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Modelling vertical migration of the cyanobacterium *Microcystis*

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Abstract

Computer models can be helpful tools to provide a better understanding of the mechanisms responsible for the complex movements of cyanobacteria resulting from changes in buoyancy and mixing of the water column in a lake. Kromkamp & Walsby (1990) developed a vertical migration model for *Oscillatoria*, that was based on the experimentally determined relationship between the rates of density change and photon irradiance in this cyanobacterium. To adapt this model to *Microcystis*, we determined related changes in carbohydrate content in cultures of *Microcystis*. Samples were incubated at various constant values of photon irradiance and then placed in the dark. The changes in carbohydrate content of the cells during these incubations were investigated. The relationship between the ratio of carbohydrate to protein and cell density in *Microcystis* was established to permit conversion of the rates of carbohydrate change to rates of density change. By plotting the calculated rates of density change against the values of photon irradiance experienced during the incubations, an irradiance-response curve of density change was established. The curve showed a distinct maximum at 278 μmol photons m^{-2} s^{-1}. At higher values of photon irradiance, the rate of density change was strongly inhibited. A positive linear correlation was found between cell density and the rates of density decrease in the dark. The validity of the use of rate equations of density change, which are based on short-term incubations at constant values of photon irradiance, to predict density changes in *Microcystis* in fluctuating light regimes was tested. This was accomplished by measuring the time course of change in carbohydrate content of two continuous cultures of *Microcystis*, which were submitted to fluctuating light regimes, and comparing the results with the changes in the carbohydrate contents of these cultures predicted by the rate equations of carbohydrate change. The results showed good agreement: the rate equations of density change were therefore introduced into the model to simulate vertical migration of *Microcystis*. The model predicts that the maximum migration depth of *Microcystis* will increase with colony size up to a maximum of 200 μm radius. The effect of colony size on the net increase in cell density during the light period was also investigated with the model. It predicts that small colonies have a higher net increase in cell density than large colonies, but are inhibited at high photon irradiances at the surface.

Introduction

In a stable water column, colony-forming cyanobacteria like *Microcystis* show a daily pattern of vertical migration that can be explained by an increase in cell density due to carbohydrate accumulation in the light and a decrease in cell density due to utilisation of carbohydrates in the dark (Kromkamp & Mur, 1984). Modelling of vertical migration can be a tool to provide a better understanding of vertical migration patterns. Kromkamp & Walsby (1990) developed a computer model to simulate vertical migration of *Oscillatoria*. To adapt this model to *Microcystis*, the relationship between density change and photon irradiance

of *Microcystis* cultures was investigated in this study. Rates of carbohydrate change can be more accurately measured than rates of density change. Since the buoyant density of cells depends on carbohydrate content in *Microcystis* (Kromkamp and Mur, 1984), rates of carbohydrate change can be converted to rates of density change. We have therefore developed a model based on measurements of changes in the carbohydrate content of the cells rather than on direct measurements of cell density. Through the relationship between carbohydrate content and cell density in *Microcystis*, which was determined in this study, the rates of carbohydrate change were converted into rates of density change.

In the model of Kromkamp & Walsby (1990), the relationship between density increase and the values of photon irradiance was assumed to be a hyperbolic function, saturated at high values of photon irradiance. The authors applied a Michaelis-Menten equation to describe this relationship. However, inhibition of photosynthesis at high values of photon irradiance has been reported for many phytoplankton species and in lakes at various latitudes (e.g. Neale & Richerson, 1987; Pierson et al., 1994; Vincent et al., 1984). Inhibition has been found in *Microcystis* (Ibelings et al., 1994; Ibelings & Mur, 1992; Köhler, 1992). To investigate the possible effects of inhibition on carbohydrate accumulation, we measured the rates of carbohydrate change at values of photon irradiance of up to 1660 μmol m^{-2} s^{-1}.

