

SHORT COMMUNICATION

Modelling the concentration of exuded dimethylsulphoniopropionate (DMSP) in the boundary layer surrounding phytoplankton cells

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Using a steady-state diffusion model, we calculated the concentration of the ecologically relevant algal metabolite dimethylsulphoniopropionate (DMSP) in the phycosphere. Incorporating diffusive losses, the concentration was predicted to be 10⁶-fold lower than previously suggested.

Dimethylsulphoniopropionate (DMSP; (CH₃)₂S⁺CH₂CH₂COO⁻) is an abundant secondary metabolite produced by many marine algae (phytoplankton and seaweeds). In a seminal paper in 1966, Fogg coined the term extracellular products to describe the vast array of substances released during normal growth of healthy phytoplankton cells (Fogg, 1966). The ecological importance of such photosynthetically derived dissolved exudates as a carbon source for planktonic bacteria has long been recognized (e.g. Cole, 1982). Recently, Fredrickson and Strom (Fredrickson and Strom, 2009) calculated that for the dinoflagellate *Heterocapsa pygmaea*, with a diameter of 8.5 µm, exudation of as little as 0.1% of intracellular DMSP results in a concentration of 49 µM within a 5 µm thick phycosphere. Their model provides an instantaneous and uniform

redistribution of DMSP within the volume of the phycosphere at a given distance from the cell edge, beyond which the concentration equals background levels. The authors acknowledged that their model does not account for losses, such as diffusion. Here, we present an alternative simple steady-state model to predict the concentration gradient of dissolved DMSP in the boundary layer around a spherical phytoplankton cell taking into account diffusive losses.

DMSP and its cleavage product dimethylsulphide (DMS; (CH₃)₂S) have attracted much research interest since DMS was proposed to affect climate (Charlson *et al.*, 1987). DMSP may account for 20% of phytoplankton carbon (Matrai and Keller, 1994) and intracellular concentrations in the range of 50–400 mM occur in the prymnesiophytes and dinoflagellates, and exceptionally

high values of 1 to 2 M have been reported (Stefels, 2000). As well as providing an important carbon and sulphur source for marine bacteria (Kiene *et al.*, 2000), DMSP has also been shown to act as a chemoattractant for bacteria (e.g. Zimmer-Faust *et al.*, 1996; Miller and Belas, 2006) and reef fish (Deboise *et al.*, 2008). Currently, there is debate over the role of DMSP in mediating phytoplankton–microzooplankton interactions with evidence suggesting that it may act as either a microzooplankton attractant (Breckels *et al.*, in preparation) or feeding inhibitor (Strom *et al.*, 2003; Strom *et al.*, 2007; Fredrickson and Strom, 2009). Despite the biogeochemical and ecological significance of DMSP, there is a lack of empirical data on the rate of exudation (Malin and Kirst, 1997) and its distribution in gradients surrounding phytoplankton cells.

Bjørnsen (Bjørnsen, 1988) estimated that healthy phytoplankton cells exude approximately 5% of their total carbon biomass each day from the low molecular weight carbon pool through passive diffusion, with the rate of cellular exudation proposed to be a function of phytoplankton surface area and biomass. Baines and Pace (Baines and Pace, 1991) dismissed passive diffusion as the main driver behind phytoplankton extracellular release, suggesting that, although it was possible for low molecular weight exudates to passively diffuse from cells, high molecular weight polysaccharide molecules which dominate phytoplankton exudates, would not conform to Bjørnsen's passive diffusion model. Nevertheless, they estimated that on average 13% of total phytoplankton carbon production is exuded as extracellular products (Baines and Pace, 1991). In the only study to-date that has aimed to model the exudation rates of DMSP, Laroche *et al.* (Laroche *et al.*, 1999) estimated that *Phaeocystis* sp. exudes between 3 and 11% and *Proocentrum minimum* exudes approximately 1% of its DMSP quota per day throughout latent, exponential and senescent growth phases. In the absence of turbulence, typical of the low Reynolds, low Péclet number environment experienced by many small phytoplankton, simple molecular diffusion is the predominant physical factor influencing the persistence of microscale nutrient patches (Seymour *et al.*, 2009). Phytoplankton exudates form diffusion-limited chemical gradients around the cell creating a layer described as the “active space” (Strickler, 1982) or “phycosphere” (Bell and Mitchell, 1972). The diffusion-limited accumulation of exudates in the boundary layer surrounding phytoplankton cells may provide infochemical cues for grazers. The ability of grazers to detect exuded infochemicals effectively increases the encounter radius of phytoplankton cells, which can substantially increase the probabilities of encounters between predators and their prey (Gerritsen and Strickler, 1977;

Buskey, 1997). The combination of grazer chemosensory threshold and the concentration gradient of the particular infochemical will dictate the encounter radius.

