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**REVIEW**

# The 100 µm length scale in the microbial ocean

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ABSTRACT: Marine microorganisms live and interact at the microscale. Yet, in a field traditionally dominated by human-scale sampling approaches, we are only just beginning to develop an appreciation for the importance of microbial microscale processes. Here, I propose that the 100 µm length scale is a useful yardstick to measure and develop an intuition for this rich microscale world, because it characterizes a range of physical, chemical and biological processes that occur in a microbe's quotidian journey through the sea.

KEY WORDS: Marine microbes · Microscale interactions · Microbial ecology · Gradients · Chemotaxis · Motility · Turbulence



## 100 µm: A FOCAL SCALE

The microbial ocean has traditionally been studied at scales of tens of liters, yet the organisms that form the subject of these studies live at scales of nanoliters (Stocker 2012). Bulk approaches are continuing to provide invaluable information on the average composition and functions of microbial populations, but they are not designed to capture the heterogeneity, within-population variability, and cell-scale dynamics that are increasingly becoming important facets of marine microbial ecology. Research in recent years has brought about an appreciation for the importance of the microscale in microbial oceanography, through *in situ* observations with techniques such as single-cell genomics (e.g. Rinke et al. 2013, Kashtan et al. 2014) and NanoSIMS (e.g. Thompson et al. 2012), as well as controlled laboratory approaches such as microfluidics (e.g. Stocker et al. 2008, Seymour et al. 2010, Rusconi et al. 2014, Yawata et al. 2014). In complementing macroscale with microscale approaches, it is important to develop an intuition for life at the microscale, to appreciate how small small is, and how microbial interactions, particularly physical ones, can be inherently different in a microscale world compared to the macroscale world to which our intuition is trained. Here, I propose that 100 µm is a useful yardstick (Fig. 1) for developing an appreciation for the microbial ocean and for life at the microscale, because it characterizes a number of important features and processes of the marine microbial world.

100 µm is intended throughout this article as a reference length scale, not an absolute value. Physicists have a notation for this concept: O(100 µm) or 'in the order of 100 µm', from several tens of micrometers to a few hundreds of micrometers. I also recognize that there is a plurality of scales that affect microorganisms, from the nanometer scale of nutrient and toxin molecules and surface appendages, to the kilometer scale of ocean mesoscale processes. This plurality of scales notwithstanding, I suggest that 100 µm represents a useful, relevant anchor point to begin to intuit the life of marine microorganisms at the scale at which they live it. Several examples are presented below.

100 µm is the characteristic minimum distance between bacteria in seawater. The concentration of bacteria in most regions of the ocean is surprisingly constant, in the order of $10^6$ bacteria $ml^{-1}$. If we