In the experiments of Kromkamp & Walsby (1990), a positive linear relationship was found between the rates of density decrease in the dark and the previously experienced values of photon irradiance. Enhanced post-illumination respiration has been found by Beardall et al. (1994); Falkowski et al. (1985); Stone & Ganf (1981) and Watanabe & Kimura (1990), and has been explained by an increase in intracellular pools of photosynthate following a period of illumination. The positive linear relationship between the respiratory rate and the carbohydrate content of the cells as found by Gibson (1975); Markager et al. (1992) and Watanabe & Kimura (1990) is consistent with this explanation. Since the increase in the carbohydrate content is dependent not only on the previously experienced values of photon irradiance but also on the duration of the exposure to light, it may be expected that the rate of carbohydrate decrease can be more accurately described as a function of the carbohydrate content of the cells rather than as a function of the previously experienced values of photon irradiance. The relationships between the rates of carbohydrate change, the values of photon irradiance and the carbohydrate content were therefore investigated by incubating samples of *Microcystis* at various values of photon irradiance and subsequently in the dark.

We have tested whether the relationships between the rates of density change and photon irradiance can be used in predicting changes in cell density in a fluctuating light climate as experienced by *Microcystis* in a mixed water column or during vertical migration. Since the rates of density change determined in this study were calculated from measured rates of carbohydrate change, the validation of the model was done by comparing measurements and calculations of changes in carbohydrate content. The time course of change in carbohydrate content was measured in continuous cultures, which were subjected to fluctuating values of photon irradiance, simulating both the absence of mixing and deep mixing in the water column. These measurements were compared with the calculated time course of change in carbohydrate content. The calculations were made with equations for rate of carbohydrate change (Passarge et al., in prep.), which were derived from results of the same experiments as the equations for rate of density change.

The vertical migration model was adapted to *Microcystis* by incorporating the irradiance-response curve of density change and an equation describing the rate of density change in the dark as a linear function of the cell density, which were determined in this study. With the adjusted model, we investigated effects of colony radius and the maximum surface photon irradiance on the migration depth of *Microcystis* and on the increase in cell density during the photoperiod.

Materials and methods

Organisms and growth conditions

A strain of *Microcystis* sp., isolated from Lake Nieuwe Meer, The Netherlands, was used. Cultures were grown with a nutrient-rich medium (Van Liere & Mur, 1978). Both the continuous cultures and the batch cultures were bubbled with air to provide a sufficient supply of carbon dioxide and to keep the cells homogeneously distributed. A medium with a low concentration of Ca (0.058 mm) was used to prevent colony formation. The temperature in the continuous cultures was kept constant at 20 °C; in the batch cultures, it varied between 20 °C and 23 °C.

For the determination of the relationship between cell density and carbohydrate content, samples from a 2-l continuous culture were taken and subsequently grown as batch cultures at different constant values of photon irradiance. Illumination of this continuous culture was provided by a Philips fluorescent tube for 12 h per day with a mean constant value of photon irradiance inside the culture vessel of 37 μmol m^{-2} s^{-1} (from hereon called block light regime).

The two continuous cultures with fluctuating light regimes were grown in flat vessels with dimensions of 237 mm × 30 mm × 450 mm (30 mm parallel to light path). Illumination was provided by a high-intensity light source (Philips HMI 1200 W).

Light regimes

Fluctuating light regimes were established by changing the photon irradiance by varying the angle of the slats of Venetian blinds, controlled by stepping motors. Specially designed software controlled the diurnal light regimes. Pneumatically operated doors provided dark conditions. The system has been described by Kroon et al. (1992). The continuous cultures received a light-dark cycle of 10:14 hours. In one of the continuous cultures the absence of mixing was simulated by applying a light regime that resembled a sine function (Z_m/Z_{eu} ratio = 0). In another continuous culture, a light regime simulating deep mixing in the water column with a circulation time of 40 minutes and exponential attenuation of photon irradiance was applied (Z_m/Z_{eu} ratio = 3.6). Z_m/Z_{eu} is the ratio between the maximum depth of the mixed layer (Z_m) and the maximum depth of the euphotic layer (Z_{eu}) in a water column. In this study, Z_{eu} was defined as the depth were the photon irradiance equals 1% of the photon irradiance at the surface of the water column. The mean values of photon irradiance in the cultures were calculated according to Lambert-Beer's law of exponential light extinction from the mean values of photon irradiance on the surface of the culture vessels, which were measured once per minute with a Li-Cor datalogger, and from the extinction coefficients of the cultures. The extinction coefficients were calculated from *in vivo* absorption spectra determined on an Aminco DW 2000 spectrophotometer, with the cuvette placed directly in front of the photomultiplier, corrected for the spectrum of the light source. The total photon dose in both continuous cultures was 2.0 ± 0.1 mol m^{-2} d^{-1}. The optical densities of the continuous cultures were kept constant at 0.08–0.09 (at a pathlength of 1 cm), measured at the start of the photoperiod on a Pharmacia-LKB Novaspec II spectrophotometer at 750 nm without collapsing the gas vesicles of the cells.

