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Disaggregation of *Microcystis aeruginosa* colonies under turbulent mixing: laboratory experiments in a grid-stirred tank

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Abstract

Samples of the cyanobacterium *Microcystis aeruginosa* from a small pond were used in laboratory experiments with a grid-stirred tank to quantify the effect of turbulent mixing on colony size. Turbulent dissipation in the tank was varied from 10^{-9} m^2 s^{-3} to 10^{-4} m^2 s^{-3}, covering the range of turbulence intensities experienced by *M. aeruginosa* colonies in the field and exceeding the maximum dissipation by two orders of magnitude. Large colonies broke up into smaller colonies during the experiments; the mass fraction of colonies with diameter less than 200 μm increased over time. Colony disaggregation was observed to increase with turbulent dissipation. The maximum stable colony diameter across all experiments was in the range 220–420 μm. The overall change in size distribution during the experiments was relatively small, and the colony size distribution remained very broad throughout the experiments. Since colony size affects migration velocity, susceptibility to grazing and surface area to volume ratios, more work is needed to determine how to best represent this broad size distribution when modelling *M. aeruginosa* populations.

Introduction

Microcystis aeruginosa Kütz. emend. Elenkin and other species of cyanobacteria can form toxic surface blooms which create major water quality problems (Reynolds, 1997a; Falconer, 1999). The formation of surface blooms by *M. aeruginosa* is facilitated by gas vesicles, which provide buoyancy to the cells. Vertical migration provides a competitive advantage for *M. aeruginosa* over negatively buoyant and slower migrating species, especially under intermittent mixing conditions (Ganf & Oliver, 1982; Humphries & Lyne, 1988; Ibelings et al., 1991b; Mitrovic et al., 2001). Colony size has a large effect on migration velocity (Hutchinson, 1957; Reynolds, 1984; Reynolds et al., 1987), grazing pressure (Oliver & Ganf, 2000) and surface area to volume ratio. In turn, surface area to volume ratio affects light attenuation (Kirk, 1975; Robarts & Zohary, 1984), growth rate (Reynolds, 1997b) and nutrient uptake. Hence any models of *M. aeruginosa* which include vertical migration require accurate values for colony size distribution (O'Brien, 2002).

Turbulent mixing can reduce the competitive advantage of *M. aeruginosa* by preventing vertical migration of the colonies, and so altering the light climate (Ibelings et al., 1991a; Wallace & Hamilton, 1999; Oliver & Ganf, 2000). Very high turbulence intensities sustained over many days can adversely effect

metabolic activity and viability of *M. aeruginosa* cells (Regel et al., 2003). Turbulence can also increase rates of both aggregation and disaggregation of phytoplankton, and lake and marine snow (e.g. Alldredge et al., 1990, Grossart & Simon, 1993, Jackson & Lochmann, 1993). The net effect of turbulence on *M. aeruginosa* colony size will depend on whether aggregation or disaggregation predominates. There is qualitative evidence for the break-up of *M. aeruginosa* colonies under turbulent conditions (Robarts & Zohary, 1984), while recent experimental work found that individual cells of *M. aeruginosa* may aggregate in turbulence (Regel et al., 2003). Naselli-Flores & Barone (2003) found that mean *M. aeruginosa* colony size was greatest when the mixing and euphotic depths were close to zero in Lake Arancio. It has been suggested that the smallest eddies in a turbulent flow (the Kolmogorov scale) place an upper limit on the size of phytoplankton in general (Reynolds, 1998). Quantifying the effect of turbulent mixing on colony diameter is essential for accurate models of *M. aeruginosa* populations and prediction of bloom formation.

It is proposed here that turbulent mixing causes a net reduction in *M. aeruginosa* colony size through disaggregation. Here, laboratory experiments are used to quantify the impact of turbulent mixing on the size distribution of *M. aeruginosa* colonies taken from a sheltered pond.

Materials and methods

Experimental procedure

Samples of *M. aeruginosa* were taken from the Reid Library Pond at the University of Western Australia on consecutive days in July 2001. The pond is a shallow (<1 m), sheltered, artificial water-body with prolific blooms of *M. aeruginosa*. In-situ samples were chosen over laboratory-cultured samples because *M. aeruginosa* is typically unicellular in continuous culture, and colonial in the field (Ibelings, 1992; Regel et al., 2003). The species was formally identified as *Microcystis aeruginosa* Kütz. emend. Elenkin. Microscopic examination verified that samples were dominated by *M. aeruginosa* (>99% cell counts).

Colonies of up to 100 mm in size were found in the Reid Pond. The large colonies were extremely fragile and broke up with minor disturbance, which was consistent with the findings of (Robarts & Zohary, 1984). Samples were removed to the laboratory with minimal disturbance and no mixing, but the maximum colony diameter measured immediately on arrival in the laboratory (referred to as sample $T = -10$ minutes) was approximately 1 mm.

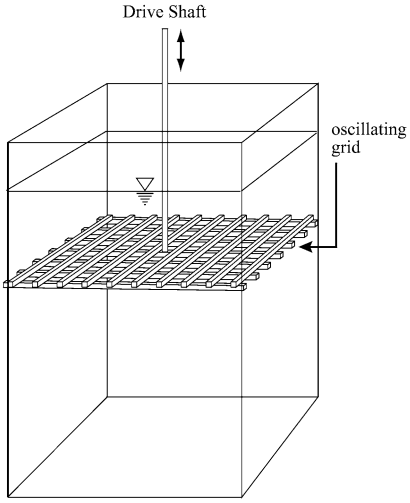

Figure 1. Grid-stirred tank. The base is square (61 × 61 cm), and tank depth is 90 cm.

