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Direct measurement of diffusivity within diatom aggregates containing transparent exopolymer particles

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Abstract

We present the first direct measurements of apparent diffusivity within diatom aggregates using a diffusivity microsensor. Transparent exopolymer particles (TEP) and aggregate dry mass (TEP and cells) were determined in the same aggregates after diffusivity measurements. Carbon in TEP comprised 8–12% of aggregate dry mass. The (wet) volume fraction of TEP in aggregates, however, was on average 7.2-fold larger than that of cells, and it decreased with increasing aggregate size similar to that of cells. The exchangeable pore-water content occupied 87–98% of the aggregate volume. The average apparent diffusivities of gases within aggregates ranged between 0.90 and 0.95 times the free diffusion coefficient in seawater. Using a diffusion-reaction model, we analyzed silicic acid concentrations within marine snow. An apparent diffusivity of silicic acid within marine snow being 0.9 times its free diffusion coefficient in seawater, and a specific net silica dissolution rate of 0.002 h^{-1} could explain the observation that concentrations of silicic acid are significantly higher within marine snow compared to that of the ambient water.

Organic aggregates >0.5 mm comprise a significant component of suspended and sinking organic matter in lakes, rivers, and in the sea (Alldredge and Silver 1988; Simon et al. 2002). These aggregates, also known as marine snow in the sea, form mainly through coagulation of phytoplankton, detritus, and transparent exopolymer particles (TEP) in the euphotic zone (Kiørboe et al. 1994; Passow et al. 2001). Marine snow is characterized by high amounts of TEP that glue the particles together (Alldredge et al. 1993). The physical and chemical microenvironments, and thus the microbial growth conditions, within marine snow are significantly different from those of the bulk. Hydrolysis and respiration rates are high on marine snow (Smith et al. 1992; Ploug et al. 1999). Concentrations of dissolved organic carbon (DOC) (Alldredge 2000), inorganic nutrients (Brzezinski et al. 1997; Shanks and Trent 1979), and oxygen (Alldredge and Cohen 1987; Ploug et al. 1999) within marine snow are significantly different from those of the surrounding water. Hence, small-scale flow and diffusion within and around marine snow appear to be key processes determining coagulation and remineralization of particles in the ocean.

The observed concentration gradients of solutes associated with marine snow have led to speculations that diffusion may be slow within these aggregates (Shanks and Reeder 1993; Brzezinski et al. 1997; Alldredge 2000). The diffusive flux in a spherical aggregate is described by Fick's first law of diffusion:

$$J = \phi D_s \frac{dC}{dr} \quad (1)$$

where J is the flux, ϕ is the porosity, D_s is the effective diffusion coefficient of the solute, and dC/dr is the radial concentration gradient of the solute. The combined

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parameter ϕD_s is referred to as the apparent diffusivity. The effective diffusion coefficient is a function of the free diffusion coefficient in the surrounding water, D_0, the porosity, and the tortuosity. The tortuosity can be interpreted as the actual distance a molecule or an ion travels through an aggregate compared to that in water per unit length of the aggregate.

Here, we present the first direct measurements of apparent diffusivity in diatom aggregates using a diffusivity microsensor. We estimated the volume fractions of cells and TEP within the same aggregates after diffusivity measurements. Composition and apparent diffusivity were analyzed in aggregate of different sizes and age. Concentration gradients of silicic acid within and around diatom aggregates were analyzed using a diffusion-reaction model.

Materials and methods

Diatom aggregates—As a model of marine snow, we used diatom aggregates formed in roller tanks (diameter: 11 cm; height: 5.5 cm) rotating at 3 rpm during 1–8 d in darkness (Shanks and Edmondson 1989). The aggregates were composed of *Skeletonema costatum* grown in F/2-medium (Guillard and Rythe 1962) during 14 d in a 12 : 12 light : dark cycle at 16°C.

