Flow and diffusion around and within diatom aggregates: Effects of aggregate composition and shape

Eva-Maria Zetsche 1,2,3*, Ann I. Larsson, 4 Morten H. Iversen, 5,6 Helle Ploug 1

1Department of Marine Sciences, University of Gothenburg, Box 461, 40530 Gothenburg, Sweden
2Analytical, Environmental and Geo-Chemistry, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium
3Royal Netherlands Institute for Sea Research, PO Box 140, 4400 AC Yerseke, The Netherlands
4Department of Marine Sciences, Tjärnö Marine Laboratory, University of Gothenburg, Strömstad, Sweden
5Helmholtz Young Investigator Group SEAPUMP, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany
6MARUM, Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany

Abstract

Diatom aggregates constitute a significant fraction of the particle flux from the euphotic zone into the mesopelagic ocean as part of the ocean’s biological carbon pump. Modeling studies of their exchange processes with the surrounding water usually assume spherical shape and that aggregates are impermeable to flow. Using particle image velocimetry, we examined flow distributions around individual aggregates of various irregular shapes formed from two different diatom species: (1) Skeletonema marinoi, known for its cell-cell stickiness, and (2) Chaetoceros affinis, exhibiting cell-TEP (transparent exopolymeric particles) stickiness. Chaetoceros aggregates formed porous, highly irregularly shaped aggregates as compared to the more compact and near-spherical Skeletonema aggregates, yet flow distributions around both types of aggregates were relatively similar at a millimeter scale. At a micrometer scale, the irregular shape of diatom aggregates caused velocity gradients and vorticity close to the surface to locally vary more than for spherical model aggregates (agar-yeast spheres). Water was depleted from the surface of all aggregate types and we found no direct evidence that flow occurred within aggregates. Digital holographic imaging and Alcian blue staining revealed a substantial presence of TEP likely clogging the interstitial pore spaces in Chaetoceros aggregates. Radial oxygen concentration distributions measured by O2 microsensors within the aggregates were similar to those modeled for aggregates and spheres impermeable to flow. Thus, transport of gases, nutrients, and solutes likely occurs by diffusion, even within large, irregularly shaped diatom aggregates during sinking.

Diatoms are one of the most abundant types of phytoplankton and contribute approximately 20% of the earth’s primary production (Nelson et al. 1995). Diatoms often follow a bloom-and-bust cycle with blooms terminated by the formation of fast-sinking aggregates (>0.5 mm) (Alldredge and Gotschalk 1988). They constitute a significant fraction of the particle flux from the euphotic zone into the mesopelagic ocean as part of the “biological carbon pump” (Volk and Hoffert 1985; Turner 2015). This particle export from the upper ocean to greater depths and the seafloor not only redistributes carbon and nutrients in the ocean, but plays a major role in the uptake of atmospheric carbon dioxide (CO2) by the ocean (Volk and Hoffert 1985; Bergkvist et al. 2018). The vertical carbon flux attenuates greatly in the ocean’s twilight zone and the fate of carbon in sinking aggregates is an ongoing research topic (Sanders et al. 2016; Siegel et al. 2016).

Aggregates sinking through the water column scavenge other particles and are at the same time remineralized by colonizing bacteria and protozoa (Simon et al. (2002) and references therein); they function as “hotspots” of microbially driven remineralization (Grossart and Ploug 2001; Grossart et al. 2007; Bižić-Ionescu et al. 2018). Aggregate-associated bacteria are often present in concentrations two to three orders of magnitude higher than that in the ambient water (Simon et al. 2002; Thiele et al. 2015) and appear to function fundamentally differently to the communities living in the ambient seawater (Delong et al. 1993; Moeseneder et al. 2001; Thiele et al. 2015). Microbes in aggregates solubilize and remineralize particulate organic

*Correspondence: eva-maria.zetsche@uantwerpen.be

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Present address: Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
matter (POM) releasing carbon dioxide as well as dissolved organic matter (DOM) and nutrients (Smith et al. 1992; Ploug and Bergkvist 2015; Bergkvist et al. 2018). Total carbon turnover in sinking aggregates is dependent on the influx and efflux of oxygen, nutrients, and DOM to and from the aggregate as determined by flow, reaction rate, and molecular diffusion as well as aggregate properties such as porosity, composition and presence of transparent exopolymeric particles (TEP), among others (Moradi et al. 2018). Under O2 saturation in the ambient water, efficient mass transfer by diffusion across the aggregate-water interface has been demonstrated using O2 microsensors (Smith et al. 1992; Ploug et al. 1997; Ploug and Jørgensen 1999; Ploug et al. 1999a). In oxygen minimum zones, however, O2 respiration within aggregates potentially becomes diffusion limited, and diatom aggregates can be microenvironments hosting various anaerobic N-transformation processes (Ploug 2001; Ploug and Bergkvist 2015; Stief et al. 2016).

Diatom aggregates have been extensively studied over the past decades with regard to a wide range of biological, chemical, and physical studies (e.g., Burd and Jackson 2009; Turner 2015). They are well known to be highly porous with porosities >99.9% (Alldredge and Gotschalk 1988; Iversen and Ploug 2010), where porosity defines the fraction of volume that is not diatom cells. A high porosity, however, does not necessarily imply a high permeability as pore spaces may in fact be occupied by TEP or are too small to admit flow through the aggregate interior (Ploug and Passow 2007). The volume fraction of TEP in diatom aggregates can be >90%. Consequently, TEP can affect aggregate hydrodynamics in such a way that aggregates containing high amounts of TEP may, in fact, behave like spheres impermeable to flow (Ploug and Passow 2007).

Model studies of mass transfer to and from sinking aggregates furthermore assume a spherical geometry of aggregates (Logan and Alldredge 1989; Kiorboe et al. 2001; Moradi et al. 2018). Many diatom aggregates, however, show irregular geometry (Alldredge and Gotschalk 1988). Few empirical investigations of the flow distribution around sinking diatom aggregates with irregular shapes have been reported (Ploug and Jørgensen 1999). This study investigated how flow distributions are affected by shape irregularities but also by the presence of TEP. We used particle image velocimetry (PIV) combined with a vertical flow system to measure flow fields around two different types of diatom aggregates: (1) dense, compact aggregates formed from the species *Skeletonema marinoi* and (2) large, flocculent aggregates formed from the species *Chaetoceros affinis*, as well as around (3) agar spheres as reference aggregates. Aggregate characteristics, including radial oxygen concentration distributions and the presence of TEP using holographic imaging and Alcian blue staining were determined for all diatom aggregates. PIV was used to study the flow distributions around sinking marine aggregates at high spatial resolution.

### Materials and methods

Diatom and model aggregates were produced in the laboratory and studied in a vertical flow chamber (Fig. 1) described in full detail in Ploug and Jørgensen (1999). In brief, the flow chamber was a 10 cm high plexiglass tube (5 cm diameter) with a net extended in the middle. Fluid flow was supplied from below and a disperser was positioned at the inlet to the lower chamber below the net. The net created a laminar flow that was relatively uniform across the upper chamber from 1 mm above the net at flow velocities >1.0 mm s⁻¹ as documented by PIV in Ploug and Jørgensen (1999). The chamber was positioned in an aquarium filled with the same water as that used for the roller-tank incubations (0.2 μm filtered seawater at 17°C with a salinity of 31). The aquarium water was maintained at a constant temperature of 17°C using a Julabo F12-ED Refrigerated/Heating Circulator (Germany). The flow chamber was used in combination with PIV. We characterized aggregates with respect to size, sinking velocity, dry weight, TEP content, and radial oxygen distribution. The experiment was carried out in a thermostated room at 17°C.