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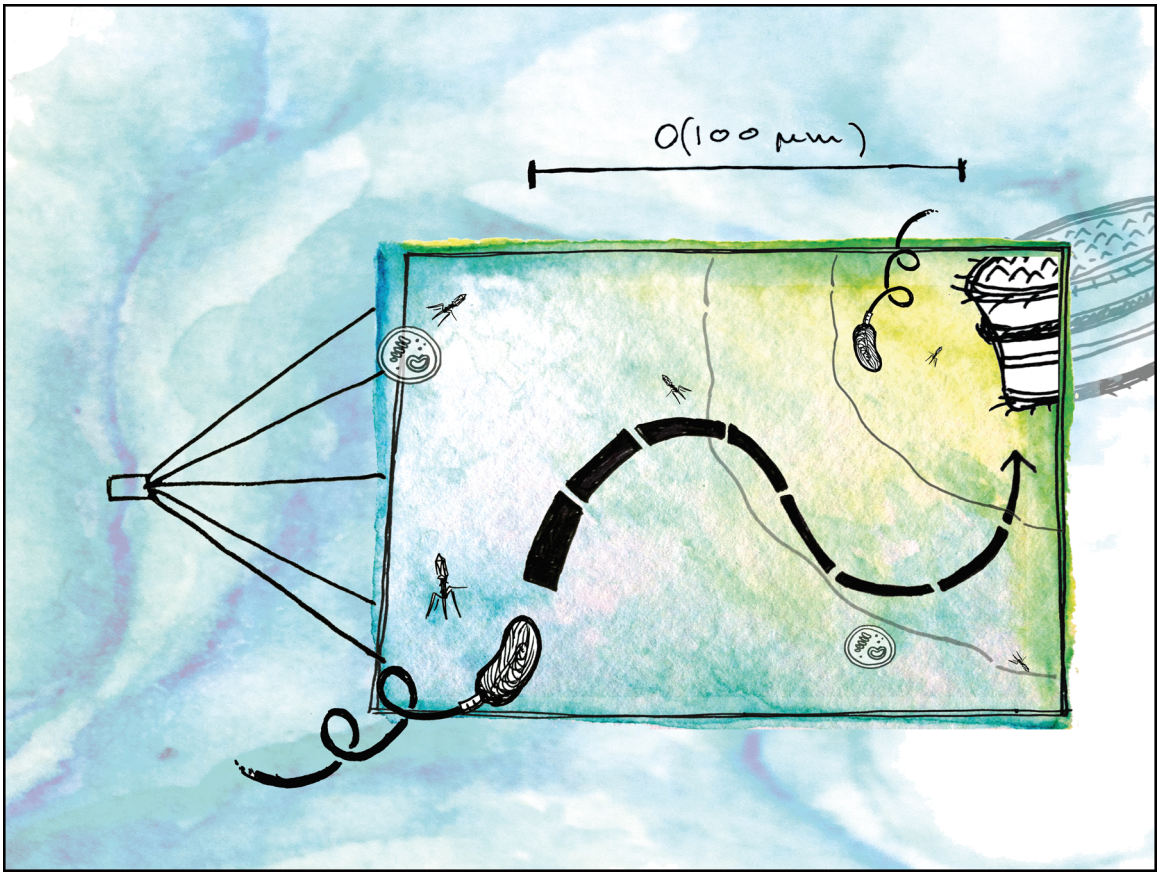


Fig. 1. 100 μm is a useful yardstick in the microscale world of marine bacteria. While environmental processes affecting microbial ecology often unfold at larger scales, as exemplified by the phytoplankton bloom depicted in blue hues in the background, it is the microscale that most immediately affects the life of marine and aquatic bacteria. Characteristic distances among bacteria, whether motile (flagellated cells) or non-motile (circular, non-flagellated cells), as well as between microbes and viruses occur over scales on the order of 100 μm, here denoted as 'O(100 μm)'. So, too, do gradients of dissolved organic matter (yellow-green coloration and grey contour lines) emanating for example from phytoplankton (diatom on the top right), to which motile bacteria can respond by directional swimming (thick dashed trajectory). Processes such as turbulence (not depicted) also contribute to spatial heterogeneity at O(100 μm) scales, further increasing the prominence of this scale in microbial ecology

arranged these bacteria uniformly in a cube with 1 cm sides (i.e. in 1 ml of seawater), the distance between a bacterium and its nearest neighbor would be 100 μm. Because this spacing is proportional to the cubic root of bacterial concentration, it is rather insensitive to variations in concentration: a 10-fold change in concentration causes only a ~2-fold (i.e. $10^{1/3}$) change in the spacing. For example, at a low concentration of $10^5$ bacteria $ml^{-1}$, the distance is 215 μm; at a high concentration of $5 \times 10^6$ bacteria $ml^{-1}$, the distance is 58 μm. If we take into account that bacteria are not arranged in a regular lattice and consider $10^6$ randomly distributed bacteria per milliliter, the distance becomes 55 μm (this is the mean distance to the nearest neighbor and is smaller than the 100 μm value because of the biased significance of smaller cell-to-cell distances over larger ones). Similar considerations apply to the distance between bacteria and viruses. For example, assuming $10^6$ bacteria $ml^{-1}$and $10^7$ viruses $ml^{-1}$, a bacterium is on average 26 μm from the nearest virus when both populations are randomly distributed (interestingly, a virus is then 55 μm from the nearest bacterium). These values are all O(100 μm). Therefore, bacteria in the ocean are typically O(100 μm) away from another bacterium or virus and this distance is relatively insensitive to variations in cell concentration.

