552
- Batoosingh E, Riley GA, Keshwar B (1969) An analysis of experimental methods for producing particulate organic matter in sea water by bubbling. *Deep-Sea Res* 16:213–219
- Benner R, Pakulski SD, McCarthy M, Hedges JI, Hatcher PG (1992) Bulk chemical characterization of dissolved organic matter in the ocean. *Science* 255:1561–1564
- Bjørnsen PK, Hansen L, Løkkegaard B, Berg L (1992) Bakterioplankton og bakteriegæsning. In: Fenchel T (ed) *Planktonodynamik og stofomsætning i Kattegat*. Miljøstyrelsen, Copenhagen, p 137–150
- Bratbak G (1985) Bacterial biovolume and biomass estimations. *Mar Environ Microbiol* 49:1488–1493
- Carlson CA, Ducklow HW, Michaels AF (1994) Annual flux of dissolved organic carbon from the euphotic zone in the northern Sargasso Sea. *Nature* 371:405–408
- Carman KR (1990) Mechanisms of uptake of radioactive labels by meiobenthic copepods during grazing experiments. *Mar Ecol Prog Ser* 68:71–83
- Copin-Montégut G, Avril B (1993) Vertical distribution and temporal variation of dissolved organic carbon in the north-west Mediterranean Sea. *Deep-Sea Res I* 40:1963–1972
- Decho AW (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanogr Mar Biol Annu Rev* 28:73–153
- Decho AW, Moriarty DJW (1990) Bacterial exopolymer utilization by a harpacticoid copepod: a methodology and results. *Limnol Oceanogr* 35:1039–1049
- Ducklow H, Mitchell R (1979) Compositions of mucus released by coral reef coelenterates. *Limnol Oceanogr* 24:706–714
- Fenchel T (1982) Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. *Mar Ecol Prog Ser* 9:35–42
- Flood PR, Deibel D, Morris CC (1992) Filtration of colloidal melanin from sea water by plankton tunicates. *Nature* 355:630–632
- Fowler SW, Knauer GA (1986) Role of large particles in the transport of elements and organic compounds through the oceanic water column. *Prog Oceanogr* 16:147–194
- Grasshof K (1976) *Methods of seawater analysis*. Verlag Chemie, Weinheim
- Guillard RL (1975) Culture of phytoplankton for feeding

- marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum Press, New York, p 29–60
- Hedges J (1987) Organic matter in sea water. *Nature* 330:205–206
- Heinbokel JF (1978) Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. *Mar Biol* 47:177–189
- Herndl GJ (1992) Marine snow in the northern Adriatic Sea: possible causes and consequences for a shallow ecosystem. *Mar Microb Food Webs* 6:149–172
- Hewes CD, Holm-Hansen O (1983) A method for recovering nanoplankton from filters for identification with the microscope: the filter-transfer-freeze (FTF) technique. *Limnol Oceanogr* 28:389–394
- Hill PS (1992) Reconciling aggregation theory with observed vertical fluxes following phytoplankton blooms. *J Geophys Res* 97:2295–2308
- Hoagland KD, Rosowski JR, Grez MR, Roemer SC (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry and physiology. *J Phycol* 29:537–566
- Jackson GA (1995) TEP and coagulation during a mesocosm experiment. *Deep-Sea Res II* 42:215–222
- Jiang Q, Logan BE (1991) Fractal dimensions of aggregates determined from steady-state size distributions. *Environ Sci Tech* 25:2031–2038
- Johnson BD, Zhou XL, Wangersky PJ (1986) Surface coagulation in seawater. *Neth J Sea Res* 20:201–210
- Keeling CD (1993) Surface ocean CO₂. In: Heimann M (ed) The global carbon cycle. Springer Verlag, Berlin, p 413–429
- Kepkay PE (1991) Surface coagulation and microbial respiration in response to local advection and sea state in the North Atlantic. *Mar Ecol Prog Ser* 69:143–147
- Kepkay PE (1994) Particle aggregation and biological reactivity of colloids. *Mar Ecol Prog Ser* 109:293–304
- Kepkay PE, Johnson BD (1988) Microbial response to organic particle generation by surface coagulation in sea-water. *Mar Ecol Prog Ser* 48:193–198
- Kepkay PE, Harrison WG, Irwin B (1990) Surface coagulation, microbial respiration and primary production in the Sargasso Sea. *Deep-Sea Res* 37:145–155
- Kepkay PE, Johnson BD (1989) Coagulation on bubbles allows the microbial respiration of oceanic dissolved organic carbon. *Nature* 385:63–65
- Kepkay PE, Niven SEH, Milligan TG (1993) Low molecular weight and colloidal DOC production during a phytoplankton bloom. *Mar Ecol Prog Ser* 100:233–244
- Kilps JR, Logan BE, Alldredge AL (1994) Fractal dimensions of marine snow determined from image analysis of *in situ* photographs. *Deep-Sea Res I* 41:1159–1169