**Fig. 1** Setup of a vertical flow chamber depicting the approximate positioning of the profiling microelectrode used for measuring oxygen concentrations, the needle occasionally used for stabilization during particle image velocimetry (PIV) measurements as well as the position of an aggregate approximately one diameter above the net. The PIV laser sheet is projected into the chamber from behind the flow chamber centered on the aggregate, and the PIV camera is facing the laser sheet from the right-hand side (modified from Ploug and Jørgensen (1999)).
Diatom aggregates

Two cultures were used: (1) *S. marinoi* (North Sea) and (2) *C. affinis* (North Sea), and are hereafter referred to as *Skeletonema* and *Chaetoceros*, respectively. Both cultures were grown at 16°C in 0.2 μm filtered seawater (salinity of 31) enriched with nutrients according to f/2 medium (Guillard 1975). The f/2 medium used for the diatoms was enriched with silicate at a molar ratio of nitrate to silicate of 2.9. The cultures were kept under a light:dark cycle (12:12 h) until they were harvested and incubated in 1.4 L Plexiglas cylinders (roller tanks) to form aggregates (Shanks and Edmondson 1989). Roller tank incubations were carried out in order to obtain aggregates formed with *Skeletonema* and *Chaetoceros* at a cell concentration of 9 × 10^6 cells L^-1 and at two higher cell concentrations of approx. 20 × 10^6 and 30 × 10^6 cells L^-1. The roller tanks were rotated on a rolling table at 1.5 rpm in the dark at 17°C.

Model aggregates

Model agar-yeast aggregates were made according to Cronenberg (1994). In brief, a suspension of baker’s yeast in 2% (w/w) agar was made in seawater and left to cool to 40°C, before it was dripped into a beaker filled with seawater and a thin layer of paraffin oil using a glass Pasteur pipette. Each drop formed an almost perfect agar-yeast sphere within the paraffin oil layer before settling into the sea water. Spheres were subsequently rinsed and stored for up to a few days in seawater. These nearly spherical, oxygen-consuming agar-yeast aggregates with diameters between 2 and 6 mm are impermeable to flow due to their small pore sizes in the range of 100–200 nm as known for such agarose gels (Narayanan et al. 2006). Thus, these agar-yeast aggregates were used as references of spheres impermeable to flow. During PIV measurements, they were fixed in the flow chamber by a thin glass needle (< 0.5 mm width). Different Reynolds numbers were achieved by changing the flow rate through the vertical flow chamber.

Aggregate dry weight

Aggregates were dried in a desiccator for 3 h before weighing on a Sartorius scale manufactured to ISO9001 standards with a sensitivity of 0.1 μg. All dry weights were made as triplicate measurements of each filter, both empty and with aggregates on.

Excess density of aggregates

Apparent excess density (Δρ) of the aggregates was calculated using the Navier–Stokes drag equation:

\[ Δρ = \frac{C_D \rho_f U^2}{\frac{4}{3} \pi ESD} \]

where \( C_D \) is the dimensionless drag force defined in Eq. (2), \( ρ_f \) is the density of sea water (1.0225 g cm\(^{-3}\), at 17°C and salinity 31), \( U \) is the measured sinking velocity in cm s\(^{-1}\), and \( g \) is the

**Fig. 2** Measured sinking velocities as a function of equivalent spherical diameter (ESD) for aggregates formed at different cell concentrations of *Skeletonema marinoi* and *Chaetoceros affinis*. 

**Size measurements**

Agar-yeast spheres with various diameters, and diatom aggregates with spherical to ellipsoid shapes with various sizes and surface topographies were selected for measurements. Aggregate size was measured as equivalent spherical diameter (ESD). For this, maximum lengths of two perpendicular axes of each aggregate were extracted from images of the PIV recording (see later). Aggregate volume was calculated by assuming an ellipsoid shape \( V = \frac{4}{3} \pi abc \) with the two measured axes \( a,b \) and the average of both for the third axis \( c \), which was then used to calculate ESD = 2(\( V / [4/3\pi] \))\(^{1/3}\).

**Sinking velocity**

The sinking velocity of single aggregates was measured in the vertical flow system (Fig. 1). Individual aggregates were gently transferred from the roller tanks to the chamber using a wide-bore pipette. Flow was adjusted using a needle valve until the aggregate remained suspended at the point where the sinking velocity of the aggregate was balanced by the upward-directed seawater flow velocity, approximately one aggregate diameter above the net of the flow chamber (Ploug et al. 2010). This was well beyond the distance from the net where micro-jets and micro-wakes occur (0-0.5 mm) and the PIV recordings are not influenced by the presence of the net as previously shown (Ploug and Jørgensen 1999; Kiørboe et al. 2001). The sinking velocity of an aggregate was calculated by dividing the volumetric flow rate by the cross-sectional area of the chamber (Ploug et al. 2010). Triplicate measurements were made for each aggregate.
gravitational acceleration of 981 cm s$^{-2}$, and ESD the equivalent spherical diameter in cm. $C_D$ was calculated using the drag equation for Reynolds numbers ($Re$) given by White (2006):

$$C_D = \frac{24}{Re} + \frac{6}{1 + Re^{0.5}} + 0.4 \quad 0 < Re \leq 2 \times 10^5$$

(2)

where $Re$ was defined as:

$$Re = \frac{Ud}{v}$$

(3)

where $d$ was the aggregate diameter and $v$ the kinematic viscosity of seawater ($1.1281 \times 10^{-2}$ cm$^2$ s$^{-1}$ at 17°C and salinity 31). $Re$ expresses the relative importance of inertial and viscous forces acting in the vicinity of a sinking particle, in this case our aggregates.

**Solid matter density and cellular volume fraction of aggregates**

The solid matter density, $\rho_s$, was calculated from the excess density ($\Delta \rho$), the dry mass ($DW$) and volume ($V$) of individual aggregates (Alldredge and Gotschalk 1988):

$$\Delta \rho = \frac{DW}{V} \times \left(1 - \frac{\rho_f}{\rho_s}\right)$$

(4)

The cellular volume fraction of aggregates was subsequently calculated according to Alldredge and Gotschalk (1988):

$$\text{cellular volume fraction} = \frac{DW/\rho_s}{V}$$

(5)