Diurnal changes in the continuous culture

During the photoperiod, samples from the continuous cultures with fluctuating light regimes were taken every forty minutes for the determination of carbohydrate and protein. The protein content of the cells was analysed in triplicate on freeze-dried samples, according to the Folin method with bovine serum albumin as the standard (Herbert et al., 1971). The carbohydrate content of the cells was determined as glucose polymer in triplicate on freeze-dried samples with the GOD-Perid method from Boehringer (Werner et al., 1970), after hydrolysis with HCl at 100 °C during 1 h and neutralisation with NaOH.

Incubations

Samples from the continuous cultures with a Z_m/Z_{eu} ratio of 0 and 3.6, which were taken at the beginning of the photoperiod, were incubated in small flat culture flasks. Illumination was provided by a high-intensity light source (Philips HMI 1200 W), with a vessel filled with water placed between the light source and the flasks to decrease heating of the sub-cultures. The content of this vessel was kept cool by partially replacing it with cold water and adding ice during the course of the experiments. The flasks were placed at various distances from the light source and some flasks were additionally shaded by neutral density filters in order to provide a wide range of values of photon irradiance. The value of photon irradiance before (I_{in}) and behind the flasks (I_{out}) was measured with a Li-Cor datalogger (at ± 20 spots on the flasks). From these measurements the mean values of photon irradiance ($\bar{I}$) in the flasks were calculated according to the following equation (Van Liere & Walsby, 1982):

$$\bar{I} = (I_{in} - I_{out})/\ln(I_{in}/I_{out}). \quad (1)$$

Incubations were performed at 22 different values of photon irradiance. From the start of the incubation in the light onwards, samples for carbohydrate analysis were taken every 15 to 20 minutes during 1.5 hours. After exposure of 4 hours to the light, the light source was switched off and samples were taken every 20 to 35 minutes during the subsequent dark period. Dark incubations with samples taken directly from the

continuous cultures without previous incubation in the light were also performed. Samples for protein analysis were also taken at the start of the incubations in the light and in the dark. Carbohydrate and protein contents of the cells were determined as described before.

The rates of carbohydrate change in the light as well as in the dark could be well described as the slopes of the linear equations determined by applying regression analysis to the time courses of the carbohydrate content of the samples. For the incubations in the light, there was a linear relationship between carbohydrate content and incubation time between the second sample (taken after 15 to 20 minutes of incubation in the light) up to the last sample that was taken during the incubation in the light. For slopes that resulted in a significance level of $p<0.1$, the hypothesis of linearity had to be rejected and the values of the slopes were set to zero. For the incubations in the dark, linear regression was applied to the first up to the last sample taken during the incubation in the dark. The slopes that were not significant at a $p<0.1$ level were rejected.

