SHORT COMMUNICATION

Hydrodynamic interactions at low Reynolds number: an overlooked mechanism favouring diatom encounters

VINCENZO BOTTE*, MAURIZIO RIBERA D'ALCALÀ AND MARINA MONTRESOR

STAZIONE ZOOLOGICA ANTON DOHRN, VILLA COMUNALE, 80121 NAPOLI, ITALY

*CORRESPONDING AUTHOR: vincenzo.botte@szn.it

Suspensions of small fibres are known to produce clusters of particles during sedimentation, independently of fluid shear and Brownian motion. This phenomenon, which is due to hydrodynamic interactions at low Reynolds number, has been predicted theoretically and observed in experiments and numerical simulations. We hypothesize that this mechanism may be at least partially responsible for the encounter and aggregation of marine diatoms.

Diatoms are a key phytoplankton group in the oceans, where they are responsible for a large fraction of primary production and play a fundamental role in the carbon and silicon biogeochemical cycles (Trèguer and LaRocha, 2013). The drawdown of these elements in the water column is largely mediated by rapid mass sinking following their growth season, which is a distinctive trait of diatom life cycles (Smetaceck, 1985; Alldredge and Gotschalk, 1989; Thornton, 2002). The possibility to aggregate and encounter other cells is a mandatory prerequisite for sexual reproduction, especially for pennate species that lack flagella to propel them through the water. Sexual reproduction is also crucial in diatom life cycles as a means of allowing genetic recombination and the restoration of maximum cell size after the progressive miniaturization induced by asexual divisions (Round *et al.*, 2005). Surprisingly, there are very few reports of diatom sexual stages in marine natural populations, but two recent publications described in detail massive and simultaneous sex events involving *Pseudo-nitzschia* species, which are needle-shaped, chain-forming pennate diatoms abundant in coastal and oceanic waters (Holtermann *et al.*, 2010; Sarno *et al.*, 2010). *Pseudo-nitzschia* cells were occasionally observed in groups of chains arranged in parallel (Fig. 1 and Holtermann *et al.*, 2010); auxospores (the zygotes produced after gamete conjugation) were also observed on contiguous cells in a chain, demonstrating that pairing between chains, or between chains and single cells, must have been a common event (Sarno *et al.*, 2010).

The mechanisms generally considered to explain the aggregation of cells are differential settling, fluid shear induced by laminar or turbulent flow and Brownian motion (Kiørboe, 2008). The first assumes different sizes or fractal dimensions, and is unlikely to apply to single cells or chains of the same length, while the latter is probably not relevant, considering the size of diatom cells and chains (Burd and Jackson, 2009). Therefore, shear-induced encounters represent the most likely mechanism for bringing pennate diatoms together. Nevertheless, aggregates have also been observed under
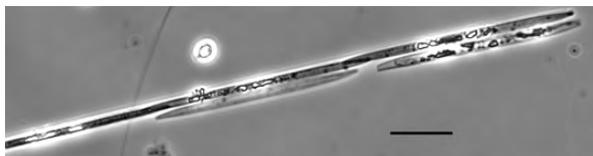
Fig. 1. Light micrograph of a chain alongside two individual cells of *Pseudo-nitzschia* cf. *delicatissima* in a natural sample collected during the sexual event described by Sarno *et al.* (Sarno *et al.*, 2010). Scale bar = 20 µm.

very calm conditions (e.g. Peinert *et al.*, 1982; Alldredge and Gotschalk, 1989), when cells float or sink slowly.

The need to improve our understanding of the mechanisms that promote association of diatom cells or chains was the motivation for the present analysis. Although our data relate to *Pseudo-nitzschia*, the principles discussed here should apply to all planktonic diatoms.

Sinking of *Pseudo-nitzschia* cells in a liquid medium under the effect of gravity is characterized by an extremely low Reynolds number ($Re = UL/\nu$, with U being the velocity, L the length of the object and ν the kinematic viscosity of the fluid), a dimensionless number that gives a measure of the ratio between inertial and viscous forces. For a sedimentation velocity of the order of a metre per day (Smayda and Boleyn, 1965) and cell/chain lengths ranging from 30 µm to hundreds of micrometres, the Reynolds number is in the order of 10^{-4}; hence inertia is very low and the motion is dominated by viscous forces.

At Reynolds numbers of this order of magnitude, the sedimentation velocity of non-spherical particles is known to depend strongly on the collective sedimentation process: each particle rotates under the influence of the fluctuations of the fluid velocity resulting from the settling of surrounding particles. In other words, for sedimentation at very low Reynolds number, a change in the orientation of a given particle produces velocity fluctuations that, in turn, induce a change in orientation and velocity fluctuations in the surrounding particles. The net result of these interactions is a force tending to unite or separate particles, leading to aggregation during the sedimentation process.