**Velocity field measurements**

Flow around the diatom and model aggregates suspended in the vertical flow chamber was studied using PIV, a technique enabling detailed spatial information to investigate the laminar flow around sinking aggregates. When aggregates were stable in their natural suspended state at least one diameter above the net within the chamber, they were sustained in their position with a thin glass needle (<0.5 mm diameter; Fig. 1) from the back to ensure stability during the PIV measurements, particularly with respect to the fragile *Chaetoceros* aggregates. Such support needles have been used in previous studies and do not produce measurable interference with the flow and diffusion fields around aggregates as compared to theoretical distributions of flow and concentrations gradients (Kiørboe et al. 2001; Ploug et al. 2002). The water was seeded with 4 μm nylon particles (TSI Incorporated, USA) and the flow chamber was illuminated from the side with a 1 mm thick laser light sheet (continuous red light diode laser, Coherent F-system, 10 W). The laser light sheet illuminated the center of each aggregate, and an area of 27 x 20 mm around the aggregate was studied. The aggregates shaded the area opposite to the laser light source and for dense particles flow around the aggregate could only fully be studied at the illuminated side. The 1600 x 1200 pixel PIV camera (Imager PRO X, LaVision) with a 50-mm Nikkor lens was placed in front of the flow chamber and recorded 1000 images per aggregate in the single frame mode at 20 Hz. Images were analyzed with the DaVis 8.2.0 software (LaVision) using time-series cross correlation and the multipass option with a final interrogation window size of 24 x 24, or, where possible, 16 x 16 pixels for higher resolution. An overlap of 50% between interrogation windows resulted in a resolution of 5–7 flow vectors mm$^{-1}$ depending on the final interrogation window size. From the resulting instantaneous vector fields, average flow fields were produced from which velocity profiles upstream, downstream, and at the equator of each aggregate were extracted in triplicate. Off-the-equator parallel profiles were extracted perpendicular to the flow direction, while for up- and down-stream profiles streamlines were followed from the pole regions in their respective directions. This allowed us to accommodate any deviations from perfectly vertical flow especially in the downstream region. The effect of aggregate shape on velocity gradients around the aggregates was further examined using the operation extract scalar field: strain in DaVis 8.2.0. For each interrogation window the horizontal shear rate ($dV_y/dx$) and the vertical shear rate ($dV_x/dy$) was calculated, where $V$ is the flow velocity in the horizontal ($x$) and vertical ($y$) direction, respectively. The total resultant shear rate along the surface of the aggregates was then approximated by taking the square root of the sum of squared shear. Furthermore, the vorticity fields around aggregates were calculated using the operation extract scalar field: rotation and shear (vorticity-z) in DaVis 8.2.0.

**Oxygen measurements**

Oxygen gradients at the aggregate-water interface and within the aggregates were measured using a Clark-type oxygen microelectrode with a tip diameter < 10 μm (Revbsbech 1989) mounted in a micromanipulator and calibrated at air saturation and under anoxic conditions (Unisense, Denmark). The electrode current was measured on a picoamperemeter (Unisense, PA2000) and read on a strip chart recorder (Kipp and Zonen) at high resolution (2 μM O$_2$ cm$^{-3}$). The 90% response time of the electrode was <1 s, and the stirring sensitivity < 0.3%. During O$_2$-measurements, aggregates were either freely suspended above the net in the vertical flow chamber or fixed with a thin glass needle during measurements. Fluid motion and solute distribution in the vicinity of the aggregates under these experimental conditions are equivalent to those in the vicinity of an aggregate sinking through the water column at a velocity equal to the water flow velocity (Kiørboe et al. 2001; Moradi et al. 2018). All measurements were done at steady state of the oxygen gradients. Oxygen distributions around and within aggregates were analyzed relative to those
predicted for aggregates impermeable to flow using an analytical diffusion–reaction model (Ploug et al. 1997). In this model, the theoretical O₂ distribution within a sphere is calculated as a function of aggregate size, apparent diffusivity, diffusive boundary layer thickness, and O₂ respiration rate. For the measured aggregate size, and the diffusive boundary layer thickness, the O₂ respiration rate can be modeled using an Excel fitting routine of measured O₂ concentration distribution, and a measured or assumed apparent diffusivity within aggregates. In the present study, we assumed an apparent diffusivity of O₂ within aggregates of 1.73 × 10⁻⁵ cm² s⁻¹ at a salinity of 30 and 17°C as measured previously in diatom aggregates (Broecker and Peng 1974; Ploug and Passow 2007). This model shows similar O₂ distributions in aggregates impermeable to the transmitted light are generated by refractive index changes between the object and the surrounding medium and thus reveals differences in optical path length (OPL), therefore allowing the visualization even of apparently “transparent” substances such as exopolymers and their quantification.

**Imaging of aggregates**

**Holographic imaging**

A differential digital holographic microscope (oLine D³HM, Ovizio Imaging Systems NV/SA, Belgium) was used to visualize diatom aggregate structure. Details of the instrument and its imaging capability are provided in Zetsche et al. (2014, 2016b). A 4x Olympus objective was used as well as a 40x Leica objective for closer observations. Aggregates were gently placed into a custom-made glass-bottom dish (5 cm × 5 cm with a well depth of 5 mm) that did not alter the structure of the aggregates while they were imaged. Holograms record both the intensity information as well as phase information when the microscope’s light beam (red LED light source [630 nm]) is passing through the objects of interest (e.g., aggregates, diatom cells, etc.). Differences in the phase of the transmitted light are generated by refractive index changes between the object and the surrounding medium and thus reveals differences in optical path length (OPL), therefore allowing the visualization even of apparently “transparent” substances such as exopolymers and their quantification.

**Alician blue staining**

Diatom aggregates were gently placed onto 0.4 μm polycarbonate filters on a filter stand, rinsed with Milli-Q water and stained with a 0.02% Alician blue solution in 0.06% acetic acid to detect the presence of TEP (Engel 2009). Staining took ~5 s before aggregates were rinsed twice gently with Milli-Q water. Filters were then placed on a drop of immersion oil on Cyctoclear slides (Sterlitech Corporation, USA), another drop of oil was added and a coverslip placed on top of the filter (Logan et al. 1994). Filters were imaged with an Olympus light microscope (BX-51) at various magnifications (4x, 10x, and 100x with oil immersion) and images captured with an attached Canon EOS 600D camera.

**Table 1.** Cell concentrations (in cells L⁻¹) used in the roller tanks to form aggregates are given, along with size (in equivalent spherical diameter (ESD)), dry weight (DW), dry weight per aggregate volume as well as calculated cellular volume fraction, apparent excess density, solid matter density, Reynolds (Re) number, the drag coefficient (C_D), and sinking velocity of the aggregates formed from *Skeletonema marinoi* and *Chaetoceros affinis*.