Density measurements

Samples taken from the continuous culture with the block light regime were incubated during different periods of time at various values of photon irradiance as well as in the dark to establish a wide range of cell densities. The gas vesicles of the cells in the sub-cultures were collapsed (at a pressure of 1.1 MPa) and drops of the samples were subsequently layered on top of Percoll gradients (in triplicate), which were obtained by centrifugating mixtures of 5 ml twice-distilled water, 1 ml 10 times concentrated medium and 4 ml Percoll (or 4 ml twice-distilled water and 5 ml Percoll for cells with a higher density) at 39 100 ***g*** for 60 minutes (Oliver et al., 1981). The gradients with the cells were recentrifugated for 10 minutes in a benchtop centrifuge with a swingout rotor at 1600 ***g***, with the result that the cells formed a distinct band in the gradient. After centrifugation, a small sample of this band was removed with a microsyringe and dropped onto a calibrated paraffin-CCl_4 gradient to determine the density of the Percoll where the cells formed the visible band, in order to determine the density of the samples. The paraffin-CCl_4 gradient was obtained by layering 10 different ratios of paraffin and CCl_4 in a 1 litre cylinder (Oliver & Walsby, 1988), resulting in a gradient that varied in density between 909.2 kg m^{-3} and 1175.6 kg m^{-3}. The gradient was calibrated with drops of distilled water and coloured salt solutions of known density. The calibration curve was linear. The resulting positions of both the calibration solutions and the Percoll bands formed by the cells in the paraffin-CCl_4 gradient were measured with an ocular. The carbohydrate contents on basis of protein of the samples were analysed as described above.

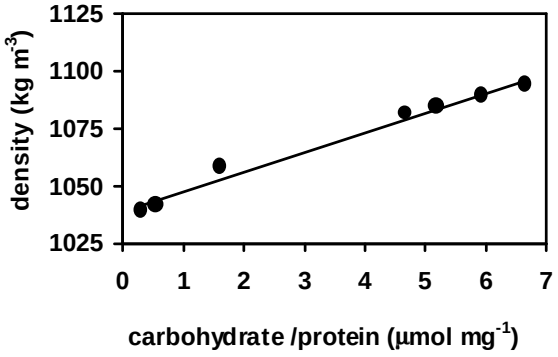

Figure 1. Cell density plotted against carbohydrate protein ratio in cells of *Microcystis*. The correlation is fitted with a linear function (solid line).

Results

Construction of the computer model

This model of vertical migration is based on the model of Kromkamp & Walsby (1990), but new equations have been used to describe the rates of density change both in the light and in the dark.

The values of photon irradiance (I_0) on the surface at different times *t* during the photoperiod are described by a sine function of the maximum value of photon irradiance at noon (I_m) and the length of the photoperiod (D_L):

$$I_0 = I_m \sin(\pi t / D_L). \quad (2)$$

The value of photon irradiance at the depth of the cyanobacterial colony (I_z) is calculated according to Lambert-Beer's law of exponential light extinction:

$$I_z = I_0 e^{-K_z}, \quad (3)$$

where *K* is the extinction coefficient of the water (m^{-1}) and *z* is the depth of the colony (m). To minimize the effect of step size, the increase in density is determined by using the mean value of photon irradiance between two steps ($\bar{I}_z$):

$$\bar{I}_z = (I_{z2} - I_{z1}) / \ln(I_{z2}/I_{z1}), \quad (4)$$

where I_{z2} and I_{z1} are the values of photon irradiance at depths z_2 andz_1, respectively (Van Liere & Walsby, 1982).

A correlation was found between the density of the cells ρ (in kg m^{-3}) and the ratio, c, of carbohydrate content (in mmol) to protein content (in g) as shown in Figure 1. The coefficients of equation 5 were fitted to the data by applying linear regression analysis ($R^2 = 0.9851$):

$$\rho = a * c + b \tag{5}$$

where $a = 8.5587$ g mmol^{-1} kg m^{-3}, $b = 1039.1$ kg m^{-3} and the units of c are mmol g^{-1}.

To obtain the rates of density change in the batch cultures incubated at different constant values of photon irradiance and in the dark, first the rates of carbohydrate change were determined by applying linear regression analysis to the time course of the carbohydrate content during the incubations. From the rates of carbohydrate change, the rates of density change were calculated with equation 5. Plotting the rates of density change against the values of photon irradiance showed an optimal value of photon irradiance of 278 μmol m^{-2} s^{-1} (Figure 2) and negative rates of density change at values of photon irradiance above 1347 μmol m^{-2} s^{-1}. The coefficients of equation 6, which was modified from Richter (1985), were fitted to the data by applying nonlinear regression analysis ($R^2 = 0.9543$).

$$d_{(I)} = (N_0/60) I \mathrm{e}^{(-I/I_0)} + e, \tag{6}$$

where $d_{(I)}$ is the rate of density change (kg m^{-3} min^{-1}) at photon irradiance I; I is the photon irradiance (μmol photons m^{-2} s^{-1}); I_0 is I where $\rho(I)$ is maximum (277.5 μmol photons m^{-2} s^{-1}); N_0 is a normative factor (0.0945 kg m^{-3} μmol^{-1} photons m^2) and e is the rate of density change at $I = 0$ (-0.0165 kg m^{-3} min^{-1}).