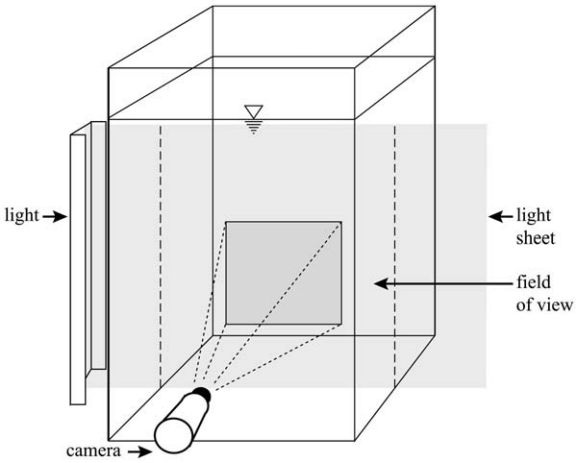

Figure 2. Perspex viewing vessel (6 × 6 × 16 cm), with vertical light sheet.

Turbulent mixing was generated in a laboratory tank by a vertically oscillating, horizontal grid. The grid was made from 1 cm square aluminium bars, each separated by 5 cm (mesh size $M = 6$ cm), and was contained within a square-based Plexiglas tank of dimensions 61 × 61 × 90 cm (Fig. 1). The mean position of the grid was 53 cm above the tank floor. The stroke length S was 4 cm, and the frequency, f, was varied between experiments (Table 1). The grid-stirred tank was cleaned prior to each experiment, and

filled to a depth of 70 cm with approximately 260 litres of filtered (<0.45 μm) tap water. Practical considerations precluded filtering this volume of pond water for each experiment. By conducting experiments at varying frequencies and hence correlating the results to turbulence intensity, it was possible to isolate the effects of turbulence on colony size from any impact of the change from pond to tap water.

The experiments were conducted in two stages, commencing within 15 minutes of sample collection from the pond. Four litres of the sample were gently added to the base of the grid-stirred tank, using a funnel and 1 cm diameter plastic tubing. The grid was oscillated at 0.5 s^{-1} for 10 minutes, after which time a depth-integrated sample of the colonies was taken by lowering an empty 1 cm diameter plastic tubing into the tank, sealing the top of the hosing and then withdrawing it from the tank. The size distribution of the colonies in this sample ($T = 0$) was then measured. After 10 minutes mixing at 0.5 s^{-1}, visual inspection indicated that the colonies were homogeneously distributed in the grid-stirred tank, and so the first stage of the experiment was complete. The first stage had three purposes. First, to ensure that the population was spatially homogeneous, and hence equally mixed through the turbulent field during Stage 2. Second, to isolate the effects of transferring from the pond to a filtered tap water environment from the second stage. Third, to determine the response of *M. aeruginosa* colonies to very low levels of mixing.

The grid oscillation was increased in the second stage, with a different frequency used in each of the six experiments (Table 1). Depth integrated samples were collected, and size distribution measured, at times $T =$ 15, 30, 60, 120, 180, 240 and 300 minutes. Since the duration of the experiments was less than 6 hours, the effect of turbulence on cell processes was considered negligible (Regel et al., 2003).

The colony size distribution of the depth-integrated samples was measured using a non-invasive video technique similar to that used by (Waite et al., 1997). A sample of approximately 400 millilitres was placed in a square-based Perspex viewing vessel of dimensions 6 × 6 × 16 cm (Fig. 2). Three 50 W halogen globes, positioned behind a vertical slot in an aluminium sheet, produced a vertical light sheet in the Perspex viewing vessel. Approximately two minutes of video footage were taken of the colonies within a 2 × 2 cm field of view in the light sheet. The vessel contents were gently hand-stirred periodically to ensure that the population remained uniformly distributed. All experiments were conducted in a dark room to maximize visibility of the colonies.

A 'frame-grabber' was used to convert the video footage to a series of still-frame images of 768 × 576 pixels. From the footage taken at each sample time in each experiment, 19 images were captured at 2 second spacing. All images were examined manually to remove any non-*M. aeruginosa* material before further analysis. The image analysis software Image Pro Plus (version 4.5, MediaCybernatics) was used on a PC, Win NT, dual processor station to calculate the size, in pixelated area, of each colony in each image. Pixelated area, A, of each colony was then converted to equivalent spherical diameter $d_e = \sqrt{\frac{4}{\pi}A}$, which is referred to hereafter simply as 'diameter'. d_e was then converted from pixel length units to μm using the calibration coefficients of Table 1, determined from calibration images taken in the viewing tank prior to each experiment. This method of measuring size distribution was validated with a sample of spherical polystyrene beads of known diameter. The size range of measured colonies was 30 μ $< d_e <$ 1000 μm. The upper bound was the limit of observed colony size. The lower bound on d_e, defined by the resolution of the imaging system, was approximately one order of magnitude above the diameter of individual *M. aeruginosa* cells.