100 μm is approximately the distance covered by motile marine bacteria in 1 s. Marine bacteria are known to be fast swimmers by comparison with *Escherichia coli*, the undisputed model organism for bacterial motility. Whereas the latter swims at 15 to 30 μm $s^{-1}$, multiple species of marine bacteria have been reported to swim at mean speeds of 60 to 80 μm

$s^{-1}$ (Mitchell et al. 1995, Stocker et al. 2008, Seymour et al. 2010, Hütz et al. 2011), with instantaneous bursts of up to a few hundred micrometers per second (Mitchell et al. 1995). Notwithstanding the existence of exceedingly fast marine bacteria clocking up to 1000 µm $s^{-1}$ (Fenchel & Thar 2004), speeds of bacteria in the ocean are thus frequently O(100 µm $s^{-1}$). Over a time period of 1 s, this results in a distance traveled of O(100 µm). The 1 s timescale used in this argument is based on the observation that the correlation time in the trajectories of bacteria is O(1 s). The correlation time is the time between marked changes in swimming direction, which are needed for example to climb chemical gradients (chemotaxis). Although the mechanisms for reorientation in bacteria are diverse — from 'tumbles' in *E. coli* (Berg 1993) to 'reversals' and 'flicks' in marine bacteria (Xie et al. 2011, Son et al. 2013) — the timescale between reorientations is often O(1 s): very close in fact to 1 s for *E. coli* (Berg 1993) and somewhat lower for marine bacteria based on the very few species for which it has been quantified (e.g. ~0.3 to 1 s for *Vibrio alginolyticus*; Xie et al. 2011, K. Son, F. Menolascina & R. Stocker unpubl. data). Therefore, the distance over which a marine bacterium swims approximately straight ('approximately' because it is always affected by small reorientations due to rotational diffusion; see Berg 1993) is O(100 µm). Interestingly then, a motile marine bacterium swims approximately straight for a distance comparable to the distance to its nearest neighbor (although of course usually not in that direction, i.e. this coincidence does not impact encounter rates).

100 µm is the typical size of the cluster of bacteria that accumulate by chemotaxis around microscale nutrient sources. As bacteria sense chemical concentrations in their environment using dedicated receptors, they can direct their otherwise random motility up resource gradients, accumulating near resource sources. Their ability to retain position in the most desired location — typically, where the resource concentration is greatest — is counteracted by the random component intrinsic in their motility. Quantitatively, the ability of bacteria to climb chemical gradients is measured by the chemotactic velocity, $V_C$, representing the component of the swimming speed directed along the gradient. $V_C$ is often a modest to moderate fraction (10 to 30%; see Ahmed & Stocker 2008, Stocker 2012) of the swimming speed, thus frequently in the range of 3 to 15 µm $s^{-1}$. Meanwhile, the random component of swimming is measured by the diffusivity $D$ associated with the bacteria's random walk, with $D$ often around 300 $\mu m^2$ $s^{-1}$ (Ahmed & Stocker 2008, K. Son, F. Menolascina & R. Stocker unpubl. data). Solution of the mathematical equation that describes the spatial distribution of chemotactic bacteria in a chemical gradient (the Keller-Segel equation) under certain simplifying assumptions reveals that the distribution of bacteria in a linear chemical concentration profile is exponential, i.e. it decays with distance $x$ from the source proportionately to $\exp(-x/L)$, where $L = D/V_C = 20$ to 100 µm is the length scale that measures the width of the accumulation of cells. Recent experiments with *V. alginolyticus* in which $L$ was measured directly from the observed distribution of bacteria in chemical gradients yielded $L = 50$ to 200 µm (K. Son, F. Menolascina & R. Stocker unpubl. data). Thus, bacterial clusters around resource hotspots in the ocean take the form of O(100 µm) thick regions or bands in which cells are substantially more concentrated than in the bulk, as also observed in some of the first experiments on microscale hotspots (Blackburn et al. 1998).