Diffusion of exuded solutes from a non-motile phytoplankton cell follows the same principle as diffusive intake (Berg and Purcell, 1977). Karp-Boss *et al.* (Karp-Boss *et al.*, 1996) calculated the flux of nutrient delivery to a cell as,

$$Q = 4\pi Da(C_\infty - C_0) \quad (1)$$

where Q is the flux of nutrients to the spherical cell, D the diffusion coefficient, a the radius of the cell, C_∞ the background concentration and C_0 the concentration at the cell surface. This equation can be reformulated to calculate the concentration of solutes at a given radius from the cell edge (C_r) using the equation presented by Mitchell *et al.* (Mitchell *et al.*, 1985) and modified by Kiørboe (Kiørboe, 2008) to take into account background concentration.

$$C_r = \frac{Q}{4\pi Dr} + C_\infty \text{ when } r \geq a \quad (2)$$

where Q is the flux of exuded solutes away from the cell. Equation (2) assumes diffusion from a point source at the centre of the cell and applies when r (radial distance from the cell centre) is greater than or equal to a (radius of the cell). The diffusion coefficient (D) of DMSP remains to be established; however, consistent with other small molecules, the diffusion coefficient was assumed to be $10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Karp-Boss *et al.*, 1996). It is important to note that equation (2) is susceptible to variations in diffusivity due to physicochemical properties of both the solute and the solvent. In our model, it is assumed that the phytoplankton cell is non-motile and in a non-turbulent regime, the rate of exudation is constant and DMSP is released evenly over the cell surface. As C_∞ is an additive term independent from cell exudation, we calculated concentration enhancement as $C_r - C_\infty$ to allow a simpler insight into the concentration enhancement of exuded DMSP. The enhancement of DMSP in the boundary layer surrounding different phytoplankton cells was modelled at a range of different exudation rates based on literature values and previous estimates.

The model predicted that dissolved DMSP (DMSP_d) in the phycosphere may be six orders of magnitude lower than previously suggested (Fredrickson and Strom, 2009). Furthermore, the results are consistent with data on the spatial distribution of amino acid exudates (Mitchell *et al.*, 1985). For example, in the case of

Table I: DMSP and cell characteristics for different species of phytoplankton used in the model (values shown are means)

Species	Cell diameter (μm)	Cell volume (μm^3)	Intracellular DMSP concentration (mM)	DMSP content (pg cell^{-1})	Reference
<i>Phaeocystis</i> sp.	6.0	113.0	88.5	1.34	Laroche <i>et al.</i> (1999)
<i>Heterocapsa pygmaea</i>	8.5	321.6	451.0	19.4	Keller <i>et al.</i> (1989)