This phenomenon was described by Koch and Shaqfeh (Koch and Shaqfeh, 1989), who predicted theoretically that hydrodynamic interactions between non-Brownian sedimenting spheroids give rise to an increase in the number of neighbouring particles in the vicinity of any given particle. An initially homogeneous suspension of non-spherical particles would thus develop dynamic clusters of particles during the sedimentation process. Their theoretical analysis assumed a Reynolds number much smaller than 1, i.e. $Re << 1$, and dilute concentrations. Sedimenting suspensions are usually characterized by another dimensionless parameter, $n(l/2)^3$, where n is the number of particles per unit volume and l is the particle length; dilute suspensions correspond to $n(l/2)^3 < 1$. Curves at constant values of $n(l/2)^3$, showing how this parameter varies as a function of particle concentration and length, are presented in Fig. 2, where the dotted area represents the dilute regime. For non-dilute solutions, direct interactions give rise to additional forces, making a theoretical analysis impossible.
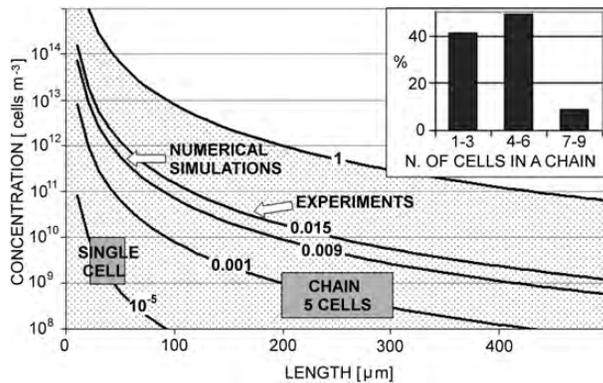
Fig. 2. Curves at constant $n(l/2)^3$ in the cell length/concentration plane. The dotted area represents the dilute regime, defined as $n(l/2)^3 < 1$, where we expect clustering to occur. The grey rectangles represent the data for single *Pseudo-nitzschia* cells and for five-cell chains (data from Sarno *et al.*, 2010; see text for details). In the inset, the percentage frequency distribution of *Pseudo-nitzschia multistriata* cells in chains of different length is presented for samples collected at the same site in 2006.
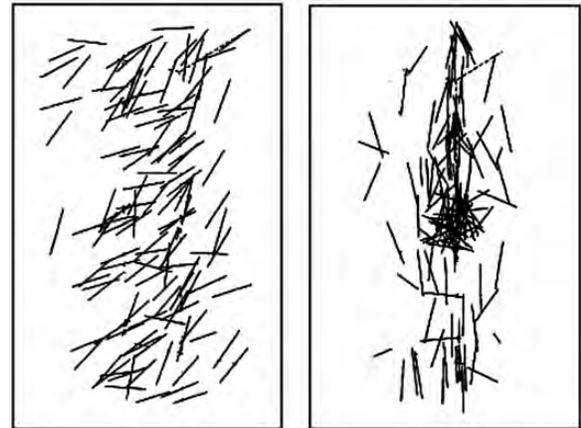
Fig. 3. Drawing illustrating the instability in a suspension of fibres as predicted theoretically and observed both experimentally and in numerical simulations: an initially homogeneous solution (left) develops cluster of fibres (right), with the cluster being elongated in the direction of gravity.

Since the pioneering work of Koch and Shaqfeh (Koch and Shaqfeh, 1989), several papers have been published where the sedimentation of spheroid-like bodies, and in particular of bodies of high aspect ratio like fibres, has been studied both experimentally (Herzhaft *et al.*, 1996; Herzhaft and Guazzelli, 1999; Metzger *et al.*, 2005, 2007) and through numerical simulations (Mackaplow and Shaqfeh, 1998; Kuusela *et al.*, 2001, 2003; Butler and Shaqfeh, 2002; Saintillan *et al.*, 2005). All these studies referred to dilute suspensions at a Reynolds number of the order of 10^{-4}. The formation of clusters of fibres under the effect of gravity alone is usually described as a concentration instability, where an initially homogeneous suspension evolves towards a very inhomogeneous state where clusters of fibres are formed, as depicted in Fig. 3. Fibres are continuously being attracted to the clusters and expelled from them, in a dynamic process. It is important to point out that this phenomenon is completely different from differential settling, which cannot be hypothesized in the case of fibres or cells of identical size.

All of the above may be summarized as follows: theoretical analysis, supported by both experiments and numerical simulations, shows that sedimenting suspensions become unstable and start to develop clusters of particles when the following three conditions are met:

(1) the sedimenting particles are elongated (such as spheroids or fibres);
(2) the Reynolds number of the sedimenting particles is much smaller than 1;
(3) the suspension is dilute, i.e. $n(l/2)^3 < 1$.