Apparent diffusivity—We used a diffusivity microsensor to directly determine apparent diffusivity (ϕD_s) within diatom aggregates. The microsensor principle is based on detection of a tracer gas diffusing away from the sensor tip (Revsbech et al. 1998). The diffusivity sensor used in the present study was based on H_2 as a tracer gas. Such sensors are very stable, which is prerequisite for high-resolution measurements. H_2 is not a completely biologically inert gas. Significant H_2 consumption, however, implies non–steady-state signals from the sensor as observed in microbial mats with high densities of algal and bacterial biomass (pers. obs.). Such signals were never observed in the present study. The microsensor was attached to a micromanipulator. The tip diameter was 100 μm, and its position was observed under a dissection microscope. Its current was measured by a picoammeter (PA2000; Unisense) connected to a strip chart recorder. The sensor was calibrated within the pore water of glass beads with diameters of 5–20 μm or of 40–60 μm, and in stagnant seawater (Revsbech et al. 1998). The signal of the free diffusion coefficient in seawater was measured in the stagnant boundary layer of water at the interface of a 2-cm-large piece of agar (1%). Measurements in the boundary layer of and within 1% agar was routinely used as a reference between measurements in aggregates. The aggregate was placed on a net, which allowed free diffusion in all dimensions during measurements (Ploug and Jørgensen 1999). The apparent diffusivity was measured at multiple positions within the aggregate under stagnant conditions. The average variation of values measured within the same aggregates was 1.4% (range: 0–4.4%). We analyzed >100 aggregates, and the same aggregates were sampled for dry weight or TEP analysis afterward. All measurements were done in a thermostated room at 16°C.

Aggregate dry weight and volume fraction of cells—The volume fraction of cells was measured in 70 aggregates. It was estimated from the aggregate dry weight and wet volume rather than by microscopy, which we found to be inaccurate. TEP comprises only ~10–15% of the dry mass in marine snow (Alldredge et al. 1998). The volume fraction occupied by diatom cells rather than by water or TEP in diatom aggregates formed from cultures may therefore be estimated by

$$F_{cells} = 0.86 \times \frac{W/\rho_s}{V} \quad (2)$$

where W is the dry weight, ρ_s is the density of the cells, and V is the aggregate volume including exchangeable pore-water, cells, and TEP. The factor 0.86 corresponds to the dry weight of cells corrected for TEP as measured in our study (see below). Aggregate wet volume was determined in a vertical flow system using a dissection microscope with a calibrated ocular micrometer scale (Ploug and Jørgensen 1999). The aggregate was turned during measurements, and its volume was calculated as for ellipsoids with three half axes (a, b, and c): $V = 4/3\pi abc$. The equivalent spherical diameter (ESD) was calculated as $ESD = 2x\ (V/(4/3\pi))^{1/3}$. For dry-weight measurements, aggregates were filtered individually onto preweighed 0.2-μm polycarbonate filters, quickly washed with distilled water, dried at 60°C, and reweighed. The sensitivity of the balance was 0.1 μg (Mettler Toledo UMX 2). The volume fraction of diatom cells was calculated from dry weight and aggregate volume (Eq. 2). The density of the diatoms was assumed to be 1.07 g cm^{-3} as estimated from Stokes law using published age-specific sizes and sinking velocities of single *S. costatum* cells (Smayda and Boleyn 1966). This density is relatively low, which gives maximum values of F_{cells}. The average value of F_{cells} was 0.009. This absolute value is low and relatively insensitive to the density of single cells when compared to the volume fraction of TEP (see below).

TEP—The volume fraction of TEP is difficult to measure by microscopy because of heterogeneity and asymmetric geometry. We therefore used the colorimetric method rather than microscopy to determine volume fractions of TEP in aggregates. Seventy aggregates with known sizes were gently filtered individually onto 0.2-μm polycarbonate filters. TEP were measured colorimetrically using the adsorption of Alcian Blue to the polysaccharide gum xanthan as a standard (Passow and Alldredge 1995). The carbon content of TEP (TEP-C) varies between 0.53 and 0.88 times the equivalent weight of gum xanthan (Engel and Passow 2001). In this study, we used the average value (0.70) to calculate TEP-C. The density of 5–80-μm-large TEP varies between 11 and 40 g C dm^{-3} (Mari 1999). The volume of TEP was here calculated as TEP-C divided by 17.4 g C dm^{-3}, which equals the carbon density of 30-μm-large TEP. The volume fraction of TEP, F_{TEP}, was calculated as the ratio of TEP volume to aggregate wet volume.