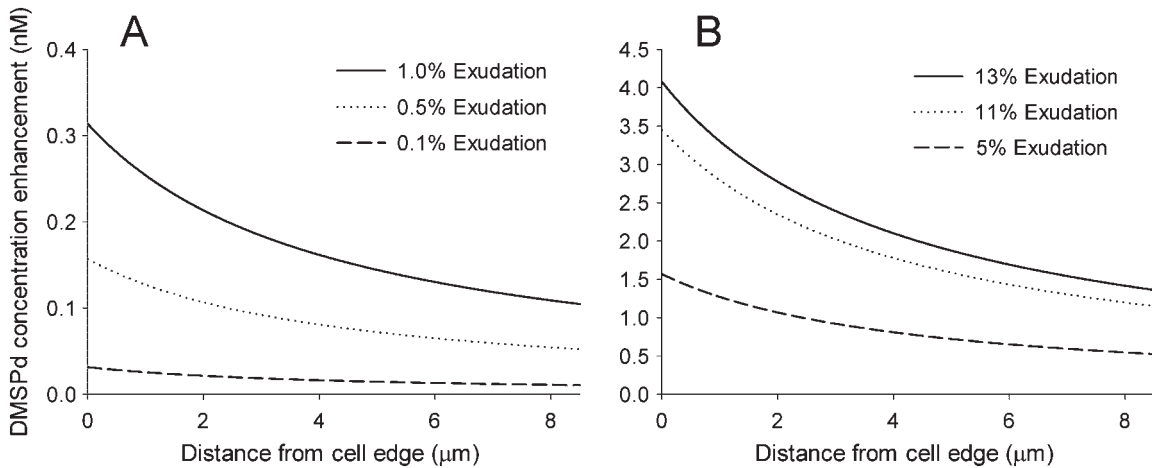


Fig. 1. Dissolved DMSP (DMSPd) concentration in the cell boundary layer of *Heterocapsa pygmaea*. (A) DMSPd concentration enhancement in the boundary layer with total DMSP exudation rates of 0.1, 0.5 and 1% day^{-1} of the total DMSP content (as in Fredrickson and Strom, 2009). (B) Concentration enhancement of DMSPd assuming DMSP is exuded at a rate of 5% day^{-1} (Björnsen, 1988), 11% day^{-1} (Laroche *et al.*, 1999) and 13% day^{-1} (Baines and Pace, 1991). X-axis extends to $8.5 \mu\text{m}$ ($r/a = 2$), cell size and DMSP data are provided in Table I.

the dinoflagellate *Heterocapsa pygmaea* (see cell size and DMSP data in Table I), exudation of just 0.1% of its DMSP content per day resulted in a concentration enhancement of only 14.4 pM above background levels at a distance of $5 \mu\text{m}$ from the cell edge (Fig. 1A) as opposed to $49 \mu\text{m}$ in the study by Fredrickson and Strom (Fredrickson and Strom, 2009). Whereas an exudation rate of 11% day^{-1} of the intracellular DMSP content resulted in DMSPd concentrations of 2.35 and 1.59 nM above the background level at distances of 2 and $5 \mu\text{m}$, respectively (Fig. 1B). Using modelled data for the exudation rate of DMSP from *Phaeocystis* sp. (Laroche *et al.*, 1999; see cell size and DMSP data in Table I), it is clear that even at high exudation rates the concentration enhancement of DMSPd does not exceed 0.4 nM close to the cell surface (Fig. 2). This is, however, a conservative estimate as the intracellular DMSP content ($1.34 \text{ pg cell}^{-1}$ equating to 88.5 mM; Table I) used in the model was recorded in early logarithmic growth. During late exponential and senescent phases, DMSP concentrations increase (Laroche *et al.*, 1999) and Stefels and van Boekel (Stefels and van Boekel, 1993) recorded intracellular DMSP concentrations during the senescent phase of around 160 mM.

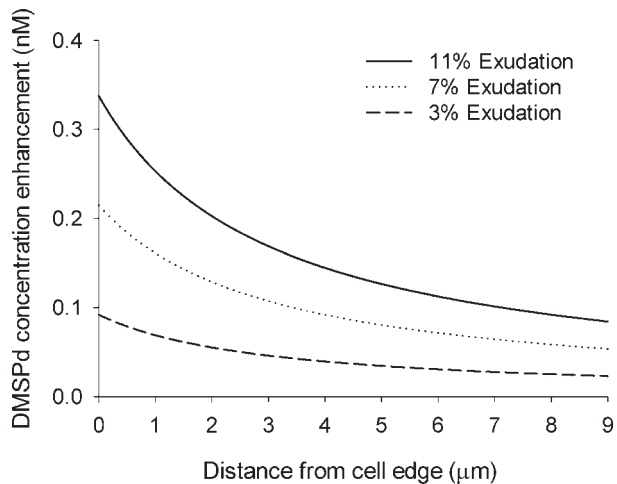


Fig. 2. DMSPd concentration enhancement in the boundary layer of *Phaeocystis* sp. across growth phases based on DMSP exudation rates between 3% and 11% day^{-1} of the DMSP quota (Laroche *et al.*, 1999). X-axis extends to $9 \mu\text{m}$ ($r/a = 3$), cell size and DMSP data are provided in Table I.

If DMSP concentrations are directly related to exudation rates, an increase in intracellular DMSP during the stationary and senescent growth phases may increase

the concentration of DMSPd in the phycosphere accordingly.