While assumption of spherical shape may have introduced some uncertainty in the results, this will be very minor. Furthermore, the conversion to volume was based on measurement of area. When used to estimate equivalent spherical diameter and colony volume, this method has less inherent uncertainty than using the lineal measurement commonly made (e.g. Brookes et al., 1999, Naselli-Flores & Barone, 2003).

Experimental analysis

Colony size data was summarised as frequency of observation in selected size ranges (i.e. bins) of d_e. The bins were separated by approximately 3 μm for 30–350 μm. Bins were combined for higher values of d_e, as there were fewer colonies in the larger size range. By calculating spherical volume, $V = \frac{1}{6}\ \pi\ d_e^3$, for each bin size, the fraction of total volume was determined. The density of *M. aeruginosa* typically ranges from 980 to 1040 kgm^{-3}, which is equivalent to a variation of $\leq \pm$ 4% from the density of water (Reynolds et al., 1987; Visser et al., 1997; Wallace & Hamilton, 1999). Hence volume is a good measure of biomass, and the volume fraction in each d_e bin is referred to as the 'mass fraction' hereafter. The experimental res-

Table 1. Summary of experimental conditions.

Expt	Grid freq. (s^{-1})	Calibration (μm/pixel)	Dissipation (Eq. 1, $m^2\ s^{-3}$) ϵ_{max} ($z = 0.12$ m)	ϵ_{min} ($z = 0.53$ m)	η_k (mm) (Eq. 2)
Stage 1	0.5		3×10^{-7}	9×10^{-10}	1.4–6.3
Stage 2					
1	0.9	32.45	2×10^{-6}	5×10^{-9}	0.9–4.1
2	1.4	32.30	7×10^{-6}	2×10^{-8}	0.7–2.9
3	1.8	32.65	2×10^{-5}	4×10^{-8}	0.5–2.4
4	2.3	32.10	3×10^{-5}	9×10^{-8}	0.5–2.0
5	2.7	30.80	5×10^{-5}	1×10^{-7}	0.4–1.8
6	3.2	32.60	9×10^{-5}	2×10^{-7}	0.4–1.6

Table 2. Fraction of total *M. aeruginosa* biomass with equivalent spherical diameter $d_e < 200$ μm. The change in mass fraction relative to $T = 0$ was averaged over all experiments for each sample time, and the level of significance of change is shown where $p < 0.10$.

Time	Mass fraction with $d_e < 200$ μm	Statistical significance of change in mass fraction with $d_e < 200$ μm, relative to time $T = 0$
Stage 1		
$T = -10$ minutes	16%	$p < 0.05$
$T = 0$	18%	n/a
Stage 2		
$T = 15$ minutes	17%	Not significant
$T = 30$ minutes	19%	$p < 0.05$
$T = 60$ minutes	21%	$p < 0.025$
$T = 120$ minutes	21%	Not significant
$T = 180$ minutes	23%	$p < 0.05$
$T = 240$ minutes	25%	$p < 0.05$
$T = 300$ minutes	26%	$p < 0.05$

ults are presented in terms of the mass fraction, since mass is conserved during particle collision and break-up (Jackson & Burd, 1998). Conservation of mass was confirmed to within 10% in the measurements for all experiments.

The diameter of the 10th percentile of mass, d_{p10}, measures the proportion of mass concentrated in the smallest colonies; by definition 90% of the total mass is found in colonies of diameter greater than d_{p10}. Thus decrease in d_{p10} is a measure of the break-up of larger colonies. Uncertainty in d_{p10} was calculated from the 19 still-frame images taken at each sample time. High frequency of observation of smaller colonies meant that random error in d_{p10} was small. For these reasons, d_{p10} was used to quantify the change in size distribution. This is analogous to the 75% area percentile used by (Hill et al., 2001) to describe flocs in the ocean, but the very low frequency of colonies with d_e greater than approximately 350 μm meant that the median and higher percentiles of both frequency and mass fraction data were subject to very high uncertainty.

The overall changes in d_{p10}, the median d_{p50} and the mass fraction with $d_e < 200$ μm were quantified over time by calculating the mean change in these parameters over all six experiments. From the mean and the standard error, the z-statistic was calculated,

and used to determine the significance level of any change.

The maximum stable diameter d_s has been used to quantify disaggregation in other studies (e.g. Parker et al., 1972, Alldredge et al., 1990). d_s is defined such that colonies with diameter $d > d_s$ break up, while those with $d < d_s$ are stable. d_s was calculated by subtracting the cumulative mass distribution at time $T = 0$ from the cumulative mass distribution at each successive sample time. The value of d_e at which this difference was greatest was the turning point in the distribution, i.e. the maximum stable diameter. While the variation in d_s was too high for comparison between experiments, an overall value of d_s was calculated across all experiments.

