Microrheology reveals microscale viscosity gradients in planktonic systems

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Microbial activity in planktonic systems creates a dynamic and heterogeneous microscale seascape that harbors a diverse community of microorganisms and ecological interactions of global significance. In recent decades great effort has been put into understanding this complex system, particularly focusing on the role of chemical patchiness, while overlooking a physical parameter that governs microbial life and is affected by biological activity: viscosity. Here we reveal spatial heterogeneity of viscosity in planktonic systems by using microrheological techniques that allow measurement of viscosity at length scales relevant to microorganisms. We show the viscous nature and the spatial extent of the phycosphere, the region surrounding phytoplankton. In ~45% of the phytoplankton cells analyzed we detected increases in viscosity that extended up to 30 μm away from the cell with up to 40 times the viscosity of seawater. We also show how these gradients of viscosity can be amplified around a lysing phytoplankton cell as its viscous contents leak away. Finally, we report conservative estimates of viscosity inside marine aggregates, hotspots of microbial activity, more than an order of magnitude higher than in seawater. Since the diffusivities of dissolved molecules, particles, and microorganisms are inversely related to viscosity, microheterogeneity in viscosity alters the microscale distribution of microorganisms and their resources, with pervasive implications for the functioning of the planktonic ecosystem.

Increasing viscosities impacts ecological interactions and processes, such as nutrient uptake, chemotaxis, and particle encounter, that occur at the microscale but influence carbon and nutrient cycles at a global scale.

Significance

At the microscopic scales at which the life of marine microbes unfolds, the physics is dominated by viscosity. Increasing viscosity slows down both the passive transport of solutes and particles and the swimming of motile microorganisms, and thus directly or indirectly affects all aspects of microbial life. Viscosity depends not only on the physical properties of water, but it also varies as a consequence of biological activity, allowing microorganisms some control over their physical environment. Our use of microrheology allows us to explore how viscosity is structured around phytoplankton cells and marine aggregates and unveils a level of spatial heterogeneity that has implications for the functioning of the microbial food web and hence of marine biogeochemical cycles.

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and mucilages that lead to the formation of transparent exopolymer particles (TEP) (12, 13). Our results reveal that the relative viscosity (defined as the ratio of local dynamic viscosity to the dynamic viscosity of artificial seawater) is on average ~2.6 times higher (along both axes analyzed; parallel to and approaching the edge of the cell or glass shard), and more variable near (at one probe-diameter’s distance from) the cell wall of diatoms than near the glass shards (Fig. 1A). In the case of the diatoms, the relative viscosity is on average ~2.2 times higher than predictions by Faxén’s law (mean along both axes), which accounts for hydrodynamic interactions of a sphere near a solid wall (Fig. 1A and see also Materials and Methods). Moreover, for viscosity measurements taken near the diatoms, we also detected anisotropy in the measurements, given as the ratio of relative viscosities in the perpendicular direction to those in the parallel direction. A value of 1 represents isotropy. Data have been jittered in the x direction by 0.1 μm to facilitate visualization.

We next used MPTM to corroborate the MOT observations, maximize the spatial coverage of the rheological measurements, and investigate different species and scenarios. Maps of relative viscosity were generated around phytoplankton cells and within aggregates, with a field of view (FoV) of 130 × 175 μm² and a spatial resolution of 2 μm. The MPTM results for healthy *C. affinis* cells (Fig. 2 and SI Appendix, Fig. S1) are in good agreement with those obtained from MOT. In 30 of the 41 *C. affinis* cells analyzed, relative viscosities near the cell wall were statistically significantly higher than those predicted by Faxén’s law. However, we did not observe any statistically significant increases in viscosity around glass shards (Fig. 3 and SI Appendix, Fig. S2, ref. 18), confirming that the high values recorded near the cells are not caused by hydrodynamic wall effects alone. As with MOT, relative viscosity values showed a tendency to decay nonlinearly away from the cell walls, suggesting exudation, diffusion, and clustering of EPS from the cell. In all explored cases, relative viscosities measured around *C. affinis* cells were highly variable, with areas showing enhanced values and others showing no notable increase. The extent of the viscous gradient around cells was also variable, ranging from 2 to more than 30 μm (Fig. 3).