The rates of density change in the dark were plotted against the values of photon irradiance and against the photon doses given during pre-incubation in the light (data not shown) and against the initial density of the cells at the time when the sub-cultures were transferred from light to dark (Figure 3). The latter showed a better correlation. The coefficients of equation 7 were fitted to the data by applying linear regression analysis ($R^2 = 0.8856$).

$$d_{(\rho)} = f_1 * \rho_i + f_2, \tag{7}$$

where $d_{(\rho)}$ is the rate of density change in the dark (kg m^{-3} min^{-1}); ρ_i is the initial density (kg m^{-3}); f_1 is the slope ($9.49 * 10^{-4}$ min^{-1}) and f_2 is the theoretical rate of density change with no carbohydrate storage in the cells (0.984 kg m^{-3} min^{-1}).

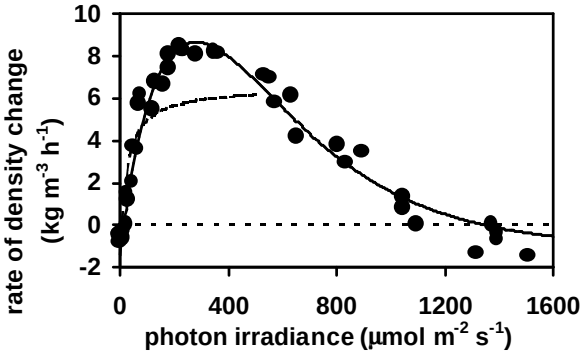

Figure 2. Rates of change in cell density of *Microcystis* plotted against photon irradiance and the curve given by equation 6 (solid line). The rates of density change in the light of *Oscillatoria* predicted by equations of Kromkamp & Walsby (1990) are shown with a dashed line.

Equation 6 was used in the model to calculate the vertical migration expected when the colony experiences values of photon irradiance above the compensation photon irradiance I_c of 10.9 μmol m^{-2} s^{-1}. I_c is given by the intersection of the irradiance-response curve (equation 6) with the X-axis at low values of photon irradiance. Equation 7 was used when the photon irradiance at the depth of the colony did not exceed I_c. The rates of density change obtained by the experiments are based on measurements of non-buoyant cell density (without gas vesicles). Therefore, the densities of equation 8 up to equation 9 are non-buoyant cell densities. In the model, however, buoyant cell densities (with gas vesicles) have to be used to calculate floating and sinking velocities (equation 10). Therefore, the non-buoyant cell density resulting from equations 8 and 9 has to be converted into buoyant cell density. This was done by adding the difference between the non-buoyant cell density of 1045 kg m^{-3} that was measured in the continuous cultures at the start of the photoperiod and the buoyant cell density of 980 kg m^{-3} that was used as a starting value for the calculations.

For values of photon irradiance above I_c, the new density ρ_2 after time interval t_x (in min) was calculated from the starting density ρ_1 and from the rate of density change $d_{(I)}$ that was calculated with equation 6. For values of photon irradiance below I_c, ρ_2 after t_x was calculated from the starting density ρ_1 and from the rate of density change $d_{(\rho)}$ that was calculated with

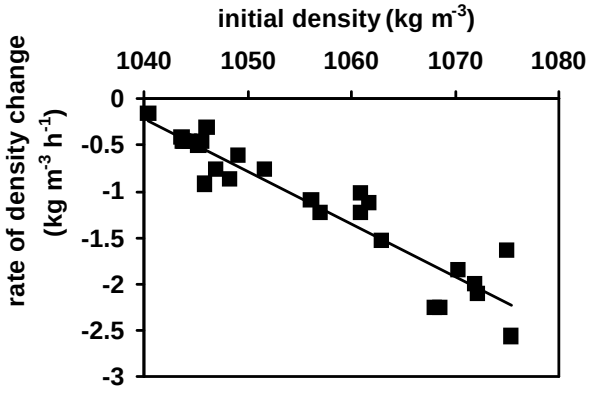

Figure 3. Rates of change in cell density of *Microcystis* in the dark after pre-incubation at various values of photon irradiance plotted against the initial density.