100 µm is the scale of many nutrient hotspots for heterotrophic marine bacteria in the water column. Phytoplankton represent an important source of dissolved organic matter (DOM) for bacteria and abundant species range in size between 10 and 100 µm. Gradual release of DOM from phytoplankton through exudation results in regions of enhanced DOM around individual phytoplankton cells (the 'phycosphere') that extend a few cell diameters from the phytoplankton surface, i.e. they are O(100 µm) in size. Rapid release of DOM from phytoplankton through lysis produces more intense and expansive phycospheres, yet the strongest accumulation of bacteria occur again in a region of O(100 µm) (S. Smriga et al. unpubl. data). Excretions by zooplankton, whose size is O(100 µm) to O(1000 µm), occur on a scale that is a fraction of the zooplankton size, hence again O(100 µm). Marine particles, which are frequently O(10 µm) to O(1000 µm) in size (the operational size cutoff for marine snow particles is 500 µm) represent ubiquitous hotspots for bacteria (Alldredge & Silver 1988). Bacteria can attach to the particles or benefit from the high DOM concentrations in their wakes (Kiørboe & Jackson 2001), which are often narrower than the particle diameter and O(100 µm) (Kiørboe et al. 2001). Droplets of crude oil, particularly those on the smaller end of the spectrum where microbial activity is presumably highest due to the large surface-area-to-volume ratio, are also O(100 µm) in size (e.g. Vilcaez et al. 2013).

100 µm is also an important scale for gradients at the ocean bottom. In many regions of the ocean, the sediment–water interface is characterized by strong

gradients in oxygen, which decreases sharply moving down from the water into the sediments. Frequently these are accompanied by opposite gradients in hydrogen sulfide, which decreases sharply moving up from the sediments into the water. Both types of gradients span several hundred micrometers and the oxygen gradient is utilized by bacteria via chemotactic or phobic responses to exquisitely retain position at the depth where chemical concentrations are optimal (Jørgensen & Revsbech 1983). In an entirely different environment at the ocean bottom, the surface of corals is characterized by diffusion boundary layers where concentrations of chemicals released from the coral, including dimethylsulfoniopropionate (DMSP), extend O(100 µm) to O(1000 µm) into the surrounding seawater, where they form gradients that bacterial pathogens follow with great speed and accuracy, presumably to find their coral host (Garren et al. 2014). Simultaneously, the coral–water interface is characterized by strong gradients in fluid velocity, as coral cilia drive vortical flows of O(100 µm) to O(1000 µm) (Shapiro et al. 2014).

100 µm is a fundamental scale for chemical gradients that bacteria experience in the ocean even when sources are smaller than 100 µm, because of the fast timescale of molecular diffusion at this length scale. Chemicals from intense sources smaller than 100 µm rapidly diffuse to O(100 µm). Molecular diffusion dictates that a point source of solutes with diffusivity $D$ expands to a size $L$ in a time $T = L^2/6D$. Thus, within only $T$ = 1.5 s a point release of small molecules, for which $D = 10^{-9}$ m$^2$ s$^{-1}$, diffuses to a size $L$ = 100 µm. The diffusing cloud of solutes progressively slows down as it grows in size — because $L = (6DT)^{1/2}$ — and as a result the cloud remains O(100 µm) in size (here interpreted as <1 mm) for almost 3 min. Similarly, viruses originating from the lysis of a cell spread in a diffusive fashion ($D \approx 10^{-12}$ m$^2$ s$^{-1}$) to form a 55 µm-radius cloud, thus on average reaching the nearest microbial neighbor in less than 10 min, illustrating that spatially heterogeneous processes at this scale can both benefit and harm bacteria, and both types of processes may affect the fitness value of motility.