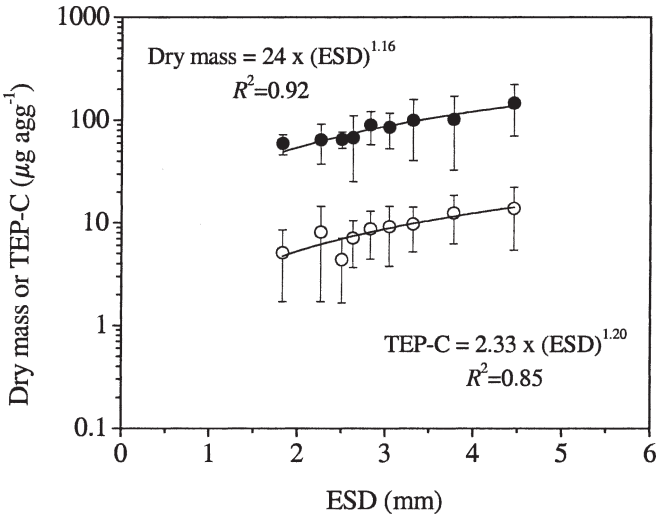

Fig. 1. Mass (closed symbols) and TEP content (open symbols) as a function of equivalent spherical diameter (ESD) of 1–8-d-old diatom aggregates. Each data point represents the mean value of 10–12 aggregates, and the error bars represent the standard deviation of the mean value.

Results and discussion

The average dry mass and carbon content of transparent exopolymer particles (TEP-C) measured in aggregates are shown (Fig. 1). TEP-C comprised on average 10% of aggregate dry mass. The distribution of dry mass in aggregates of different sizes was described by the power function: dry mass = 24 × (ESD)$^{1.16\pm013}$ (R^2 = 0.92), whereas that of TEP-C was described as TEP-C = 2.33 × (ESD)$^{1.20\pm0.20}$ (R^2 = 0.85), where dry mass and TEP-C are measured as μg agg^{-1} and the equivalent spherical diameter (ESD) is measured in mm. The relative distribution of dry mass in aggregates of different sizes—that is, the power:

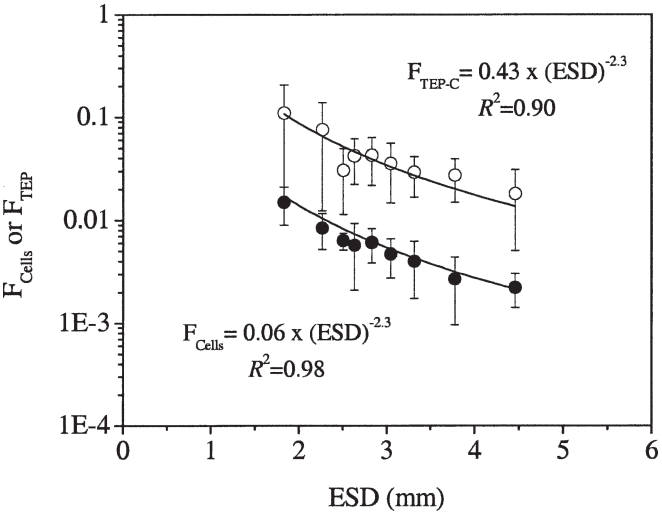

Fig. 2. Volume fraction of cells (closed symbols) and TEP (open symbols) as a function of equivalent spherical diameter (ESD) of 1–8-d-old diatom aggregates. Each data point represents the mean value of 10–12 aggregates, and the error bars represent the standard deviation of the mean value.