A sedimenting suspension of *Pseudo-nitzschia* would satisfy all three conditions: the needle-shaped *Pseudo-nitzschia* cells are very similar to fibres and tend to join end-to-end to form long-stepped chains; the Reynolds number achieved during sedimentation is of the order of 10^{-4}; and their concentration in the sea gives $n(l/2)^3 < 1$. Using the data reported in Sarno *et al.* (Sarno *et al.*, 2010) for the two *Pseudo-nitzschia* species involved in sexual reproduction, $n = 10^6$ cells $L^{-1} = 10^9$ cells m^{-3}, and $l = 50 \ \mu m = 5 \times 10^{-5}$ m, so that the order of magnitude of $n(l/2)^3$ is 10^{-5}, which is well within the dilute regime. Hence we hypothesize that *Pseudo-nitzschia* cells may form clusters during sedimentation, simply as a result of hydrodynamic interactions at low Reynolds number.

None of the experiments conducted so far has dealt with the cell length and concentration range of a natural suspension of *Pseudo-nitzschia in situ*. However, we can estimate how well a suspension of diatoms approximates to the suspensions of fibres where clustering has been observed experimentally. For single *Pseudo-nitzschia* cells, $n(l/2)^3$ is three orders of magnitude below the lowest values, i.e. 10^{-2}, of the experimental set-ups reported in the literature. However, if chains are considered, $n(l/2)^3$ increases rapidly and, for a chain consisting of five cells, it approaches values at which clustering has been both observed experimentally and predicted through numerical simulations (Fig. 2). It is important to point out that, although in the existing literature values of $n(l/2)^3$ smaller than 10^{-2} have not been considered so far, there are no theoretical lower limitations to the values of $n(l/2)^3$ at which instability can occur. In other words, we can expect clustering of fibres to occur

throughout the dilute regime, and certainly for cell suspensions where $n(l/2)^3 < 1$ (dotted area in Fig. 2).

Unfortunately, a theoretical analysis of the efficiency of this process, i.e. the quantification of the encounter rate for a given cell/chain, is not possible, as the mathematical formulation presented by Koch and Shaqfeh (Koch and Shaqfeh, 1989) and adopted in all successive investigations evaluates only the probability of a density increase around a single particle and does not provide formulas that could be used for simple calculations.

Information on the actual time required for a cell to meet another cell by this mechanism would be important in deciding whether this is an effective means of encountering partners. There are no specific data on the time needed to form a cluster in the published literature, but it is possible to infer an upper boundary from the experimental data of Herzhaft and Guazzelli (Herzhaft and Guazzelli, 1999) for a suspension with $n(l/2)^3 = 0.009$. An initially homogeneous suspension showed well-developed clusters of fibres after an interval of about 60 Stokes times, where the Stokes time is defined as the time required to fall half the fibre length with the average settling velocity. We will use this value for a suspension of diatoms, even if it relates to a higher value of $n(l/2)^3$, as it is the only information available. For a *Pseudo-nitzschia* chain of five cells, the distance travelled during an interval of 60 Stokes times is in the order of 10 mm. With a sedimentation velocity of several meters per day (Smayda and Boleyn, 1965), it would take a few minutes to cover such a distance.

This last result can be used to give a very approximate idea of the length and time scales involved in the aggregation of diatoms during sedimentation. Intuitively, we expect that the longer the diatoms are allowed to sediment in calm water, the higher the probability of forming aggregates, although we are unable to demonstrate this from existing theory. At the same time, it is worth noting that the process is highly unstable, as indicated by theory, and aggregates may once again become separated by hydrodynamic forces after being formed.

In summary, data published for fibres show that they tend to form dynamic clusters in the absence of mixing and differential settling, only requiring the interactions among flow fields generated by their sinking. We speculate that under similar conditions the sedimentation of needle-shaped diatoms will produce clusters of cells. Clearly, proof of the existence of such a phenomenon would require direct observation. We believe that an experimental set-up, rather than attempting to follow the sedimentation of individual cells or chains, should aim to observe cluster formation in an initially homogeneous suspension of diatoms. However, this appears to be a relatively complex task and direct visualization of clusters seems unlikely; in experiments carried out with glass rods, this was achieved by silvering the fibres to observe the structure of the suspension (Herzhaft and Guazzelli, 1999). Instead, *in situ* laser scattering and transmissometry (LISST) could be used to detect the presence of aggregates in the suspension, as suggested by Rzadkowolski and Thornton (Rzadkowolski and Thornton, 2012). LISST can measure diatom aggregation by detecting changes in the particle size distribution, and would allow the aggregation hypothesis to be tested in calm water at different concentrations, although it would probably not be feasible to visualize the clusters with this technique.