DMSP is a hydrophilic zwitterion and, as in the case of amino acids, the diffusion coefficient is likely to be inversely related to its concentration in aqueous solution. Ma *et al.* (Ma *et al.*, 2005) empirically measured the diffusion coefficient of different amino acids at varying concentrations from 10 to 900 mM and found that as the molecular volume and polarity of amino acids increased, the diffusion coefficient decreased. Across the range of amino acids and concentrations examined, the diffusion coefficient varied from $\sim 0.65 \times 10^{-5}$ to $1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Ma *et al.*, 2005). In the case of DMSP diffusing from a phytoplankton cell, the concentrations concerned are significantly lower than those used in the study by Ma *et al.* (Ma *et al.*, 2005). Hence, the diffusion coefficient for DMSP is likely to be closer to the upper end of the range. However, the model presented here is subject to changes in diffusivity and due to the suggested importance of DMSP as an infochemical signalling molecule, further research is required to establish its diffusion coefficient. Additionally, phytoplankton extracellular polymeric substances (EPS) rich in high molecular weight polysaccharides (10–30 kDa) (Bhaskar and Bhosle, 2005) may reduce the diffusivity of exuded low molecular weight molecules such as DMSP and amino acids, resulting in localized concentration enhancements. However, the extent of EPS production and its composition is highly species specific, and depends on the physicochemical properties of the water and the phytoplankton growth phase. Hence, the influence of these substances on the diffusivity of low molecular weight exudates remains to be established.

The amount of exuded DMSP required to mediate trophic interactions will depend on the species-specific chemosensory threshold necessary to elicit a behavioural response. A concentration threshold of between 10 and 100 nM has been shown to elicit positive chemotaxis in *Alcaligenes* strain M3A bacteria (Zimmer-Faust *et al.*, 1996) and in the heterotrophic dinoflagellate *Oxyrrhis marina* (Breckels *et al.*, in preparation). These experiments used microcapillary assays and it is likely that the threshold concentration required to elicit a response is probably lower than the concentration of the source solution because of diffusive losses during the formation of the chemical gradient. Furthermore, Miller and Belas (Miller and Belas, 2006) observed DMSP-induced positive chemotaxis of *Roseobacter* to the dinoflagellate *Pfiesteria piscicida* CCMP 1830. *Pfiesteria piscicida* has a cell diameter of 10–12 μm (<https://ccmp.bigelow.org/>) and an internal DMSP content of $0.49 \text{ pg cell}^{-1}$ (Miller and Belas, 2004), the DMSPd enhancement 5 μm from the

cell edge is approximately only 32 pM above background levels, even when assuming a relatively high DMSP exudation rate of $10\% \text{ day}^{-1}$. This indicates that natural threshold concentrations required to elicit a response to DMSPd may lie in the pM concentration range. In addition, phytoplankton exude a vast array of other compounds throughout their life cycle (e.g. Barofsky *et al.*, 2009) and the interactive effect of a synergistic “infochemical bouquet” may provide a stronger, more reliable signal to other organisms (Hay, 2009). Furthermore, the signal to noise ratio rather than the absolute concentration of the chemical stimuli may be more important in eliciting a behavioural response. Using a revised sampling approach to analyse DMSPd concentrations, Kiene and Slezak (Kiene and Slezak, 2006) found that across a broad range of ocean water types and particulate DMSP concentrations DMSPd concentrations were $\leq 2.8 \text{ nM}$. Given that the highest background concentration of DMSPd may often be in the low nM range, it is possible that exuded DMSP in the boundary layer surrounding phytoplankton cells can provide concentrations that are elevated high enough above the background concentration to act as infochemical signals, particularly in the low DMSP oligotrophic waters. However, our results also suggest that concentrations of dissolved DMSP in the boundary layer surrounding phytoplankton cells are unlikely to reach the concentrations required to inhibit microzooplankton grazing observed by Fredrickson and Strom (Fredrickson and Strom, 2009).

The simple model we have used here takes into account diffusive losses of dissolved DMSP exuded from phytoplankton cells at steady state and suggests that previous results may have overestimated phycosphere DMSPd concentrations. The model does not incorporate losses due to bacterial consumption of DMSPd, the possible presence of extracellular DMSP-lyase enzymes, or cell motility (either due to self propulsion or differences in density between the cell and the water). All of which would further reduce the concentrations of DMSP in the phycosphere. Cell size and velocity will influence the physicochemical properties acting on the distribution of dissolved solutes, resulting in modifications of the solute distribution (Kjørboe *et al.*, 2001). However, little is known about the effect of motility on the distribution or uptake of dissolved solutes (but see Magar *et al.*, 2003; Magar and Pedley, 2005). The model used here may provide a useful tool for estimating the concentration of DMSPd and similar molecules in the phycosphere especially for small, slow swimming phytoplankton inhabiting a low Reynolds, low Péclet number environment. However, to gain a fuller understanding of phytoplankton boundary layer conditions,

further investigation is needed to ascertain DMSP loss rates and provide empirical data for exudation rates.

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