To assess the prevalence of these viscous phycospheres, which we call “viscospheres,” we next performed MPTM experiments on the diatoms *Cylindrotheca fusiformis* and *Skeletomena pseudocostatum*, on the dinoflagellates *Alexandrium minutum* and *Ostreopsis cf. ovata*, and on colonies of *Phaeocystis globosa*, a haptophyte whose blooms are often associated with submesoscale (~1 km) increases in bulk-phase viscosity (19). We detected viscosity gradients around 45% of all cells analyzed, and in all species except for *C. fusiformis*. The presence of a viscous phycosphere or its spatial extent, contrary to our expectations based on diffusive rates (5), did not correlate with cell size, while there was a high degree of intra- and interspecific variability (Fig. 3). The spatial extent of the viscosphere for the entire dataset appeared to be negatively exponentially distributed (SI Appendix, Fig. S3), with large viscospheres being less frequent than small ones. Coefficients of variance of the two-dimensional (2D) viscosity maps ranged from 8 to >2,000 in all MPTM experiments.

Fig. 1. MOT measurements of relative viscosity against distance from the boundary of the objects (phytoplankton cells or glass shards). Orange dots are individual measurements around *C. affinis* cells. Black diamonds are average ± SD of measurements around glass shards. Dashed black lines represent predictions from Faxén’s laws (Materials and Methods). (A) Relative viscosity in the direction perpendicular to the edge of the object. (B) Anisotropy in the measurements, given as the ratio of relative viscosities in the perpendicular direction to those in the parallel direction. A value of 1 represents isotropy. Data have been jittered in the x direction by 0.1 μm to facilitate visualization.

![Fig. 1](https://example.com/image1)

![Fig. 2](https://example.com/image2)

**Fig. 2.** Viscosity changes around a *C. affinis* cell (A) before and (B) 1 h after light-induced cell lysis. (A and B) 2 × 2 μm MPTM viscosity maps. (C) Phase-contrast image at the start of the experiment. Cell boundaries are drawn in black in A and B, and yellow in C. (D) Viscosity estimates against the minimum distance to the boundary of the cell before (blue) and after (red) the lysis. Colored lines are a moving average with a 2-μm window. Dashed black line represents Faxén’s law for motion perpendicular to a solid boundary (Eq. 2).
cells are hotspots of microbial activity, as chemotactic bacteria swarm around them (27). Therefore, we expect the same mechanisms at play in a viscous phyccosphere to be magnified in a lysing cell, leading to a more efficient use of resources by chemotactic bacteria.

The last scenario we explored was of viscosity gradients generated by aggregates of phytoplankton. The formation of aggregates, TEP, and marine snow is facilitated by EPS acting as a loose adhesive (12). We used MPTM to map relative viscosities inside and around shear-induced aggregates of the three diatom cultures. We observed increases in viscosity of more than one order of magnitude inside the aggregates (Fig. 4), with areas of enhanced viscosity largely overlapping areas stained by Calcofluor white (specific for β-d-glucopyranose polysaccharides). We regard these estimates as conservative because the spatial coverage of the MPTM maps inside the aggregates is limited by the capacity of the microspheres to penetrate the aggregates and by self-shading. Nonetheless, given the important role that aggregates play as hotspots for zooplankton foraging and microbial activity and the importance of diffusive processes in sinking of porous particles in stratified water columns (28), we believe maps of viscosity such as those we present here will allow a more accurate characterization of processes influencing the biological carbon pump, such as the flux of chemicals that controls remineralization rates inside the aggregates (29) and the sinking speed of marine snow.

The implications of microscale viscosity gradients are grounded in fundamental physics and are pervasive, as viscosity impacts virtually all processes and interactions occurring in the microbial world. A quantitative assessment of these implications is still not possible, given current knowledge on the functional responses of relevant processes to viscosity (SI Appendix, Discussion for an in-depth treatment of this topic). However, we can be certain that increasing viscosity decreases the diffusivity of dissolved substances, small passive particles, and organisms, and slows motile plankton and sinking particles. Therefore, changes in viscosity not only affect the distribution of organisms and their resources, but also slow ecological rates, resulting in a cascade of effects at different ecological levels and scales (SI Appendix, Discussion). Increasing viscosity around osmotrophs decreases nutrient uptake rates, impacting primary and bacterial productivities. Similar reductions in encounter rates between predators (or viruses) and prey (or hosts), along with uncertain effects on the formation and sedimentation of aggregates, ultimately influence the transfer of carbon across trophic levels and the strength of the biological carbon pump. In summary, the inclusion of viscosity adds a layer of complexity to the current paradigm of microscale heterogeneity. The use of microrheological techniques, capable of delivering maps of viscosity with spatial resolution relevant to microorganisms, will allow a quantitative exploration of how these ideas unfold from the microscale upwards.