Another possible approach would be to allow the sedimentation in calm water of a relatively large number of diatom cultures with different concentrations and cell/chain lengths, and to examine the spatial distribution of the cells deposited on the floor, with the aim of detecting some inhomogeneity in the patterns that could substantiate the hypothesis of cluster formation. This last approach would not necessarily be conclusive, however, as theory shows that clusters of cells are continuously created and destroyed in the sedimentation process.

REFERENCES

Alldredge, A. L. and Gotschalk, C. C. (1989) Direct observations of the mass flocculation of diatom blooms: characteristics, settling velocities and formation of diatom aggregates. *Deep-Sea Res.*, **36**, 159–171.

Burd, A. B. and Jackson, G. A. (2009) Particle aggregation. *Ann. Rev. Mar. Sci.*, **1**, 65–90.

Butler, E. and Shaqfeh, E. S. G. (2002) Dynamic simulations of the inhomogeneous sedimentation of rigid fibres. *J. Fluid Mech.*, **468**, 205–237.

Herzhaft, B., Guazzelli, É., Mackaplow, M. B. *et al.* (1996) Experimental investigation of the sedimentation of a dilute fiber suspension. *Phys. Rev. Lett.*, **77**, 290–293.

Herzhaft, B. and Guazzelli, É. (1999) Experimental study of the sedimentation of dilute and semi-dilute suspensions of fibres. *J. Fluid Mech.*, **384**, 133–158.

Holtermann, K. E., Bates, S. S., Trainer, V. L. *et al.* (2010) Mass sexual reproduction in the toxigenic diatoms *Pseudo-nitzschia australis* and *P. pungens* (Bacillariophyceae) on the Washington coast, USA. *J. Phycol.*, **46**, 41–52.

Kiørboe, T. (2008) *A Mechanistic Approach to Plankton Ecology.* Princeton University Press, 228 p.

Koch, D. L. and Shaqfeh, E. S. G. (1989) The instability of a dispersion of sedimenting spheroids. *J. Fluid Mech.*, **209**, 521–542.

Kuusela, E., Höfler, K. and Schwarzer, S. (2001) Computation of particle settling speed and orientation distribution in suspensions of prolate spheroids. *J. Eng Math.*, **41**, 221–235.

Kuusela, E., Lahtinen, J. M. and Ala-Nissila, T. (2003) Collective effects in settling of spheroids under steady-state sedimentation. *Phys. Rev. Lett.*, **90**, 094502.

Mackaplow, M. B. and Shaqfeh, E. S. G. (1998) A numerical study of the sedimentation of fibre suspensions. *J. Fluid Mech.*, **376**, 149–182.

Metzger, B., Guazzelli, É. and Butler, J. E. (2005) Large-scales streamers in the sedimentation of a dilute fiber suspension. *Phys. Rev. Lett.*, **95**, 164506.

Metzger, B., Butler, J. E. and Guazzelli, É. (2007) Experimental investigation of the instability of a sedimenting suspension of fibres. *J. Fluid Mech.*, **575**, 307–332.

Peinert, R., Saure, A., Stegmann, P. *et al.* (1982) Dynamics of primary production and sedimentation in a coastal ecosystem. *Neth. J. Sea Res.*, **16**, 276–289.

Round, F. E., Crawford, R. M. and Mann, D.G. (eds.) (2005) *The Diatoms: Biology and Morphology of the Genera.* Cambridge University Press, p. 747.

Rzadkowolski, C.E. and Thornton, D. (2012) Using laser scattering to identify diatoms and conduct aggregation experiments. *Eur. J. Phycol.*, **47**, 30–41.

Saintillan, D., Darve, E. and Shaqfeh, E. S. G. (2005) A smooth particle-mesh Ewald algorithm for Stokes suspension simulations: the sedimentation of fibers. *Phys. Fluids.*, **17**, 033301.

Sarno, D., Zingone, A. and Montresor, M. (2010) A massive and simultaneous sex event of two *Pseudo-nitzschia* species. *Deep-Sea Res. II.*, **57**, 248–255.

Smayda, T. J. and Boleyn, B. J. (1965) Experimental observations on the flotation of marine diatoms. I. *Thalassiosira cf. nana, Thalassiosira rotula*, and *Nitzschia seriata. Limnol. Oceanogr.*, **10**, 499–509.

Smetaceck, V. S. (1985) Role of sinking in diatom life-history cycles: ecological, evolutionary and geological significance. *Mar. Biol.*, **84**, 239–251.

Thornton, D. (2002) Diatom aggregation in the sea: mechanisms and ecological implications. *Eur. J. Phycol.*, **37**, 149–161.

Trèguer, P.J. and De La Rocha, C.L. (2013) The world ocean silica cycle. *Ann. Rev. Mar. Sc.*, **5**, 477–501.