| Aggregate type and ID | Cell concentration (× 10⁶ cells L⁻¹) | ESD (mm) | DW (μg) | DW vol⁻¹ (in μg mm⁻³) | Cellular volume fraction | Apparent excess density (g cm⁻³) | Solid matter density (g cm⁻³) | Re | C_D | Sinking velocity (m d⁻¹) |
|----------------------|--------------------------------------|---------|---------|-----------------------|--------------------------|---------------------------------|-------------------------------|----|-----|------------------------|
| **Chaetoceros**      |                                      |         |         |                       |                          |                                 |                               |    |     |                        |
| Ch1                  | 9                                    | 4.5     | 87.2    | 1.8                   | 0.002                    | 3.72 × 10⁻⁵                  | 1.0415                        | 1.2 | 22.9 | 25.5                   |
| Ch2                  | 9                                    | 4.5     | 152.7   | 3.1                   | 0.003                    | 2.15 × 10⁻⁴                  | 1.0948                        | 5.5 | 6.5  | 114.9                  |
| Ch3                  | 9                                    | 4.8     | 879.4   | 14.8                  | 0.014                    | 1.74 × 10⁻⁴                  | 1.0323                        | 5.6 | 6.6  | 106.7                  |
| Ch4                  | 9                                    | 4.0     | 224.9   | 6.6                   | 0.006                    | 2.91 × 10⁻⁴                  | 1.0670                        | 5.2 | 6.8  | 124.0                  |
| Ch5                  | 9                                    | 2.8     | 490.3   | 44.7                  | 0.044                    | 1.72 × 10⁻⁴                  | 1.0241                        | 1.2 | 21.9 | 43.9                   |
| Ch6                  | 20                                   | 6.7     | 93.4    | 0.6                   | 0.001                    | 8.73 × 10⁻⁵                  | 1.1936                        | 6.9 | 5.6  | 96.3                   |
| Ch7                  | 20                                   | 6.1     | 218     | 1.8                   | 0.002                    | 9.15 × 10⁻⁵                  | 1.0741                        | 5.9 | 6.3  | 88.6                   |
| **Skeletonema**      |                                      |         |         |                       |                          |                                 |                               |    |     |                        |
| Sk1                  | 30                                   | 3.2     | 439.1   | 26.7                  | 0.025                    | 7.57 × 10⁻⁴                  | 1.0499                        | 6.2 | 5.9  | 190.1                  |
| Sk2                  | 30                                   | 2.7     | 56.9    | 5.3                   | 0.004                    | 1.67 × 10⁻³                  | 1.4892                        | 8.2 | 4.8  | 292.0                  |
| Sk3                  | 30                                   | 2.0     | 62.6    | 14.4                  | 0.012                    | 1.90 × 10⁻³                  | 1.1749                        | 4.6 | 7.6  | 212.5                  |
| Sk4                  | 20                                   | 3.5     | 190.4   | 8.3                   | 0.007                    | 1.57×10⁻³                   | 1.2577                        | 13.9 | 3.3  | 385.3                  |
| Sk5                  | 20                                   | 3.7     | 297.8   | 11.4                  | 0.01                     | 9.88×10⁻⁴                   | 1.1170                        | 11.1 | 3.9  | 287.3                  |
| Sk6                  | 20                                   | 2.8     | 580.9   | 49.8                  | 0.047                    | 1.88×10⁻³                   | 1.0601                        | 9.8 | 4.3  | 331.8                  |
| Sk7                  | 20                                   | 2.4     | 166.3   | 22.8                  | 0.019                    | 3.25×10⁻³                   | 1.1897                        | 10.2 | 4.1  | 412.1                  |
Statistical analysis

Differences between aggregate measurements were statistically analyzed using the statistical analysis routines incorporated into SigmaPlot 14.0 (Systat Software Inc., USA) with a significance level of $p < 0.05$. Where assumptions of normality and/or homogeneity of variance failed, nonparametric Mann Whitney U tests were used instead of $t$-tests.

Results

Aggregate formation

Aggregates of *Chaetoceros* cells formed at the cell concentration of $9 \times 10^6$ cells L$^{-1}$ and were flocculent and porous but stable enough for experimental handling. Generally, they were highly irregular in shape, i.e., nonspherical. At the two higher cell concentrations ($20–30 \times 10^6$ cells L$^{-1}$), however, aggregates were very fragile and consistently fell apart during handling except for two aggregates. In contrast, *Skeletonema* formed aggregates only at these higher cell concentrations ($20–30 \times 10^6$ cells L$^{-1}$). They were compact and near-spherical in shape.

Aggregate characterization

Aggregate size (ESD) ranged from 2 to 3.7 mm for *Skeletonema*, averaged 2.9 mm, and was significantly smaller than those measured for the *Chaetoceros* aggregates, which ranged from 2.8 to 6.7 mm and averaged 4.8 mm ($t$-test, $t = 4.047$, df = 14, $p < 0.001$). Generally *Skeletonema* aggregates were more compact and dense as reflected by their higher sinking velocities (range 158–412 m d$^{-1}$) compared to the *Chaetoceros* aggregates (13–124 m d$^{-1}$) ($t$-test, $t = -7.264$, df = 19, $p < 0.001$) (Fig. 1).

However, no significant difference was observed in their DW, with averages of 261 μg and 282 μg for *Skeletonema* and *Chaetoceros*, respectively (Mann–Whitney U-test, $p > 0.05$). Consequently, dry weight per aggregate volume was significantly higher in *Skeletonema* compared to *Chaetoceros* (Table 1). Yet, there was a large variability for aggregate dry weight per volume within a species that did not seem to have an influence on the drag coefficient. Aggregates with similar size and drag coefficients within each species could, however, have a very wide range of dry weights per volumes. This variability of dry weight per volume as well as low excess density and sinking velocity may partly be explained by convoluted shape, which includes areas devoid of cells and TEP in the periphery of *Chaetoceros* aggregates. Apparent excess density was an order of magnitude higher in *Skeletonema* aggregates compared to those of *Chaetoceros*, but both solid matter density and cellular volume fraction were relatively similar in

Fig. 3 Velocity distribution images of different aggregates formed from *Chaetoceros affinis* (A–C) or *Skeletonema marinoi* (D–F) cells, with different Reynolds ($Re$) numbers. Model aggregates with corresponding $Re$ numbers are shown in G–K. The gray squares are masked-out areas due to shadowing of the laser sheet. To ensure clarity of the image, only 1/4 of the vectors are displayed in the x-direction and 1/8 in the y-direction.
Skeletonema and Chaetoceros (Table 1). Excess density of agar spheres (0.0011 g cm\(^{-3}\)) has previously been determined in Ploug et al. (2010).

Particle image velocimetry measurements

The trajectories of the PIV tracer particles allowed the calculation of the flow fields around the various types of aggregates. The resulting streamlines showed that water was essentially deflected from the aggregate surface, also for highly irregular geometries (Fig. 3). Although streamlines approached the aggregates at almost perpendicular angles upstream of the aggregate surface, no particles were observed to enter the aggregates, that is, passed through them or were scavenged in the aggregate interior. Flow velocities in the downstream wake were substantially slower several diameters away from the surface independent of aggregate geometry. After passing around the aggregates, the flow converged toward the centerline in the downstream flow field and further away from the aggregate returned to a simple structure with uniform streamlines parallel to the centerline. No recirculation of flow in the downstream wake was detected for any of the aggregates and Re numbers tested. Skewed (either to the left or right) downstream streamlines at low Re numbers were an artifact of the flow chamber, which only had a relatively uniform flow field across the upper chamber when mean flow velocities were > 1.0 mm s\(^{-1}\) (Ploug and Jørgensen 1999). At higher Re, the skewness may have been an artifact of an unevenness of flow leaving the eight outlets of the flow chamber. Uneven background flow was more pronounced for Chaetoceros aggregates due to the lower flow rates compared to Skeletonema and model aggregates.