Materials and Methods
Phytoplankton Species and Cultures: Formation of Aggregates.
Growth conditions. We performed our experiments on the diatoms C. affinis (CCAP 1010/27), C. fusiformis (CCAP 1017/2), and S. pseudocostatum (CCAP 1077/7), the dinoflagellate A. minutum (CCAP 119/15), and O. ovata (GOMP17), and the haptophyte P. globosa (strains K-1321 and K-1322, Norwegian Culture Collection of Algae). Diatoms were grown in f/2 + Si medium (30) in 33.5 g/L artificial seawater (ASW, Aquarium Systems Instant Ocean Salt). A. minutum and P. globosa K-1323 were grown on L1 medium (31) in 30 g/L ASW. P. globosa K-1321 was grown on TL medium (Norwegian Culture Collection of Algae) in 30 g/L ASW. All species, except O. ovata, were grown at 19 °C without shaking in an algae incubator (Algaetron AG 230, Photon Systems Instruments) with a 12:12-h illumination cycle. The light intensity was 100 μmol photons m⁻² s⁻¹ for A. minutum and P. globosa. O. ovata was grown with L1 medium in 38.5 g/L ASW and kept at laboratory temperature (20 °C) on a windowsill to ensure it received enough illumination. We performed our experiments on the diatoms A. minutum (CCAP 119/15), and O. ovata (GOMP17), and the haptophyte P. globosa (strains K-1321 and K-1322, Norwegian Culture Collection of Algae). Diatoms were grown in f/2 + Si medium (30) in 33.5 g/L artificial seawater (ASW, Aquarium Systems Instant Ocean Salt). A. minutum and P. globosa K-1323 were grown on L1 medium (31) in 30 g/L ASW. P. globosa K-1321 was grown on TL medium (Norwegian Culture Collection of Algae) in 30 g/L ASW. All species, except O. ovata, were grown at 19 °C without shaking in an algae incubator (Algaetron AG 230, Photon Systems Instruments) with a 12:12-h illumination cycle. The light intensity was 100 μmol photons m⁻² s⁻¹ for A. minutum and P. globosa. O. ovata was grown with L1 medium in 38.5 g/L ASW and kept at laboratory temperature (20 °C) on a windowsill to ensure it received enough illumination. C. affinis cultures for the MOT experiments were also grown at room temperature higher than water (11). To test this hypothesis, we used MPTM to map viscosity around C. affinis cells before and after light-induced lysis (27), as well as around dead S. pseudocostatum, A. minutum, and O. ovata cells. We detected statistically significant increases in background viscosities of between 4 and 29% after exposure to UV and bright light in eight out of nine C. affinis samples, most likely because all cells within the FoV were lysed. Additionally, we observed localized and persistent patches of high viscosity around lysed cells (Fig. 2), as well as steep viscosity gradients around dead cells. Lysing and dead
Aggregates of cells were formed from C. affinis, C. fusiformis, and S. pseudocostatum cultures by incubating newly refreshed monocultures on a roller table (32). The roller was on the windowsill with natural light conditions at 20 °C. Within a few hours, aggregates were visible. Individual aggregates were collected very gently with a capillary tube to restrict disruption and prepared for MPTM analysis using the same protocol as for phytoplankton below.

**MOT.** We performed MOT to measure viscosity around C. affinis and around inert glass shards that mimicked the siliceous frustule of a diatom. MOT uses single, optically trapped microspheres as probes for measuring the rheological properties of the material surrounding them. Each microsphere is held by a tightly focused laser beam and can be manipulated in three-dimensional space, allowing it to be placed at specific locations around an object of interest (e.g., a phytoplankton cell). A microsphere confined within an optical trap will move with amplitudes on the nanometer scale due to the thermal fluctuations of the molecules of the surrounding material. The time-dependent mean-squared displacement calculated from the residual motion of a trapped microsphere can be used to compute the rheological properties of the fluid around it as well as the strength of the optical trap (33–36). The relative viscosity (defined as the ratio of local absolute viscosity to that of ASW) around C. affinis cells and glass shards was calculated by plotting the microsphere’s normalized position autocorrelation function against time (34). Details on our use of MOT are given in SI Appendix, Materials and Methods.