Turbulence statistics

Published calibrations exist for the turbulent velocities and length scales produced in a grid-stirred tank (Fernando & DeSilva, 1993; Noh & Fernando, 1993; DeSilva & Fernando, 1994). Using these equations, the turbulent dissipation ϵ can be calculated from the overall rms velocity q and length scale ℓ (cf. Tennekes & Lumley, 1994):

$$\epsilon = \frac{q^3}{\ell} = \frac{1}{\beta}\left(\frac{2C_1^2 + C_2^2}{3}\right)^{\frac{3}{2}} \frac{M^{\frac{3}{2}} S^{\frac{9}{2}} f^3}{z^4} = \alpha \frac{f^3}{z^4} \qquad (1)$$

The values of the constants are $\beta = 0.1$, $C_1 = 0.18 \pm 0.04$, $C_2 = 0.22 \pm 0.05$, (Fernando & DeSilva, 1993; Noh & Fernando, 1993; DeSilva & Fernando, 1994). For grid mesh size $M = 6$ cm and stroke length $S = 4$ cm, $\alpha = 5.5 \times 10^{-10}$ m^6. f is the grid frequency and z is the distance from the grid.

Equation (1) is only valid for $z > 2$ M. Closer to the grid, the turbulent flow field is not fully developed in the sense that the individual wakes being shed from adjacent bars in the oscillating grid do not merge together (e.g. Kit et al., 1997). Equation (1) indicates that dissipation is a strong function of both distance from the grid z and frequency f. Hence as colonies are stirred throughout the tank, they will experience a range of dissipations, and accordingly the minimum and maximum dissipations for each experiment are shown in Table 1. Since the dissipation is a strong function of frequency (of f^3, in fact) in each location, the frequency was varied in each experiment (Table 1), and the relationship between d_{p10} and f^3 used to determine the dependence of colony size on dissipation.

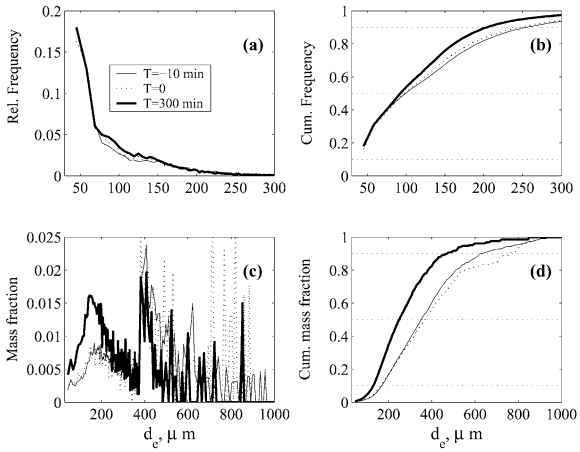

Figure 3. Size distribution of *M. aeruginosa* colonies. Relative frequency of observation (a), cumulative frequency of observation (b), relative mass fraction (c) and cumulative mass fraction (d) of *M. aeruginosa* colonies, plotted against equivalent spherical diameter d_e at times $T = -10$, 0, 300 minutes for Experiment 4 ($f = 2.3$ s^{-1}). The grid lines at 10%, 50% and 90% indicate values of d_{p10}, d_{p50}, d_{p90} respectively.

The Kolmogorov scale, η_k, was calculated from the turbulent dissipation and the kinematic viscosity ν using Equation (1) (cf. Tennekes & Lumley, 1994):

$$\eta_k = \left(\frac{\nu^3}{\epsilon}\right)^{\frac{1}{4}} = \left(\frac{\nu^3}{\alpha}\right)^{\frac{1}{4}} \frac{z}{f^{\frac{3}{4}}} \qquad (2)$$

The minimum Kolmogorov length scale in the tank was calculated from Equation (2) for each experiment (Table 1).

The relationship between colony size (d_{p10}) and turbulent dissipation (Equation (1)) was quantified through least-squares regression analysis. The significance of the relationship was determined by comparing $F = \frac{(n-2)R^2}{1-R^2}$ with the critical F-value from statistical tables, (e.g. Hogg & Ledolter, 1987), where R^2 is the coefficient of determination and $n - 2$ is the degrees of freedom in the regression.

Results

A typical colony size distribution (Expt 4) is plotted against d_e in Figure 3. The data are plotted at three sample times: prior to Stage 1 (T $= -10$ minutes), at the end of Stage 1 ($T = 0$ minutes), and at the end of Stage 2 ($T = 300$ minutes). The frequency distribution of colony diameter (Figs 3a, b) shows that $d_e < 300$ μm for greater than 90% of the colonies.

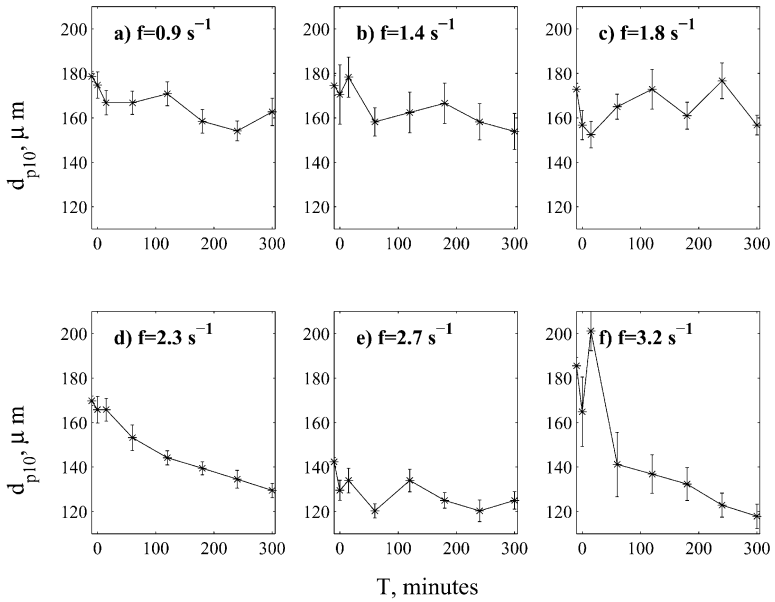

Figure 4. M. aeruginosa colony diameter at the 10th mass percentile (d_{p10}) plotted against time for each experiment: (a) Expt 1; (b) Expt 2; (c) Expt 3; (d) Expt 4; (e) Expt 5; (f) Expt 6.