Detailed velocity distributions of the equatorial, upstream, and downstream regions of aggregates with irregular geometry formed by Chaetoceros cells (Fig. 4A–C) revealed similar distributions to the compact, dense, and generally spherical aggregates formed by Skeletonema (Fig. 4D–F). Observed differences between the two species reflected the higher sinking velocities of the Skeletonema aggregates. The approximated shear rates due to velocity gradients around selected model and diatom aggregates with similar sizes and Re numbers are visualized in Fig. 5. As expected, the shear rates were highest along the edges of the upstream–downstream axis of the aggregates. Compared to the spherical model aggregates (Fig. 5B, D), the shear rates at the surface of irregularly shaped diatom aggregates (Fig. 5A, C) varied more, and both local maxima with steeper velocity gradients and local minima with less steep gradients were formed. Note that the shear rate was not depicting well the measured velocity gradient at the poles where flow was normal to the aggregate surface and was decelerated or accelerated (compare with

![Fig. 4](image-url) Measured velocity profiles up- and down-stream of the aggregate surface and off the aggregates’ equators at various Reynolds (Re) numbers for three Chaetoceros affinis (A–C), three Skeletonema marinoi aggregates (D–F) and five model aggregates (G–I). Triplicate profiles around individual aggregates measured in parallel are depicted. Corresponding images of the aggregates are shown in Fig. 3.  

Zetsche et al. Flow and diffusion around diatom aggregates
Figs. 3 and 4). Here, the velocity gradient is determined by the strain rate.

For all aggregates investigated, vorticity was associated with small-scale curvatures along the aggregate surface and its distribution was affected by aggregate shape (Fig. 6). The vorticity in the wake behind the aggregates was low at these relatively low Re numbers. At Re of approximately 1, the vorticity in the wake behind the irregular Chaetoceros aggregate was slightly higher than for the spherical model aggregate (Fig. 6A,B). However, this was only apparent on the right side of the aggregate. At Re of approximately 6, there was no obvious difference in wake vorticity between the Skeletonema aggregate and the model aggregate (Fig. 6C,D).

Aggregate imaging

The holographic D³HM imagery (Fig. 7) and light microscopy (Fig. 8) also confirmed the compact nature of the Skeletonema aggregates compared to the smaller volume fraction of cells in Chaetoceros aggregates. In the holograms, it is clearly noticeable how tightly aggregated the cells in Skeletonema aggregates were since very little light penetrated the main body of the aggregate as shown by the darkened areas from all the cells overlapping each other (Fig. 7A,B). In contrast, Chaetoceros aggregates clearly showed interstitial spaces between the cells and were never so densely aggregated in their layers going through the aggregate that the overlap of all the layers completely blocked light passing through the aggregates.
Lower OPL values were also observed in the interstitial spaces compared to the seawater surrounding the aggregates suggesting that a material different from seawater was present in these spaces (Fig. 7F,H). However, the difference in OPL values was very small (< 0.1 μm) implying that the interstitial substance was of a watery consistency, that is, that the refractive indices of the two substances were very similar.

TEP particles were observed in both Skeletonema as well as Chaetoceros aggregates, but the staining pattern was distinctly different between the two algal species (Fig. 8). In Skeletonema aggregates, staining was predominantly observed around the edges of the aggregates (Fig. 8A,B) with only some minor intrusions into the aggregates themselves, whereas Alcian blue-stained polysaccharide material in Chaetoceros aggregates was found as a dominant component within the aggregates' interstitial spaces (Fig. 8C,D). At higher magnifications it became clear that the cell walls and spines of Chaetoceros cells were covered by TEP (Fig. 8E,F).

**Oxygen measurements**

Oxygen distributions within Chaetoceros aggregates were difficult to measure due to their floculent nature and stickiness. Often the O2 microsensor would stick to the aggregate at some point and hence distort further measurements. An example of the radial oxygen concentrations within both Skeletonema and Chaetoceros aggregates, respectively, are shown (Fig. 9). The
oxygen concentration distributions within aggregates relative to that in the ambient water within the aggregates were similar to those modeled for suspended aggregates impermeable to flow and those measured and modeled in similar aggregates previously (Ploug 2001; Moradi et al. 2018). However, the measured oxygen concentrations and gradients in the wake downstream of the sinking aggregates were lower than those modeled without flow in both the downstream and in the upstream region, and reflect that advective transport was more important than diffusion outside the aggregates. These deviations were higher for the *Skeletonema* aggregate with a sinking velocity of 287 m d$^{-1}$, as compared to the *Chaetoceros* aggregate with a sinking velocity of 25.5 m d$^{-1}$. The *Skeletonema* aggregate was 3.7 mm (ESD) large while the *Chaetoceros* aggregate was 4.5 mm (ESD) large. The lower O$_2$ concentrations within *Skeletonema* aggregates demonstrate a higher respiration rate per aggregate volume relative to that of the *Chaetoceros* aggregate.

**Discussion**

The scavenging of small particles and the uptake or release of gases, nutrients, and solutes by fast-sinking marine snow are important in regulating the CO$_2$ concentration in the earth’s atmosphere. Models and experimental studies of flow distributions around sinking particles and aggregates, such as diatom aggregates, have to date focused on spherical geometry (Kiørboe et al. 2001; Moradi et al. 2018). Aggregates in the field, however, are seldom spherical. Using PIV, we obtained high-resolution flow distributions for diatom aggregates with various geometries and uneven surfaces as well as spherical model aggregates. In combination with holographic imaging, O$_2$ microsensors and modeling, we investigated the impact of aggregate composition on flow and diffusion within and around aggregates.

Two different types of diatom aggregates were studied: (1) nearly spherical and compact aggregates formed by *Skeletonema* cell chains, and (2) predominantly nonspherical and more flocculent aggregates formed by *Chaetoceros* cell chains, and further compared to almost perfectly spherical nonpermeable model aggregates. *Skeletonema* formed aggregates only at higher cell concentrations relative to *Chaetoceros*. This was most likely due to the presence of *Chaetoceros'* entanglement-enhancing spines (Alldredge and Gotschalk 1989) and its high TEP production, which was also observed.

---

**Fig. 7** Digital holographic microscopy images of diatom aggregates. Extracted intensity images showing the edges of dense compact *Skeletonema marinoi* aggregates (**A, B**) compared with *Chaetoceros affinis* aggregates (**C, D**) that have numerous interstitial spaces between cells within the aggregates. Intensity images (**E, G**) and the respective falsely colored phase images (**F, H**) of edges of two different *Chaetoceros* aggregates are shown where interstitial spaces (indicated with arrows) were observed to have different optical heights than the surrounding seawater (SW), suggesting the presence of transparent exopolymeric particles (TEP). The color scheme for the phase images was chosen to clearly depict differences in optical height. Shown scale bars are 200 μm for images **B, D, E** and 100 μm for **A, C, G**.
on the spines (Kiørboe and Hansen 1993; Crocker and Passow 1995). Chaetoceros aggregates were fluffy in appearance, never similar in shape, and frequently revealed various protrusions (Fig. 2A–C). It has previously been shown that Chaetoceros coagulation depends on TEP-TEP or TEP-cell stickiness (Kiørboe and Hansen 1993; Passow et al. 1994; Logan et al. 1995) while that for Skeletonema depends on cell–cell stickiness (Kiørboe and Hansen 1993; Ziervogel and Forster 2005). With the Alcian blue staining we observed a general lack of TEP within Skeletonema aggregates, but it was highly present within Chaetoceros aggregates (Fig. 8). Here, TEP was present between the cell chains as well as around individual cells along the cell walls and spines (Fig. 8). TEP is sticky (Alldredge et al. 1993; Passow 2002; Mari et al. 2017) and thus creates a matrix of cell chains and spines that are glued together. The presence of low density TEP in Chaetoceros aggregates most likely also decreased the size-specific sinking velocities of Chaetoceros aggregates (13–124 m d\(^{-1}\)) compared to the fast-sinking, dense Skeletonema aggregates (range 158–412 m d\(^{-1}\)) (Engel and Schartau 1999) as some recent studies have