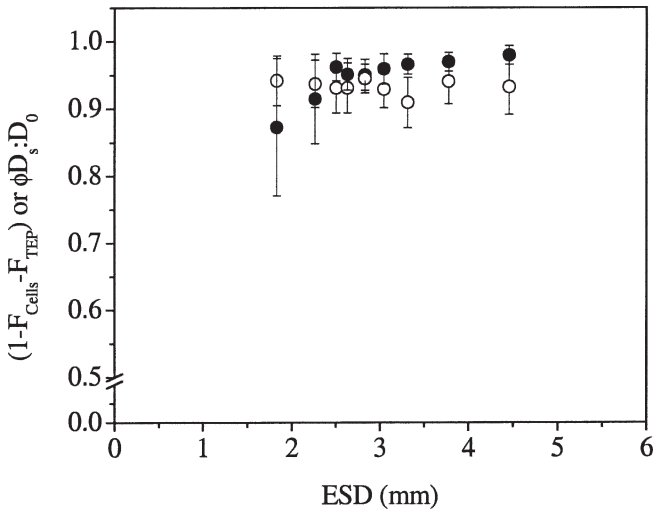

Fig. 3. Exchangeable pore-water fraction (1–F_{cells}–F_{TEP}) (closed symbols) and the apparent diffusivity relative to the free diffusion coefficient in seawater (open symbols) measured in the same aggregates as a function of equivalent spherical diameter (ESD) of 1–8-d-old diatom aggregates. Each data point represents the mean value of 10–12 aggregates, and the error bars represent the standard deviation of the mean value.

1.16 ± 0.13—was similar to that of TEP-C (1.20 ± 0.20). The relative distribution of dry mass in aggregates of different sizes has been used as an estimate of the fractal dimension of marine snow and other aggregates. The fractal dimension of marine snow (D_3) in general has been estimated to be 1.39 ± 0.15, whereas that of 7–20-mm-large diatom snow was 1.52 ± 0.19 (Logan and Wilkinson 1990). The fractal dimensions of our aggregates were thus also close to those of natural marine snow. The average size-specific aggregate dry mass, however, was 2.8 times larger than the average values of similar-sized marine snow (Alldredge and Gotschalk 1988). The size-specific density of marine snow varies approximately 10-fold, and the density of our diatom aggregates thus fell within the observational range of the more compact in situ aggregates (Alldredge and Gotschalk 1988). The average volume fractions of cells and TEP within aggregates are shown as a function of aggregate size (Fig. 2). The volume fraction occupied by cells was described by F_{cells} = 0.06 × (ESD)$^{-2.30}$ (R^2 = 0.98). The volume fraction of TEP in aggregates was on average 7.2-fold higher than that estimated for cells. Averaged values decreased with increasing aggregate size: F_{TEP} = 0.43 × (ESD)$^{-2.30}$ (R^2 = 0.90). We estimated the exchangeable pore-water content (porosity) in aggregates to equal (1 − F_{cells} − F_{TEP}). The values of the apparent diffusivity relative to the free diffusion coefficient in seawater were close to the values of the estimated exchangeable pore-water fraction in aggregates (Fig. 3). A close correlation between averaged values of the apparent diffusivity and pore water, however, was not observed. TEP is highly hydrated, and it consists of ~99% bound water by weight (Characklis and Gooksey 1983). The effective diffusion coefficients of oxygen and glucose (D_s) in agar and alginate gels, which also contain >95% bound water, are similar to their respective diffusion

Table 1. Physical characteristics of aggregates.