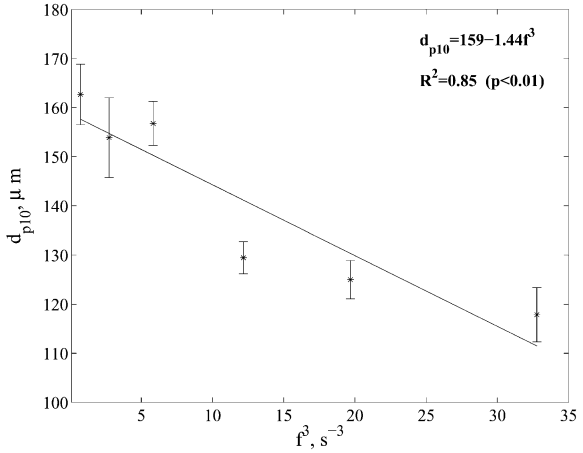

Figure 5. Colony diameter at the 10th mass percentile (d_{p10}) plotted against f^3 (turbulent dissipation), averaged for all experiments at $T = 300$ minutes.

However, the very wide size distribution of the colonies (30–1000 µm) meant that a large proportion of the biomass was concentrated in a small number of large colonies: approximately half of the biomass in colonies with $d_{\mathrm{e}} > 300$ µm (Fig. 3d). The mean colony diameter at time $T = 0$ in Expt 4 was 124 µm (SD = 96 µm).

Across all experiments, the mass fraction of colonies with $d_{\mathrm{e}} < 200$ µm increased during Stage 1 and Stage 2 (Table 2). This can be observed in the increase in the number of smaller colonies during both stages of experiment 4 (Fig. 3), most obviously in Stage 2. The number of larger colonies correspondingly decreased in Stage 2, but appeared to increase in Stage 1 of that experiment (Fig. 3d). This was caused by a small increase in the number of observations of very large colonies (Fig. 3c), which have a disproportionate effect on the mass fraction. This illustrates why d_{p10} was chosen as a measure of change in colony size; because the very large number of observations of small colonies (Fig. 3a) makes this measurement more robust.

d_{p10} generally decreased over time (Fig. 4), and this decrease was found to be significant in both stages of the experiments. Observation of Figure 4 suggests that this effect was greatest for experiments with frequency greater than 1.8 s^{-1}. The median also decreased significantly during both stages, although the skewed size distribution meant that the level of statistical confidence was lower (Table 3). The maximum stable diameter was 220–420 µm during Stage 2.

The diameter of the 10th mass percentile was negatively correlated to dissipation, as measured by f^3, as shown for $T = 300$ minutes in Figure 5 ($R^2 = 0.85$, $p < 0.01$). The development of this relationship is shown by the increase in the coefficient of determination R^2 over time (Fig. 6). The regression was significant ($p < 0.05$) for samples taken at times

Table 3. Decrease in d_{p10} (10th mass percentile) and d_{p50} (median) over time, averaged over all six experiments at each sample time. Level of significance is noted in brackets where $p < 0.10$.

	Mean decrease in d_{p10}		Mean decrease in d_{p50}	
Stage 1				
$T = 0$ minutes	10 μm	($p < 0.01$)	15 μm	
Stage 2				
$T = 15$ minutes	−6 μm		−30 μm	
$T = 30$ minutes	4 μm	($p < 0.10$)	10 μm	
$T = 60$ minutes	10 μm	($p < 0.05$)	25 μm	($p < 0.01$)
$T = 120$ minutes	7 μm		24 μm	
$T = 180$ minutes	13 μm	($p < 0.05$)	42 μm	($p < 0.10$)
$T = 240$ minutes	16 μm	($p < 0.10$)	55 μm	($p < 0.10$)
$T = 300$ minutes	20 μm	($p < 0.05$)	54 μm	($p < 0.10$)

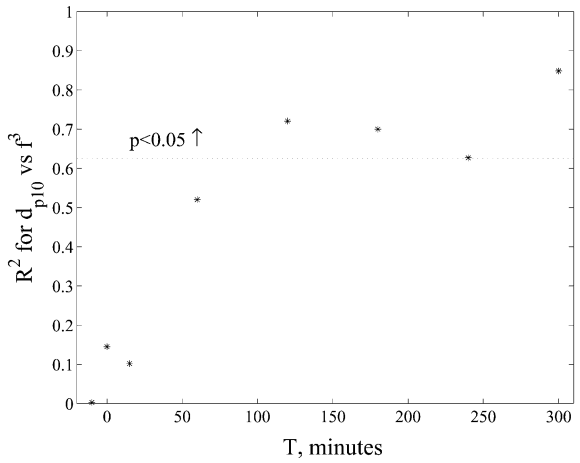

Figure 6. Coefficient of determination R^2 calculated for the relationship between the diameter at the 10th mass percentile (d_{p10}) and the turbulent dissipation (f^3), plotted against sample time T. The relationship is statistically significant ($p < 0.05$) for $T \geq 120$ minutes.