**Fig. 8 (A, B)** Aggregates formed by Skeletonema marinoi cells showed Alcian blue staining and thus the presence of transparent exopolymeric particles (TEP) predominantly on the edges of aggregates. In contrast, TEP formed a dominant component of the interstitial spaces in aggregates formed by Chaetoceros affinis (C, D). Particularly, the spines and cell walls of C. affinis showed Alcian blue staining (E, F). (A–D): 4× objective, (E, F) 100× oil objective.
demonstrated positive buoyancy of TEP (Azetsu-Scott and Pascow 2004; Mari et al. 2017).

Overall, all measured sinking velocities were within the expected range for marine snow (Alldredge and Gotschalk 1988); however, the sinking velocity of larger Chaetoceros aggregates was slower than for smaller Skeletonema aggregates, and there was no relationship between the dry weight of the aggregates and their sinking velocity (linear regression, r = 0.03) confirming observations by Alldredge and Gotschalk (1988). Aggregate dry weight per volume was higher in Skeletonema as compared to that of Chaetoceros aggregates but the large variability within a diatom species prevents any clear relationships with the drag coefficient and ultimately sinking velocity. We expect that shape may also affect the observed differences in sinking velocity. Drag and therefore settling velocity of an aggregate are functions of its shape (Komar and Reimers 1978), and nonspherical aggregates are expected to sink slower than spherical ones (Simpson 1982; Alldredge and Gotschalk 1988). Our limited observations within one species do not allow for such conclusions. However, the nonspherical Chaetoceros indeed showed slower sinking velocities and lower apparent excess densities as compared to those of the nearly spherical Skeletonema aggregates.

We are not aware of other studies that have investigated irregularly shaped diatom aggregates with PIV to provide high spatial resolution of the flow field around the aggregates as a function of Re. Viscous forces dominate water movement at Re < 0.1, while inertial forces dominate at higher Re. As demonstrated in the present study and earlier, streamlines change from being symmetrical upstream and downstream of a sinking aggregate at Re < 0.1 (i.e., Stokes “creeping” flow) to become increasingly asymmetrical at higher Re (Kiørboe et al. 2001). PIV clearly depicted the curvilinear feature of the streamlines in a wake with retarded fluid flow downstream in all aggregate types similar to those modeled in previous studies with spherical geometries (Kiørboe et al. 2001; Moradi et al. 2018). In our study we observed very similar flow around both types of diatom aggregates as well as the model spheres at a millimeter scale. Vorticity measured around spherical aggregates was similar to that predicted by theory and previously modeled by Kiørboe et al. (2001). At Re ~1, the trail of vorticity downstream of a sinking, highly irregularly shaped diatom aggregate tends to extend further than for a spherical model aggregate (Fig. 6A,B). At Re ~6, however, there was no obvious difference to the downstream vorticity field of the nearly spherical diatom aggregate compared to that of the spherical model (agar) aggregate (Fig. 6C,D). The streamline images (Fig. 3) demonstrated that no flow separation occurred in the wake behind aggregates (i.e., downstream) at these Re.

Whereas velocity gradients along the surface of spherical model aggregates smoothly change along the upstream-downstream axis as predicted by theory (Kiørboe et al. 2001), the velocity gradients varied more along the surface of irregularly shaped diatom aggregates. Irregular surfaces of sinking diatom aggregates produced local maxima and minima of shear rates and vorticity relative to the smoother variation around sinking spheres (Figs. 5, 6). High shear rates imply steep velocity gradients in flow tangential to the surface. Encounter and thus potential coagulation with particles and colonization by bacteria from the ambient water are highest where the velocity gradients are steepest. Thus local growth of the aggregates via scavenging is expectedly highest in these regions.

Diatom aggregates are fractal and the cellular volume fraction decreases significantly with increasing aggregate size (Alldredge and Gotschalk 1988). Model calculations have
indicated that fluid flow in the order of 26 to 160 μm s⁻¹ may occur in large (7–20 mm diameter), sinking diatom aggregates with cellular volume fractions <0.0001 (Logan and Alldredge 1989). However, that model did not include TEP to be part of the aggregate matrix as TEP was only discovered a few years later (Alldredge et al. 1993). The aggregates in our study were all smaller and had more than 10-fold higher volume fractions of cells as compared to the aggregates studied by Logan and Alldredge (1989). In our study, the O₂ distributions within both Skeletonema and Chaetoceros aggregates were similar to those previously reported for these types of diatom aggregates and similar to those of spheres and other aggregates impermeable to flow (Ploug et al. 1997; Ploug et al. 1999b; Ploug and Bergkvist 2015; Moradi et al. 2018). The O₂ concentrations within Skeletonema aggregates normalized to size were lower than those in Chaetoceros, and imply that the O₂ consumption per volume aggregates was higher in the compact Skeletonema aggregates with more dry weight (cells) per volume as compared to those formed by Chaetoceros. These observations suggest that the interstitial spaces observed in Chaetoceros cells were indeed largely filled with TEP. What appears as empty space by eye, in fact, is a matrix of cells and TEP. Digital holographic microscopy has the ability to detect certain polymeric substances (Zetsche et al. 2016a; Zetsche et al. 2016b), and the imaging of Chaetoceros aggregates suggests that a substance different in its optical properties was present in the interstitial spaces compared with the surrounding seawater (Fig. 7). The OPL differences were very small, yet support what is known for TEP: It has very similar properties to stagnant water (Ploug et al. 2008). The wake with O₂-depleted water in the downstream region is due to respiration within aggregates and a consequence of the retarded flow in the wake of the aggregate as measured and modeled earlier (Kiorboe et al. 2001; Ploug 2001; Moradi et al. 2018). A wake with concentration gradients of nutrients and solutes leaking from sinking aggregates was hypothesized almost two decades ago as a consequence of high hydrolytic activities by aggregate-attached bacteria (Smith et al. 1992; Azam and Long 2001). Almost at the same time, the wake behind sinking aggregates impermeable to flow was demonstrated for aggregates with Re numbers between 1 and 10 (Kiorboe et al. 2001; Ploug 2001). Since then, experimental studies and models have shown that bacterial chemotaxis helps bacteria to locate and colonize the wake behind sinking aggregates (Stocker et al. 2008), and even zooplankton localize and find aggregates due to the long wake with retarded fluid motion and concentration gradients of solutes (Jackson and Kiørboe 2004; Lombard et al. 2013). Hence, the wake behind sinking aggregates as demonstrated here and in other studies appears to be crucial for both bacteria and zooplankton during their degradation of sinking aggregates. Most studies have focused on chemotaxis as the key foraging mechanism for bacteria and zooplankton to locate sinking aggregates. The composition of leaking chemical compounds in the wake of aggregates, however, may largely vary across different aggregate sources and also change during degradation. Our study demonstrates the retarded flow in the wake. This physical signature by a sinking aggregate in the water is expectedly more stable during microbial aggregate degradation and suggests rheotaxis as an alternative strategy used by foraging zooplankton.