- King LK, Parker BC (1988) A simple, rapid method for enumerating total viable and metabolically active bacteria in groundwater. *Appl Environ Microbiol* 54:1630–1631
- Kjørboe T, Hansen JLS (1993) Phytoplankton aggregate formation: observations of patterns and mechanisms of cell sticking and the significance of exopolymeric material. *J Plankton Res* 15:993–1018
- Kjørboe T, Nielsen TG (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. I. Copepods. *Limnol Oceanogr* 39:493–507
- Kjørboe T, Andersen KP, Dam HG (1990) Coagulation efficiency and aggregate formation in marine phytoplankton. *Mar Biol* 107:235–245
- Kjørboe T, Tiselius P, Mitchell-Innes B, Hansen JLS, Visser AW, Mari X (1998) Intensive aggregate formation but low vertical flux during an upwelling induced diatom bloom. *Limnol Oceanogr* 43:104–116
- Koike I, Hara S, Terauchi T, Kogure K (1990) Role of sub-micrometer particles in the ocean. *Nature* 345:242–244
- Kranck K, Milligan TG (1988) Macroflocs from diatoms: *in situ* photography of particles in Bedford Basin, Nova Scotia. *Mar Ecol Prog Ser* 44:183–189
- Lee SH, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Appl Environ Microbiol* 53:1298–1303
- Logan BE, Hunt JR (1987) Advantage for microbes of growth in permeable aggregates in marine systems. *Limnol Oceanogr* 32:1034–1048
- Logan BE, Wilkinson DB (1990) Fractal geometry of marine snow and other biological aggregates. *Limnol Oceanogr* 35:130–136
- Logan BE, Kilps JR (1995) Fractal dimensions of aggregates formed in different fluid mechanical environments. *Water Res* 29:443–453
- Logan BE, Passow U, Alldredge AL, Grossart HP, Simon M (1995) Rapid formation and sedimentation of large aggregates is predictable from coagulation rates (half-life) of transparent exopolymeric particles (TEP). *Deep-Sea Res* 42:203–214
- Mari X, Burd A (1998) Seasonal size spectra of transparent exopolymeric particles (TEP) in a coastal sea and comparison with those predicted using coagulation theory. *Mar Ecol Prog Ser* 163:63–76
- Mari X, Kjørboe T (1996) Abundance, size distribution and bacterial colonization of transparent exopolymeric particles (TEP) during spring in the Kattegat. *J Plankton Res* 18:969–986
- McCave IN (1984) Size spectra and aggregation of suspended particles in the deep ocean. *Deep-Sea Res* 31:329–352
- Moal J, Martin-Jezequel V, Harris RP, Samain JF, Poulet SA (1987) Interspecific and intraspecific variability of the chemical composition of marine phytoplankton. *Oceanol Acta* 10:339–346
- Mopper K, Zhou J, Sri Ramana K, Passow U, Dam HG, Drapeau DT (1995) The role of surface-active carbohydrates in the flocculation of a diatom bloom in a mesocosm. *Deep-Sea Res II* 42:47–73
- Morel FMM, Gschwend PM (1987) The role of colloids in the partitioning of solutes in natural waters. In: Stumm W (ed) Aquatic surface chemistry. John Wiley & Sons, New York, p 405–422
- Mullin MM, Sloan PR, Eppley RW (1966) Relationship between carbon content, cell volume, and area in phytoplankton. *Limnol Oceanogr* 11:307–311
- Mykkestad S (1974) Production of carbohydrates by marine planktonic diatoms. I. Comparison of nine different species in culture. *J Exp Mar Biol Ecol* 15:261–274
- Mykkestad S (1977) Production of carbohydrates by marine planktonic diatoms. II. Influence of the N/P ratio in the growth medium on the assimilation ratio, growth rate, and production of cellular and extracellular carbohydrates by *Chaetoceros affinis* var. *Willei* and *Skeletonema costatum*. *J Exp Mar Biol Ecol* 29:161–179
- Mykkestad S, Haug A (1972) Production of carbohydrates by the marine diatom *Chaetoceros affinis* var. *Willei*. I. Effect of the concentration of nutrients in the culture medium. *J Exp Mar Biol Ecol* 9:125–136
- Nagata T, Watanabe Y (1990) Carbon- and nitrogen-to-volume ratios of bacterioplankton grown under different nutritional conditions. *Appl Environ Microbiol* 56:1303–1309
- Niven SEH, Kepkay PE, Bugden JBC (1997) The role of TEP in ²³⁴Th scavenging during a coastal diatom bloom. *Radio-protection-Colloques* 32:213–490
- Norrmann B, Zweifel UL, Hopkinson CS, Fry B (1995) Produc-

- tion and utilization of dissolved organic carbon during an experimental bloom. *Limnol Oceanogr* 40:898–907
- Obernosterer I, Herndl GJ (1995) Phytoplankton extracellular release and bacterial growth: dependence on the inorganic N:P ratio. *Mar Ecol Prog Ser* 116:247–257