**Edge effects.** In all our calculations we accounted for the hydrodynamic effects caused by the proximity to a solid object. A moving sphere experiences an increase in hydrodynamic drag at close proximity to a solid surface. Since the hydrodynamic drag coefficient \( \gamma \) is proportional to the dynamic viscosity \( \eta \) of the fluid, this effect translates into an apparent increase in the viscosity of the fluid near the boundary. This increase in \( \gamma \) has been estimated by Faxén’s law (37, 38) and depends on the radius of the sphere \( a \), its distance from the surface \( s \), and the viscosity of the material in which the sphere is suspended. Faxén’s effect experienced by a microsphere moving parallel to the surface is given by (39)

\[
\gamma^\parallel = \frac{\gamma}{1 - (9/16)(a/s) + (1/8)(a/s)^2},
\]

where \( \gamma^\parallel \) is the correct hydrodynamic drag coefficient for a sphere moving parallel to the surface, \( \gamma \) is the hydrodynamic drag coefficient far from the surface, which for a sphere is \( \gamma = 6 \times a \eta \). Similarly, for the microsphere’s motion perpendicular to the surface Faxén’s effect is

\[
\gamma^\perp = \frac{\gamma}{1 - (9/8)(a/s) + (1/2)(a/s)^2},
\]

where \( \gamma^\perp \) is the corrected hydrodynamic drag coefficient for a sphere moving perpendicular to the surface. The apparent increase in relative viscosity is purely due to hydrodynamic effects close to a boundary wall, in the parallel and perpendicular directions, is given by the ratio of the drag coefficients \( \gamma^\parallel/\gamma \) and \( \gamma^\perp/\gamma \), respectively.

**Anisotropy.** We assessed the anisotropy of the local relative viscosity measured using MOT by calculating the ratio of the relative viscosity measured from the particle motion perpendicular to the edge of the cell or glass shard by the relative viscosity measured from the particle motion parallel to the edge of the cell or glass shard. Fig. 18 includes Faxén’s effect and hence the effects purely due to the hydrodynamics near a solid wall by plotting \( \gamma^\perp/\gamma^\parallel \) for varying distances from the edge of the object.

**Passive MPTM.** To assess spatial variability in viscosity at the microscale, we used passive MPTM (40). Briefly, the sample of interest was seeded with fluorescent microspheres of known diameter and density. The microspheres were tracked under the microscope as they underwent free Brownian motion. From analyses of their trajectories, and given a known and constant temperature, it is possible to estimate the local dynamic viscosity of the fluid into which the microspheres were suspended. We partitioned the 2D field of view into squares of given length and estimated average dynamic viscosity from analysis of all tracks registered within each square. This procedure allowed us to generate maps of relative viscosity with a resolution of 2 μm, around individual phytoplankton cells as well as around, and to a certain extent within, aggregates. All our maps have been published without any preselection in a Figshare collection (18). Details on the sample preparation and visualization and the calculation of viscosity are given in SI Appendix, Materials and Methods.
Lysis of individual phytoplankton cells was induced by exposing them to ultraviolet (UV) and white light at maximum intensities for ~10 min, following Smriga et al. (27). The field of illumination was narrowed to minimize effects on nearby cells. In the case of diatoms, the lysing procedure was deemed successful when motile bacteria started to aggregate around the cell (signaling release of material). To avoid hydrodynamic interactions of swimming bacteria with the microscopes, we waited until the cloud of bacteria had dissipated before starting the recordings, which could take at least 10 min but no more than 1 h. In the case of dinoflagellates, which are motile, we immobilized the cells by exposing them to UV and white light at the maximum intensity for a short period. Upon exposure to intense light A. minutum cells would first lose the lateral flagellum and then the polar one. If the light were left on longer, a "blob" of material would be seen coming out of the cell.

**Exopolymer staining.** Simultaneously with the MPTM of aggregates we assayed the presence of several labelings commonly used to visualize different exopolymers. Of the other stains, Con A and CFW produced the highest signal. In fluorescence microscopy, these stains revealed an intricate network of polysaccharides within our samples, but with Alexa 488, CFW, and Alcian blue. Alcian blue 752 (to S.H. and Ó.G). M.T., A.J.W., and T.M. acknowledge support through Engineering and Physical Sciences Research Council/Biotechnology and Biological Sciences Research Council/Medical Research Council Grants (EP/R035067/1, EP/R035563/1, and EP/R035156/1).