In conclusion, overall similar flow distributions at a millimeter scale were evident around both spherical aggregates and irregularly shaped aggregates. However, the irregular shape caused heterogeneity of velocity gradients and vorticity distributions on a micrometer scale along the aggregate surface with local minima and maxima as compared to those along spherical model aggregates. Chaetoceros chains formed non-spherical porous aggregates, most likely due to their TEP-coated spines, but also featured a strong presence of TEP within their interstitial spaces which most likely caused aggregates to behave in a similar way to the less permeable and more spherical Skeletonema aggregates. To our knowledge, fluid flow has never been empirically demonstrated with in diatom aggregates. Theoretical models of interstitial flow through diatom aggregates were adapted to models developed from observations in model aggregates composed of steel threads before TEP was discovered (Adler 1981; Logan and Alldredge 1989). Therefore, its applicability as a model for fragile diatom aggregates is, in fact, questionable. Our study demonstrates that while diffusion can be the major mechanism of mass transfer within aggregates, aggregate topography is significant for local variability in small-scale velocity gradients along the surface potentially enhancing both coagulation with other particles as well as colonization by microbiota as compared to ideally spherical aggregates with a smooth surface.

References

Adler, P. M. 1981. Streamlines in and around porous particles. J. Colloid Interface Sci. 81: 531–535. https://doi.org/10.1016/0021-9797(81)90434-3

Alldredge, A. L., and C. Gotschalk. 1988. In situ settling behavior of marine snow. Limnol. Oceanogr. 33: 339–351. doi:10.4319/lo.1988.33.3.0339
Alldredge, A. L., and C. C. Gotschalk. 1989. Direct observations of the mass flocculation of diatom blooms: Characteristics, settling velocities and formation of diatom aggregates. Deep-Sea Res. Pt. A 36: 159–171. doi:10.1016/0198-0149(89)90131-3

Alldredge, A. L., U. Passow, and B. E. Logan. 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. Deep-Sea Res. Pt. I 40: 1131–1140. doi:10.1016/0967-0637(93)90129-q

Azam, F., and R. A. Long. 2001. Sea snow microcosms. Nature 414: 495. doi:10.1038/35107174

Azetsu-Scott, K., and U. Passow. 2004. Ascending marine particles: Significance of transparent exopolymer particles (TEP) in the upper ocean. Limnol. Oceanogr. 49: 741–748. doi:10.4319/lo.2004.49.3.0741

Bergkvist, J., I. Klawonn, M. J. Whitehouse, G. Lavik, V. Brüchert, and H. Ploug. 2018. Turbulence simultaneously stimulates small-and large-scale CO_2 sequestration by chain-forming diatoms in the sea. Nat. Commun. 9: 3046. doi:10.1038/s41467-018-05149-w

Bizić-Ionescu, M., D. Ionescu, and H.-P. Grossart. 2018. Organic particles: Heterogeneous hubs for microbial interactions in aquatic ecosystems. Front. Microbiol. 9: 2569. doi:10.3389/fmicb.2018.02569

Broeker, W. S., and T.-H. Peng. 1974. Gas exchange rates between air and sea. Tellus 26: 21–35. doi:10.3402/tellusa.v26i1.2973

Burd, A. B., and G. A. Jackson. 2009. Particle aggregation. Ann. Rev. Mar. Sci. 1: 65–90. doi:10.1146/annurev.marine.010908.163904

Crocker, K. M., and U. Passow. 1995. Differential aggregation of diatoms. Mar. Ecol. Prog. Ser. 117: 249–257. doi:10.3354/meps117249

Cronenberg, C. C. H. 1994. Biochemical engineering on a micro-scale: Biofilms investigated with needle-type glucose sensors. PhD. Van’t Hoff Institute for Molecular Sciences (HIMS). University of Amsterdam, The Netherlands.

Delong, E. F., D. G. Franks, and A. L. Alldredge. 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. 38: 924–934. doi:10.4319/lo.1993.38.5.0924

Engel, A. 2009. Determination of marine gel particles, p. 125–142. In O. Wurl [ed.], Practical guidelines for the analysis of seawater. CRC Press.

Engel, A., and M. Schartau. 1999. Influence of transparent exopolymer particles (TEP) on sinking velocity of Nitzschia closterium aggregates. Mar. Ecol. Prog. Ser. 182: 69–76. doi:10.3354/meps182069

Grossart, H.-P., and H. Ploug. 2001. Microbial degradation of organic carbon and nitrogen on diatom aggregates. Limnol. Oceanogr. 46: 267–277. doi:10.4319/lo.2001.46.2.0267

Grossart, H.-P., K. W. Tang, T. Kjørboe, and H. Ploug. 2007. Comparison of cell-specific activity between free-living and attached bacteria using isolates and natural assemblages. FEMS Microbiol. Lett. 266: 194–200. doi:10.1111/j.1574-6968.2006.00520.x

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29–60. In Culture of marine invertebrate animals. Springer. Boston, MA. doi:10.1007/978-1-4615-8714-9_3

Iversen, M. H., and H. Ploug. 2010. Ballast minerals and the sinking carbon flux in the ocean: Carbon-specific respiration rates and sinking velocity of marine snow aggregates. Biogeoosciences 7: 2613–2624. doi:10.5194/bg-7-2613-2010

Jackson, G. A., and T. Kiørboe. 2004. Zooplankton use of chemodetection to find and eat particles. Mar. Ecol. Prog. Ser. 269: 153–162. doi:10.3334/meps269153

Kjørboe, T., and J. L. S. Hansen. 1993. Phytoplankton aggregate formation: Observations of patterns and mechanisms of cell sticking and the significance of exopolymeric material. J. Plankton Res. 15: 993–1018. doi:10.1093/plankt/15.9.993

Kjørboe, T., H. Ploug, and U. H. Thygesen. 2001. Fluid motion and solute distribution around sinking aggregates I: Small-scale fluxes and heterogeneity of nutrients in the pelagic environment. Mar. Ecol. Prog. Ser. 211: 1–13. doi:10.3354/meps211001

Komar, P. D., and C. Reimers. 1978. Grain shape effects on settling rates. J. Geol. 86: 193–209. doi:10.1086/649674

Li, X.-Y., and Y. Yuan. 2002. Collision frequencies of microbial aggregates with small particles by differential sedimentation. Environ. Sci. Technol. 36: 387–393. doi:10.1021/es010681d

Logan, B. E., and A. L. Alldredge. 1989. Potential for increased nutrient uptake by flocculating diatoms. Mar. Biol. 101: 443–450. doi:10.1007/bf00541645

Logan, B. E., H.-P. Grossart, and M. Simon. 1994. Direct observation of phytoplankton, TEP and aggregates on polycarbonate filters using brightfield microscopy. J. Plankton Res. 16: 1811–1815. doi:10.1093/plankt/16.12.1811