Table 1. Variables used in the computer model

Variable	Unit	Value
Extinction coefficient (*K*)	m^{-1}	-2
Density of water (ρ')	kg m^{-3}	998
Starting density	kg m^{-3}	980
Minimum density	kg m^{-3}	920*
Maximum density	kg m^{-3}	1065*
Form resistance (Φ)		1*
Cell volume / colony volume (*A*)		1
Time interval (*P*)	min	5

* from Reynolds et al. (1987)

equation 7.

$$\text{for } I_z \geq I_c: \quad \rho_2 = \rho_1 + t_x * d_{(I)}; \tag{8}$$

$$\text{for } I_z < I_c: \quad \rho_2 = \rho_1 + t_x * d_{(\rho)}. \tag{9}$$

The new density is introduced into a modified Stokes's equation to calculate the new sinking velocity u (in m s^{-1}):

$$u = 2\,\boldsymbol{g} r^2 (\rho - \rho') A/(9\Phi n), \tag{10}$$

where $\boldsymbol{g}$ is the gravitational acceleration (9.8 m s^{-2}), r the effective radius of the colony (in m), ρ and ρ' the densities of the cyanobacterium and water (998.2 kg m^{-3}), respectively, A is the proportion of cell volume relative to colony volume, Φ the form resistance and n the viscosity of the water (10^{-3} kg m^{-1} s^{-1}). The new velocity is used to calculate how far the colony sinks or floats during the next time interval:

$$z_2 = z_1 + 60 * u * t_x, \tag{11}$$

where z_1 is the starting depth at $t = 1$, z_2 is the new depth at $t = 2$ and t_x is the time interval (in min) between $t = 2$ and $t = 1$. The constants used in the model are given in Table 1.

Validation of the model equations describing the rates of density change

In Figures 4 and 5, the diurnal changes in the values of photon irradiance and in the ratio of carbohydrate and protein content in the continuous cultures with fluctuating light regimes are shown. The light regime with $Z_m = 0$ (Figure 4) simulated the absence of mixing, whilst the light regime with $Z_m/Z_{eu} = 3.6$ (Figure 5) simulated deep mixing in the water column. Although the total daily photon dose was identical in both continuous cultures, the accumulation of carbohydrate was much lower in the culture with $Z_m/Z_{eu} = 3.6$. On basis of the time course of photon irradiance in the cultures ($\bar{I}$), the rates of carbohydrate change in the continuous cultures were calculated with the irradiance-response curve for rates of carbohydrate change for values of $\bar{I} \geq I_c$, while a linear relationship between the rates of carbohydrate decrease and the carbohydrate content of the cells was used to calculate the carbohydrate changes at values of $\bar{I} < I_c$. For the equations for the rates of carbohydrate change, see Passarge et al. (in prep.). The calculations of the time course of rates of carbohydrate change were initialized by using the carbohydrate content that was measured in the continuous cultures at the start of the photoperiod as a start value for the calculations. The calculated time course of change in carbohydrate content in both continuous cultures agreed very well with the measured values (Figures 4 and 5), except for a slight overestimation of the carbohydrate content during the second part of the photoperiod.