$T \geq 120$ minutes. Regression of $\log(d_{p10})$ vs f^3 was also significant, but with lower R^2 values, indicating that d_{p10} was best described by a linear relationship with turbulent dissipation.

Discussion

Overall, there was a small but significant decrease in colony size with turbulent mixing in both the first and second stages of these laboratory experiments, as shown using two different measures of size distribution. The dissipation was $\epsilon = 9 \times 10^{-10}$ m^2 s^{-3} to 3 $\times 10^{-7}$ m^2 s^{-3} in Stage 1. The minimum dissipation in the tank for Stage 2 ranged from 5×10^{-9} m^2 s^{-3} to 2×10^{-7} m^2 s^{-3}, and the maximum dissipation ranged from 2×10^{-6} m^2 s^{-3} to 9×10^{-5} m^2 s^{-3}. Since the dissipation found in lakes is 10^{-11} m^2 s^{-3} to 10^{-6} m^2 s^{-3} (Wüest & Lorke, 2003), the maximum dissipation in these experiments exceeded the maximum value experienced by *M. aeruginosa* in the field.

When the samples from the pond were left in collection vessels in the absence of any mixing or stirring, the colonies congregated on the surface. The video method was not able to determine the size of individual colonies under these conditions, and any other method for size measurement would have involved disturbing the colonies congregated on the surface. For this reason, it was not possible to estimate the size distribution of a 'control' sample in the absence of turbulent mixing. To overcome this problem, the experiments were conducted over a range of turbulence intensities.

The relationship between turbulent dissipation (f^3) and d_{p10} in Stage 2 was statistically significant ($p < 0.05$) for $T \geq 120$ minutes. This confirmed that the break-up of *M. aeruginosa* colonies in the grid-stirred tank was caused by the turbulence, rather than by differences between laboratory conditions and the pond where the samples originated. The relationship was only significant ($p < 0.05$) for $T \geq 120$ minutes because it was only when disaggregation produced colonies at the lower end of the size distribution that a change in d_{p10} could be detected. Hence the short delay in the development in the relationship between

d_{p10} and dissipation suggests a delay in the formation of the smallest size fractions, rather than a delay in the response of disaggregation to turbulence.

Regel et al. (2003) reported some aggregation when a unicellularculture of *M. aeruginosa* was exposed to turbulence in a grid-stirred tank. It is possible that some aggregation of the smallest colonies did occur in the experiments reported here, and was undetected due to the resolution of the imaging system. The broad size distribution and the high concentration of biomass in larger colonies mean that aggregation of very small colonies would have a minimal effect on the population, however.

Naselli-Flores & Barone (2003) found an order of magnitude increase in the mean size of *M. aeruginosa* colonies in Lake Arancio when the euphotic and mixing depths fell to almost zero, suggesting the presence of a surface bloom. There is other evidence to suggest that very large *M. aeruginosa* colonies form at the surface under calm conditions (Ganf, 1974; Robarts & Zohary, 1984). Since turbulence had a relatively minor effect on colony size distribution in these experiments, it seems possible that the large colonies which form in *M. aeruginosa* surface blooms are broken up when the colonies are re-entrained into the water column, e.g. during a wind event. Once this has occurred, the effect of subsequent turbulent mixing on colony size distribution may be relatively minor, as found in these experiments. These dynamics need to be investigated in further field work, in order for *M. aeruginosa* colony size distributionto be accurately parameterized in models.

In the formation of surface scums, differential motion of colonies, both horizontal and vertical, and high concentrations are likely to contribute to aggregation (cf. Jackson & Lochmann, 1993). *M. aeruginosa* colonies might usefully be compared with chemical flocs, which also form in the absence of turbulence, and break-up in its presence (e.g. Parker et al., 1972, Smith & Kitchener, 1978). This is quite different from marine aggregates or lake snow, where turbulence promotes aggregation (e.g. Grossart & Simon, 1993, Jackson & Lochmann, 1993, Hill, 1992), and disaggregation dominates only under very intense mixing, corresponding to waves and intense storm events (Alldredge et al., 1990; Hill et al., 2001).

The range of the maximum stable diameter across all experiments during Stage 2 was 220–420 μm, and this has two main implications. Firstly, when exposed to the range of ϵ similar to and greater than that experienced in the field, d_s varied over only a small fraction of the full range of colony size. Hence while a relationship may exist between d_s and turbulent dissipation for *M. aeruginosa* colonies, as found in studies of other aggregates (e.g. Parker et al., 1972, Alldredge et al., 1990), this may not lead to large differences in size distributions during different mixing regimes in the field. Secondly, since $d_s \leq \eta_k$ in the experiments, the results do not contradict the hypothesis of (Reynolds, 1998) that η_k places an upper limit on colony size.