Age (d)	Number in sample	Diameter (mm)	F_{cells}	F_{TEP}	$\phi D_s : D_0$	Mass (μg agg^{-1})	TEP-C (μg C agg^{-1})	TEP-C : mass (μg : μg)
1 d*	19	3.6 ± 0.8	0.003 ± 0.004	0.013 ± 0.007	0.93 ± 0.04	65.8 ± 22.7	6.5 ± 3.5	0.10
3 d*	12	3.8 ± 1.8	0.009 ± 0.010	nd†	0.91 ± 0.05	182 ± 154	nd	nd
5–6 d*	18	2.9 ± 0.4	0.005 ± 0.003	0.022 ± 0.006	0.95 ± 0.01	64.2 ± 36.8	5.0 ± 2.1	0.08
8 d*	22	2.8 ± 0.5	0.004 ± 0.002	0.025 ± 0.008	0.90 ± 0.03	49.8 ± 22.6	4.2 ± 1.7	0.08
3–4 d	35	3.0 ± 0.6	0.008 ± 0.004	0.044 ± 0.040	0.95 ± 0.02	113 ± 43.6	11.5 ± 6.5	0.10
3–4 d	80	2.7 ± 0.6	0.009 ± 0.004	0.058 ± 0.049	nd	78.5 ± 28.6	9.5 ± 5.1	0.12

* Samples belong to the same time series.
† nd, not determined.

coefficients in water (Libicki et al. 1988). The apparent diffusivity in TEP may therefore also be close to that in water, and it can explain that the apparent diffusivity was relatively uniformly distributed within aggregates of different sizes.

The carbon and nitrogen content of diatom aggregates and the activities of attached bacteria change during the first week after their formation (Grossart and Ploug 2000). Data were, therefore, sorted by age to analyze if such changes are reflected in mass, TEP, and diffusivity. Average values of aggregate size and age, mass and TEP-C content, and apparent diffusivity relative to the free diffusion coefficient in seawater are shown in Table 1. The average volume fraction occupied by cells varied between 0.003 and 0.011, whereas that of TEP ranged between 0.013 and 0.058. The TEP-C : mass ratio (w : w) varied between 0.08 and 0.12. The averaged values of the apparent diffusivity in diatom aggregates ranged between 0.90 and 0.95 times the free diffusion coefficient in seawater. The aggregate composition and diffusion characteristics did not show any significant changes with the age of aggregates, but variations were large.

The high value of diffusivity of small molecules reported here compares well with values obtained for small as well as large molecules in biofilms with low biomass (cells and TEP) volume fractions (Libicki et al. 1988; Stewart 1998). Our measurements also confirm previous studies where fine-scale oxygen gradients measured within aggregates could be modeled assuming a diffusion coefficient of oxygen >90% of the free diffusion coefficient in seawater (Ploug et al. 1997). The effective diffusion coefficients (D_s) for small and large solutes relative to their respective values in seawater differ on average by ~10% in biofilms. Combining microinjection and optical microsensor techniques, the effective diffusion coefficient of large, charged solutes, such as HPTS (8-hydroxypyrene-1,3,6 trisulfonic acid), within diatom aggregates has been shown to equal the free diffusion coefficient in water (D. de Beer, unpubl. data). The effective diffusion coefficients of ionic species in biofilms relative to their free diffusion coefficients in water, however, can be 50–66% lower than those for small and large solutes (Libicki et al. 1988; Stewart 1998).

Steep concentration gradients of gases, nutrients, and other solutes in marine snow are sometimes interpreted as a reflection of low diffusivity of solutes within marine snow. Sampling of "pore water" in aggregates to measure concentrations of DOC, silicic acid, and other nutrients has been approximated by measuring total amount of the chemical species within an aggregate slurry subtracted by that in the bulk and divided by the aggregate volume (Shanks and Trent 1979; Brzezinski et al. 1997; Alldredge 2000). Sampling of "pore water" in aggregates, however, is complicated by the fact that concentration gradients not only occur inside the aggregate but also extend far into the surrounding water. The volume of water containing concentration gradients of solutes adjacent to sinking aggregates may be 100-fold larger than the aggregate itself (Kiørboe et al. 2001).