100 µm is a fundamental scale for chemical gradients that bacteria experience in the ocean even when sources are larger than 100 µm, because of turbulent stirring, which can rapidly create filaments and sheets of O(100 µm). Whereas molecular diffusion is unavoidable and its magnitude is dictated almost exclusively by the chemical in question, the presence and intensity of turbulence are site-dependent. Yet, in many regions of the ocean, turbulence is prevalent, and its effect in stirring chemicals is rather insensitive to its intensity. The most immediate action of turbulence — and a fundamental precursor to its long-term, intuitive effect of mixing — is to stir what is suspended in water. This stirring produces finer and finer concentration features, down to a smallest scale — the Batchelor scale — below which molecular diffusion takes over and obliterates chemical gradients into homogeneity. Stirring thus reshapes heterogeneities in the distribution of nutrients into a tangled web of nutrient sheets and filaments (Guasto et al. 2012, Taylor & Stocker 2012). The smallest and most prevalent sheets and filaments occur at the Batchelor scale, given by $L_B = (\nu D^2/\varepsilon)^{1/4}$, where $\nu \sim 10^{-6}$ m$^2$ s$^{-1}$ is the kinematic viscosity of seawater and $\varepsilon$ is the turbulent kinetic energy, a measure of the strength of turbulence. For many regions of the ocean, $\varepsilon = 10^{-6}$ to $10^{-10}$ W kg$^{-1}$. Therefore, for small molecules with $D = 10^{-9}$ m$^2$ s$^{-1}$, $L_B$ = 30 to 300 µm, i.e. $L_B$ = O(100 µm).

100 µm is thus a focal scale in the microbial ocean, as it characterizes a multitude of processes relevant for or caused by marine bacteria. Bacteria are separated by a distance of O(100 µm) and can cover this distance in about 1 s; nutrient hotspots and chemical gradients that bacteria exploit often occur at scales of O(100 µm), both in the water column and near the ocean bottom, and bacteria are capable of forming strong accumulations around these hotspots, with a characteristic thickness of O(100 µm); and both molecular diffusion and turbulent stirring drive gradients towards the 100 µm length scale. It is clear that microbial processes unfold over a much broader spectrum of scales: to cite but one example, for the biochemical processes underpinning adhesion of cells to surfaces or adherence of viruses to hosts a scale of <1 µm is more relevant. Yet, I propose that if one length scale were to be emphasized within the continuum of scales that characterize microbial life in the ocean in order to help anchor our intuition for the elusive microworld, that length scale is 100 µm, in view of its importance in microbial encounter processes, the effects of fluid flow, and the role of chemical gradients.

## THE MICROSCALE ROAD AHEAD

More and more, marine microbial ecology is moving towards a single-cell understanding of the lives of microbes. Molecular tools including single-cell genomics as well as chemical characterization tools at the subcellular level such as NanoSIMS are yielding unprecedented information on the identity,

molecular activities and chemical activities of individual bacteria. Atomic force microscopy at the single-cell level is revealing unexpected conjoined lifestyles of microbes, highlighting the importance of single-cell encounter processes. Microfluidic tools are providing access to microbial behavior at the level of individual cells. Sea-going devices, including pneumatically operated syringes for sampling, fluorescent and holographic cameras for direct visualization, and microfluidic devices for behavioral quantification, are opening up the ocean for microscale exploration. Simultaneous — albeit not yet convergent — to this shift towards single cells is a strong and growing emphasis in microbial ecology on cell individuality, phenotypic heterogeneity, and the importance of considering variability among individual cells to understand population functions (Ackermann 2015). A focus on single cells underscores the importance of a cell's immediate microenvironment as one determinant of individuality. Yet, to a large extent our conceptual frameworks for interpreting single-cell molecular and chemical information are still those developed from decades of bulk-scale approaches.

Encounter rates with other cells, microscale chemical gradients, small-scale turbulence, as well as fluid forces and torques are the quotidian features of an individual bacterium's journey through the ocean, yet rarely are they taken into account, sometimes for lack of experimental frameworks, but most times for lack of conceptual frameworks.

I argue that this fascinating time in microbial ecology, where the single bacterium is becoming the lead character, will be the more fruitful in fostering new understanding the more we can integrate modern characterization efforts of a single cell with an understanding of that cell's immediate, microscale environment. Achieving this will require new experimental tools both in the laboratory and in the field, as well as a broader appreciation of conceptual frameworks for what the microscale environment looks like to a microbe. In this spirit, the 100 µm length scale represents a useful starting point to avoid getting lost in the cascade of scales, because it represents the convergence of multiple biological, chemical and physical processes in marine and aquatic environments.

We have only just begun to appreciate how rich the microscale world of marine bacteria is. An intuitive understanding of the microscale processes determining the lives of individual microbes can be a powerful guide in providing new answers — as well as shaping new questions — in marine microbial ecology.

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