The video method used in this study allowed non-intrusive measurements of very large numbers of *M. aeruginosa* colonies, up to 10 000 per sample time. This is the first time such extensive measurements of *M. aeruginosa* colony size distribution have been published, and the results show that the size distribution of colonies is extremely broad and non-normal. This suggests that mean and standard deviation of colony size do not provide a good description of the population. A large fraction of total biomass is concentrated in a small number of large colonies, and hence large sample sizes are required to adequately define the size distribution of a *M. aeruginosa* population. Since migration velocity is strongly affected by colony size, this broad size distribution needs to be accurately characterized in models of *M. aeruginosa*. Shape can also effect the rising and sinking velocity of phytoplankton (Padisák et al., 2003), and more work is required to parameterize this effect for modelling populations of *M. aeruginosa* colonies. More detailed measurements of a small number of colonies stained with Indian-ink would allow more detailed investigation of the role of mucilage in the aggregation and disaggregation of colonies.

A grid-stirred tank was used in these experiments because it provided an environment similar to that experienced by *M. aeruginosa* colonies in the field. Couette cylinders have been successfully used to quantify the impact of mean shear on phytoplankton 'stickiness', (e.g. Waite et al., 1997) and growth (e.g. Thomas & Gibson, 1990, Gibson & Thomas, 1995). While they have also been used to investigate the impact of shear on cell and colony morphology (Moisander et al., 2002), three-dimensional turbulence is needed to fully investigate and understand the implications of turbulent mixing on break-up of cyanobacterial colonies in the wild.

Grid-stirred tanks produce three-dimensional turbulent fields, but the dissipation, and hence shear, varies over depth (Equation (1)). Alldredge et al. (1990) measured disaggregation in a grid-stirred tank, using video technology to observe individual aggregates.

Since turbulent dissipation is a function of distance from the grid, particle disaggregation was correlated to the turbulent dissipation at the position of the measurement. This resulted in detailed measurements of a small number of aggregates. A different approach is used in this study, where the change in size is measured for a representative sample of the population which had been circulated through the full range of dissipation in the tank. This approach produced measurements for a larger numbers of colonies, with corresponding statistical parameters for the population. Furthermore, if the time for disaggregation is similar to or greater than the mixing time, a history of dissipation will provide a better measure of colony break-up than the instantaneous value of dissipation (Hill et al., 2001).

Conclusions

This study has used quantitative experiments and statistical analysis to verify that *M. aeruginosa* colonies do break up under turbulent mixing, although this effect is small even for the highest turbulence intensities likely to be experienced by *M. aeruginosa* in the field. The very broad size distribution measured here is unlikely to be accurately represented by using a single value of colony diameter in models of vertical migration. More work is required to determine how best to characterize the size distribution of *M. aeruginosa* in such models. It would also be useful to investigate whether nutrient availability affects colony size distribution.

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References

Alldredge, A. L., T. C. Granata, C. C. Gotschalk & T. D. Dickey, 1990. The physical strength of marine snow and its implications for particle disaggregation in the ocean. Limnology & Oceanography 35: 1415–1428.

Brookes, J. D., G. G. Ganf, D. Green & J. Whittington, 1999. The influence of light and nutrients on buoyancy, filament aggregation and flotation of *Anabaena circinalis*. Journal of Plankton Research 21: 327–341.

DeSilva, I. P. D. & H. J. S. Fernando, 1994. Oscillating grids as a source of nearly isotropic turbulence. Physics of Fluids 6: 2455–2464.

Falconer, I. R., 1999. An overview of problems caused by toxic blue-green algae (Cyanobacteria) in drinking and recreational water. Environmental Toxicology 14: 5–12.

Fernando, H. J. S. & I. P. D. DeSilva, 1993. Note on secondary flows in oscillating-grid, mixing-box experiments. Physics of Fluids A 5: 1849–1851.

Ganf, G. G., 1974. Diurnal mixing and the vertical distribution of phytoplankton in a shallow equatorial lake (Lake George, Uganda). Journal of Ecology 62: 611–629.

Ganf, G. G. & R. L. Oliver, 1982. Vertical separation of light and available nutrients as a factor causing replacement of green algae by blue-green algae in the plankton of a stratified lake. Journal of Ecology 70: 829–844.

Gibson, C. H. & W. H. Thomas, 1995. Effects of turbulence intermittency on growth inhibition of a red tide dinoflagellate, *Gonyaulax polyedra* Stein. Journal of Geophysical Research 100: 24841–24846.

Grossart, H. & M. Simon, 1993. Limnetic macroscopic organic aggregates (lake snow): occurrence, characteristics, and microbial dynamics in Lake Constance. Limnology & Oceanography 38: 532–546.

Hill, P. S., 1992. Reconciling aggregation theory with observed vertical fluxes following phytoplankton blooms. Journal of Geophysical Research 97: 2295–2308.

Hill, P. S., G. Voulgaris & J. H. Trowbridge, 2001. Controls on floc size in a continental shelf bottom boundary layer. Journal of Geophysical Research 106: 9543–9549.

Hogg, R. V. & J. Ledolter (eds), 1987. Engineering Statistics. Macmillan Publishing Company, New York.

Humphries, S. E. & V. D. Lyne, 1988. Cyanophyte blooms: the role of cell buoyancy. Limnology & Oceanography 33: 79–91.

Hutchinson, G. E., 1957. A Treatise on Limnology. John Wiley & Sons Inc., New York.

Ibelings, B. W., 1992. Cyanobacterial waterblooms: the role of buoyancy in watercolumns of varying stability. PhD thesis, The University of Amsterdam.