Using the approach of pore-water sampling as described above and assuming that concentration gradients do not change considerably within the first hours after sampling, it has been suggested that the diffusion coefficient of silicic acid in diatom aggregates may be up to two orders of magnitude lower than that in seawater (Brzezinski et al., 1997). Such a low diffusivity could be expected to lead to a high export of silicate and silicic acid from surface water into the deep ocean by sinking diatom aggregates. Silicic acid is dominated by its neutral form, $Si(OH)_4$, at pH <8.2 (Stumm and Morgan 1981), and its diffusion coefficient in seawater is 7.5×10^{-6} cm^2 s^{-1} at 15°C (Wollast and Garrels 1971; Li and Gregory 1974). The mean time for silicic acid to diffuse from the center toward the surface of a 3-mm-large aggregate can be calculated as $t = r_0^2/D = (0.15 \text{ cm})^2/(0.01 \times 7.5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1} \times 8{,}640 \text{ s d}^{-1}) =$ 3.4 d, if the diffusivity of silicic acid in the aggregate is only 0.01 times that in (stagnant) seawater. However, the diffusion time is 54 min only if the diffusivity of silicic acid is 0.9 times that in seawater as suggested by our study. Gradients will thus change considerably within an hour after sampling in the ocean, when the diffusivity inside aggregates is close to that in seawater. We used a diffusion-reaction model at steady state to examine theoretical concentration gradients of silicic acid within a stagnant diatom aggregate with a diffusivity of silicic acid of 0.9 times that in seawater (Ploug et al. 1997). This condition implies a maximum estimate of the concentrations within marine snow, and it resembles the hydrodynamic condition for an aggregate stored in a container (no sinking) 1–2 h after it has been collected in the ocean until processing in the laboratory. In the model, it is assumed that no sorption occurs and that the dissolution rate is evenly distributed within the aggregates. Radial distribution of silicic acid concentration within aggregates was calculated with a specific net dissolution rate of 0.002 h^{-1} (Bidle and

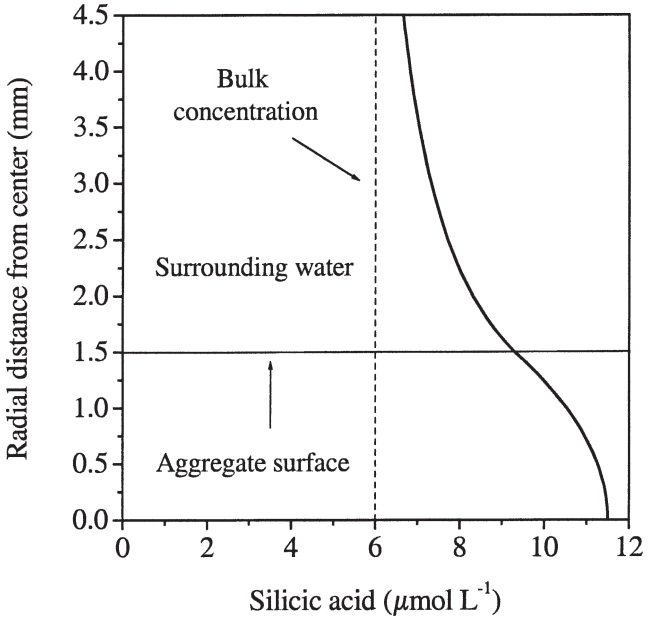

Fig. 4. Modeled distributions of silicic acid (full line) within and around a diatom aggregate. The concentration in the ambient water is indicated (dashed line). See text for details.

Azam 1999), an aggregate radius of 1.5 mm, an ambient silic acid concentration of 6 μmol L^{-1}, and a biogenic silicate content in the aggregate of 100 nmol agg^{-1} (Brzezinski et al. 1997). Calculated silicic acid concentrations ranged from 9.3 to 11.5 μmol L^{-1} within aggregates, and the gradients extended far into the surrounding water (Fig. 4). Assuming all silicic acid above the ambient concentration of 6 μmol L^{-1} within the 4.5-mm distance from the center ($3r_0$) to occur inside the aggregate results in an apparent concentration of 41 μmol L^{-1} inside the aggregate. To calculate this, we considered the aggregate and its surrounding water as an array of concentric shells:

$$C_\infty + \left(\frac{4}{3}\pi r_{sphere}^3\right)^{-1} \times \sum_{n=0}^{n=3r_a} 0.5 \times (C_{n+1} + C_n) \times \left(\frac{4}{3}\pi(r_{n+1}^3 - r_n^3)\right) \quad (3)$$

where r_{sphere} is the radius of the aggregate and C_n is the modeled concentration above that of the ambient water at the radial distance, r_n, and C_∞ is the bulk concentration. This apparent concentration within aggregates is 6.8-fold higher than ambient concentrations and similar to values estimated by Brzezinski et al. (1997). This value, however, is biased by the fact that the larger fraction of silicic acid occurs in the surrounding boundary layer rather than within the aggregate itself. A diffusion coefficient of silicic acid within aggregates close to that in seawater and a specific dissolution rate of 0.002 h^{-1} can thus also explain the apparent concentrations of silicic acid measured within diatom aggregates in slurries. The specific dissolution rate of silicic acid in diatom detritus varied between 0.001 and 0.008 h^{-1} (Bidle and Azam 1999). A higher dissolution rate or a lower apparent diffusion coefficient compared to the values used in our model implies proportionally steeper concentration gradients within aggregates. The efflux to the surrounding water, however, is the product of the apparent diffusivity and the concentration gradient of the chemical species at the surface (Eq. 1). A lower diffusion coefficient of a chemical species produced within aggregates does, therefore, not necessarily reduce the efflux to the surrounding water.

The present study demonstrates that the volume of diatom aggregates is dominated largely by water rather than by TEP or by cells. Apparent diffusivities close to that of (stagnant) seawater inside marine snow may be explained by the high water content of these aggregates. The size-specific dry weights of our aggregates were on average higher than the average value of similar-sized marine snow formed in the sea. The diffusivity of gases, nutrients, and other solutes in natural marine snow may thus be higher than in our model aggregates. Nutrient starvation would be greatly enhanced in aggregates dominated by living phytoplankton cells, and anoxic aggregates would be common in the sea if the apparent diffusivity of gases and nutrients in aggregates was 0.01 times that of the free diffusion coefficients in seawater only. Experimental results, however, have shown that aggregates seldom are anoxic unless they occur in oxygen minimum zones (Ploug et al. 1997, 1999; Ploug 2001). The diffusion time of oxygen in 3-mm aggregates is generally ~20 min (pers. obs.), which also implies that the diffusivity of oxygen within aggregates is close that in seawater (see above). We therefore trust our measurements also to be valid for other types of aggregates.

Concentration gradients of solutes develop at small scale where diffusion is fast and advective flow is slow. The mass transfer coefficient of diffusion, K, is described by the diffusion coefficient (D_0) divided by the boundary thickness at the aggregate–water interface. For oxygen, the mass transfer coefficient of diffusion across the boundary layer at the aggregate–water interface is $\sim 2.0 \times 10^{-5}$ cm^2 s^{-1} : 200×10^{-4} cm = 10 μm s^{-1} in 1–4-mm-large aggregates sinking >50 m d^{-1} at 20°C (Ploug 2001). Theoretical models have shown that advective flow in the order of 24–160 μm s^{-1} may occur within 7–20-mm-large, sinking diatom aggregates with a porosity ranging between 0.99939 and 0.99987 (Logan and Alldredge 1989). That study was performed before TEP was discovered within marine snow. Since then, it has been directly demonstrated that oxygen gradients occur within sinking aggregates and that mass transfer often appears to be dominated by diffusion within these aggregates (Ploug et al. 1999, 2002; Ploug 2001). Advective flow within aggregates thus appears to be very low or absent. Our study shows that the exchangeable pore-water content within diatom aggregates is considerably lower than the porosity estimated from the volume fraction of cells only because TEP occupies the larger fraction of the aggregate volume. A high-volume fraction of TEP may limit advective flow rather than diffusion rates within marine snow. This means that the presence of TEP may be prerequisite for concentration gradients of solutes to develop within marine snow.

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