- Olesen M, Lundsgaard C (1995) Seasonal sedimentation of autochthonous material from the euphotic zone of a coastal system. *Estuar Coastal Shelf Sci* 41:475–490
- Painter TJ (1983) Algal polysaccharides. In: Aspinall GO (ed) *The polysaccharides*. Academic Press, London, p 196–285
- Parker BC, Diboll AG (1966) Alcian stains for histochemical localization of acid and sulfated polysaccharides in algae. *Phycologia* 6:37–46
- Passow U, Alldredge AL (1994) Distribution, size and bacterial colonization of transparent exopolymeric particles (TEP) in the ocean. *Mar Ecol Prog Ser* 113:185–198
- Passow U, Alldredge AL, Logan BE (1994) The role of particulate carbohydrate exudates in the flocculation of diatom blooms. *Deep-Sea Res I* 41:335–357
- Porter GK, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* 25:943–948
- Putt M, Stoecker DK (1989) An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. *Limnol Oceanogr* 34:1097–1103
- Sambrotto RN, Langdon C (1994) Water column dynamics of dissolved inorganic carbon (DIC), nitrogen and O₂ on Georges Bank during April, 1990. *Cont Shelf Res* 14:765–789
- Sambrotto RN, Savidge G, Robinson C, Boyd P, Takahashi T, Karl DM, Langdon C, Chipman D, Marra J, Codispoti L (1993) Elevated consumption of carbon relative to nitrogen in the surface ocean. *Nature* 363:248–250
- Schuster S, Herndl GJ (1995) Formation and significance of transparent exopolymeric particles in the northern Adriatic Sea. *Mar Ecol Prog Ser* 124:227–236
- Schuster S, Arrieta JM, Herndl GJ (1998) Adsorption of dissolved free amino acids on colloidal DOM enhances colloidal DOM utilization but reduces amino acid uptake by orders of magnitude in marine bacterioplankton. *Mar Ecol Prog Ser* 166:99–108
- Shimeta J (1993) Diffusional encounter of submicrometer particles and small cells by suspension feeders. *Limnol Oceanogr* 38:456–465
- Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Mar Ecol Prog Ser* 51:201–213
- Smith SV, Hollibaugh JT (1993) Coastal metabolism and the oceanic organic carbon balance. *Rev Geophys* 31:75–89
- Smith DC, Simon M, Alldredge AL, Azam F (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* 359:139–141
- Smith DC, Steward GF, Long RA, Azam F (1995) Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. *Deep-Sea Res II* 42:75–95
- Stachowitsch M, Fanuko N, Richter M (1990) Mucus aggregates in the Adriatic Sea: an overview of stages and occurrences. *Mar Ecol* 11:327–350
- Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol Oceanogr* 12:411–418
- Strickland JDH, Parsons TR (1972) *A practical handbook of seawater analysis*, 2nd edn. *Bull J Fish Res Bd Can* 167, p 310
- Sutcliffe WH, Baylor ER, Menzel DW (1963) Sea surface chemistry and Langmuir circulation. *Deep-Sea Res* 10:233–243
- Sutherland IW (1972) Bacterial exopolysaccharides. *Adv Microbiol Physiol* 8:143–213
- Søndergaard M, Middelboe M (1993) Measurements of particulate organic carbon: a note on the use of glass fiber (GF/F) and Anodisc filters. *Arch Hydrobiol* 127:73–85
- Toggweiler JR (1993) Carbon overconsumption. *Nature* 363:210–211
- Tranvik CJ, Sherr EB, Sherr BF (1993) Uptake and utilization of 'colloidal DOM' by heterotrophic flagellates in seawater. *Mar Ecol Prog Ser* 92:301–309
- Verity PG, Robertson CY, Tronzo CR, Andrews MG, Nelson JR, Sieracki ME (1992) Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol Oceanogr* 37:1434–1446
- Wells ML, Goldberg ED (1991) Occurrence of small colloids in sea water. *Nature* 353:342–344
- Wells ML, Goldberg ED (1992) Marine submicron particles. *Mar Chem* 40:5–18
- Wells ML, Goldberg ED (1993) Colloid aggregation in seawater. *Mar Chem* 41:353–358
- Wells ML, Goldberg ED (1994) The distribution of colloids in the North Atlantic and Southern Oceans. *Limnol Oceanogr* 39:286–302
- Williams PJLeB (1995) Evidence for the seasonal accumulation of carbon-rich dissolved organic material, its scale in comparison with changes in particulate material and the consequential effect on net C/N assimilation ratios. *Mar Chem* 51:17–29
- Wilson JO, Valiada I, Swain T (1986) Carbohydrate dynamics during decay of litter of *Spartina alterniflora*. *Mar Biol* 92:277–286
- Worm J, Søndergaard M (1998) Alcian Blue-stained particles in an eutrophic lake. *J Plankton Res* 20:179–186
- Zweifel UL, Wikner J, Hagström Å (1995) Dynamics of dissolved organic carbon in a coastal ecosystem. *Limnol Oceanogr* 40:299–305

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