Logan, B. E., U. Passow, A. L. Alldredge, H.-P. Grossart, and M. Simont. 1995. Rapid formation and sedimentation of large aggregates is predictable from coagulation rates (half-lives) of transparent exopolymer particles (TEP). Deep-Sea Res. Pt. II 42: 203–214. doi:10.1016/0967-0645(95)00012-F

Lombard, F., M. Koski, and T. Kiørboe. 2013. Copepods use chemical trails to find sinking marine snow aggregates. Limnol. Oceanogr. 58: 185–192. doi:10.4319/lo.2013.58.1.0185

Mari, X., U. Passow, C. Migon, A. B. Burd, and L. Legendre. 2017. Transparent exopolymer particles: Effects on carbon cycling in the ocean. Prog. Oceanogr. 151: 13–37. doi:10.1016/j.pocean.2016.11.002

Moeseneder, M. M., C. Winter, and G. J. Herndl. 2001. Horizontal and vertical complexity of attached and free-living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA fingerprints. Limnol. Oceanogr. 46: 95–107. doi:10.4319/lo.2001.46.1.0095
Moradi, N., B. Liu, M. Iversen, M. M. Kuypers, H. Ploug, and A. Khallili. 2018. A new mathematical model to explore microbial processes and their constraints in phytoplankton colonies and sinking marine aggregates. Sci. Adv. 4: eaa1991. doi:10.1126/sciadv.aat1991

Narayanan, J., J.-Y. Xiong, and X.-Y. Liu. 2006. Determination of agarose gel pore size: Absorbance measurements vis a vis other techniques. J. Phys.: Conference Series. 28: 83–86. doi:10.1088/1742-6596/28/1/017

Nelson, D. M., P. Tréguer, M. A. Brzezinski, A. Leynaert, and B. Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Occulation of diatoms by attached bacteria. Limnol. Oceanogr. 37: 98–104. doi:10.4319/lo.1992.37.1.0098

Narayanan, J., J.-Y. Xiong, and X.-Y. Liu. 2006. Determination of agarose gel pore size: Absorbance measurements vis a vis other techniques. J. Phys.: Conference Series. 28: 83–86. doi:10.1088/1742-6596/28/1/017

Nelson, D. M., P. Tréguer, M. A. Brzezinski, A. Leynaert, and B. Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Occulation of diatoms by attached bacteria. Limnol. Oceanogr. 37: 98–104. doi:10.4319/lo.1992.37.1.0098

Nelson, D. M., P. Tréguer, M. A. Brzezinski, A. Leynaert, and B. Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Occulation of diatoms by attached bacteria. Limnol. Oceanogr. 37: 98–104. doi:10.4319/lo.1992.37.1.0098

Nelson, D. M., P. Tréguer, M. A. Brzezinski, A. Leynaert, and B. Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Occulation of diatoms by attached bacteria. Limnol. Oceanogr. 37: 98–104. doi:10.4319/lo.1992.37.1.0098

Nelson, D. M., P. Tréguer, M. A. Brzezinski, A. Leynaert, and B. Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Occulation of diatoms by attached bacteria. Limnol. Oceanogr. 37: 98–104. doi:10.4319/lo.1992.37.1.0098

Ploug, H., W. Stolte, and B. B. Jørgensen. 1999b. Diffusive boundary layers of the colony-forming plankton alga, Phaeocystis sp.-implications for nutrient uptake and cellular growth. Limnol. Oceanogr. 44: 1959–1967. doi:10.4319/lo.1999.44.8.1959

Ploug, H., A. Terbrüggen, A. Kaufmann, D. Wolf-Gladrow, and U. Passow. 2010. A novel method to measure particle sinking velocity in vitro, and its comparison to three other in vitro methods. Limnol. Oceanogr. Methods 8: 386–393. doi:10.4319/lom.2010.8.386

Revsbech, N. P. 1989. An oxygen microsensor with a guard cathode. Limnol. Oceanogr. 34: 474–478. doi:10.4319/lo.1989.34.2.0474

Sanders, R. J., et al. 2016. Controls over ocean mesopelagic interior carbon storage (COMICS): Fieldwork, synthesis, and modeling efforts. Front. Mar. Sci. 3: 1–10. doi:10.3389/fmars.2016.00136

Shanks, A. L., and E. W. Edmondson. 1989. Laboratory-made artificial marine snow: A biological model of the real thing. Mar. Biol. 101: 463–470. doi:10.1007/bf00541648

Sheng, G.-P., H.-Q. Yu, and X.-Y. Li. 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. Biotechnol. Adv. 28: 882–894. doi:10.1016/j.biotechadv.2010.08.001

Siegel, D. A., et al. 2016. Prediction of the export and fate of Global Ocean net primary production: The EXPORTS science plan. Front. Mar. Sci. 3: 1–10. doi:10.3389/fmars.2016.00022

Simon, M., H.-P. Grossart, B. Schweitzer, and H. Ploug. 2002. Microbial ecology of organic aggregates in aquatic ecosystems. Aquat. Microb. Ecol. 28: 175–211. doi:10.3354/ame028175

Simpson, W. 1982. Particulate matter in the oceans-sampling methods, concentration, size distribution and particle dynamics. Oceanogr. Mar. Biol. 20: 119–172.

Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359: 139–142. doi:10.1038/359139a0

Stief, P., A. Kamp, B. Thamdrup, and R. N. Glud. 2016. Anaerobic nitrogen turnover by sinking diatom aggregates at varying ambient oxygen levels. Front. Microbiol. 7: 98. doi:10.3389/fmicb.2016.00098

Stockner, R., J. R. Seymour, A. Samadani, D. E. Hunt, and M. F. Polz. 2008. Rapid chemotactic response enables marine bacteria to exploit ephemeral microscale nutrient patches. PNAS 105: 4209–4214. doi:10.1073/pnas.0709765105

Thiele, S., B. M. Fuchs, R. Amann, and M. H. Iversen. 2015. Colonization in the photic zone and subsequent changes during sinking determine bacterial community composition in marine snow. Appl. Environ. Microbiol. 81: 1463–1471. doi:10.1128/aem.02570-14

Turner, J. T. 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean’s biological pump. Prog.
Zetsche et al. 2016. Digital holographic microscopy as a novel tool to detect and classify nanoplanktonic organisms. Limnol. Oceanogr. Methods 12: 757–775. doi:10.4319/lom.2014.12.757

Zetsche, E.-M., A. El Mallahi, and F. J. R. Meysman. 2016b. Digital holographic microscopy: A novel tool to study the morphology, physiology and ecology of diatoms. Diatom Res. 31: 1–16. doi:10.1080/0269249X.2016.1140679

Zetsche, E., A. El Mallahi, F. Dubois, C. Yourassowsky, J. Kromkamp, and F. J. R. Meysman. 2014. Imaging-in-flow: Digital holographic microscopy as a novel tool to detect and classify nanoplanktonic organisms. Limnol. Oceanogr. Methods 12: 757–775. doi:10.4319/lom.2014.12.757

Zetsche et al. Flow and diffusion around diatom aggregates

Submitted 14 May 2019
Revised 11 November 2019
Accepted 08 January 2020

Associate editor: Thomas Kiørboe