Ibelings, B. W., L. R. Mur, R. Kinsman & A. E. Walsby, 1991a. *Microcystis* changes its buoyancy in response to the average irradiance in the surface mixed layer. Archiv für Hydrobiologie 120: 385–401.

Ibelings, B. W., L. R. Mur & A. E. Walsby, 1991b. Diurnal changes in buoyancy and vertical distribution in populations of *Microcystis* in two shallow lakes. Journal of Plankton Research 13: 419–436.

Jackson, G. A. & A. B. Burd, 1998. Aggregation in the marine environment. Environmental Science & Technology 32: 2805–2814.

Jackson, G. A. & S. Lochmann, 1993. Modelling coagulation of algae in marine ecosystems. In Buffle, J. & H. P. van Leeuwen (eds), Environmental Particles, Vol. 2. Lewis Publishers, FL: 387–414.

Kirk, J. T. O., 1975. A theoretical analysis of the contribution of algal cells to the attenuation of light within natural waters. II. spherical cells. New Phytologist 75: 21–36.

Kit, E. L. G., E. J. Strang & H. J. S. Fernando, 1997. Measurement of turbulence near shear-free density interfaces. Journal of Fluid Mechanics 334: 293–314.

Mitrovic, S. M., L. C. Bowling & R. T. Buckney, 2001. Quantifying potential benefits to *Microcystis aeruginosa* through disentrainment by buoyancy within an embayment of a freshwater river. Journal of Freshwater Ecology 16: 151–157.

Moisander, P. H., J. L. Hench, K. Kononen & H. W. Paerl, 2002. Small-scale shear effects on heterocystous cyanobacteria. Limnology & Oceanography 47: 108–119.

Naselli-Flores, L. & R. Barone, 2003. Steady-state assemblages in a Mediterranean hypertrophic reservoir. The role of Microcystis ecomorphological variability in maintaining an apparent equilibrium. Hydrobiologia 502: 133–143.

Noh, Y. & H. J. S. Fernando, 1993. The role of molecular diffusion in the deepening of the mixed layer. Dynamics of Atmospheres & Oceans 17: 187–215.

O'Brien, K. R., 2002. The effects of turbulent mixing on the vertical distribution and biomass of phytoplankton populations. PhD thesis, The University of Western Australia.

Oliver, R. L. & G. G. Ganf, 2000. Freshwater blooms. In Whitton, B. A. & M. Potts (eds), The Ecology of Cyanobacteria. Kluwer Academic Publishers, Dordrecht: 149–194.

Padisák, J., É. Soróczki-Pintér & Z. Rezner, 2003. Sinking properties of some phytoplankton shapes and the relation of form resistance to morphological diversity of plankton – an experimental study. Hydrobiologia 500: 243–257.

Parker, D. S., W. J. Kaufman & D. Jenkins, 1972. Floc breakup in turbulent flocculation processes. Journal of the Sanitary Engineering Division of the American Society of Civil Engineers 98: 79–99.

Regel, R. H., J. D. Brookes, G. G. Ganf & R. W. Griffiths, 2004. The influence of experimentally generated turbulence on the Mash01 unicellular *Microcystis aeruginosa* strain. Hydrobiologia 517: 107–120.

Reynolds, C. S., 1984. The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge, UK.

Reynolds, C. S., 1997a. The control and management of cyanobacterial blooms. In Australian Society of Limnologists 36th Congress Symposia: 6–22.

Reynolds, C. S., 1997b. Vegetation Processes in the Pelagic: A Model for Ecosystem Theory. Ecology Institute, Germany.

Reynolds, C. S., 1998. Plants in motion: Physical-biological interaction in the plankton. In Imberger, J. (ed.), Physical Processes in Lakes and Oceans. American Geophysical Union Washington, U.S.A.: 535–560.

Reynolds, C. S., R. L. Oliver & A. E. Walsby, 1987. Cyanobacterial dominance: the role of buoyancy regulation in dynamic lake environments. New Zealand Journal of Marine & Freshwater Research 21: 379–390.

Robarts, R. D. & T. Zohary, 1984. *Microcystis aeruginosa* and underwater light attenuation in a hypertrophic lake (Hartbeesport Dam, South Africa). Journal of Ecology 72: 1001–1017.

Smith, D. K. W. & J. A. Kitchener, 1978. The strength of aggregates formed in flocculation. Chemical Engineering Science 33: 1631–1636.

Tennekes, H. & J. L. Lumley, 1994. A First Course in Turbulence, 2nd edn. MIT Press, Boston, USA.

Thomas, W. H. & C. H. Gibson, 1990. Effects of small scale turbulence on microalgae. Journal of Applied Phycology 2: 71–77.

Visser, P. M., J. Passarge & L. R. Mur, 1997. Modelling vertical migration of the cyanobacterium *Microcystis*. Hydrobiologia 349: 99–109.

Waite, A. M., S. Gallagher & H. G. Dam, 1997. New measurements of phytoplankton aggregation in a flocculator using videography and image analysis. Marine Ecology Progress Series 155: 77–88.

Wallace, B. B. & D. P. Hamilton, 1999. The effect of variations in irradiance on buoyancy regulation in *Microcystis aeruginosa*. Limnology & Oceanography 44: 273–381.

Wüest, A. & A. Lorke, 2003. Small-scale hydrodynamics in lakes. Annual Review of Fluid Mechanics 35: 373–412.