Simulations

In Figure 6, the vertical migration patterns during 24 hours, calculated by the model for various colony radii are shown. The maximum depth of the colonies increased with colony size for colonies ≤ 200 μm (Figure 6a and Figure 6b). For colonies>200 μm (Figure 6b), the relationship between maximum depth and colony radius was less clear. In Figure 7, the dependence of the maximum depth on the values of photon irradiance on the surface is shown for various small colony radii (≤ 100 μm). The depth of migration initially increased with an increase in the values of photon

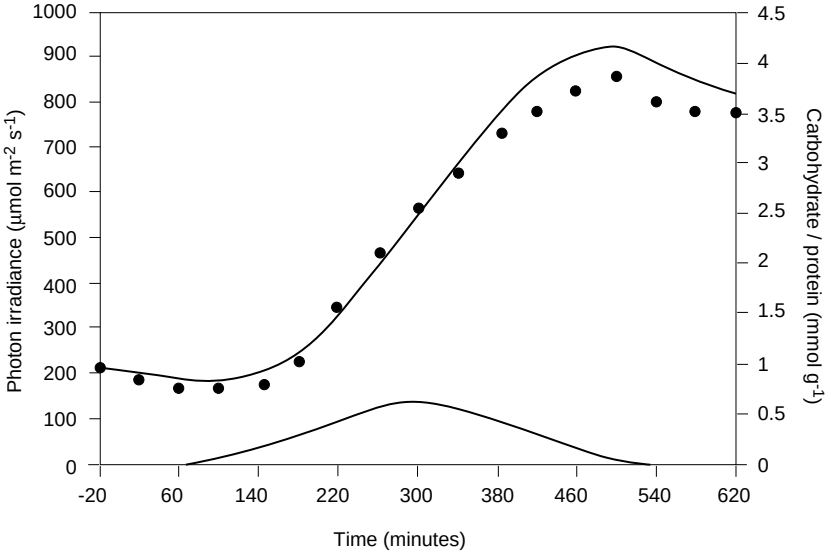

Figure 4. Diurnal changes in carbohydrate protein ratio, measured (dots) and calculated (bold line), and in the values of photon irradiance (regular line) in a continuous culture of *Microcystis* with a light regime simulating $Z_m/Z_{eu} = 0$. The photoperiod started at t = 0.

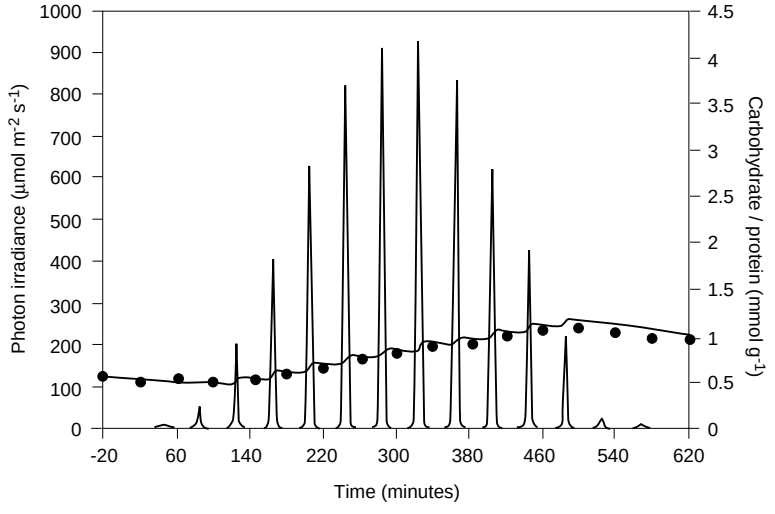

Figure 5. Diurnal changes in carbohydrate protein ratio, measured (dots) and calculated (bold line), and in the values of photon irradiance (regular line) in a continuous culture of *Microcystis* with a light regime simulating $Z_m/Z_{eu} = 3.6$. The photoperiod started at t = 0.

irradiance on the surface, but decreased again when the values of photon irradiance exceeded a certain value. This value was lower for small colonies than for larger colonies.

The impact of colony radius and of the values of photon irradiance at the surface on the net increase in density during the photoperiod were also investigated (Figure 8). The smaller colonies had a higher net increase in density during the photoperiod than the larger colonies. Due to their shallower migration, the photon dose received by the small colonies was apparently higher. At high values of photon irradiance (≥ 800 μmol m^{-2} s^{-1}), the net increase of the small colonies decreased as a result of inhibition at high values of photon irradiance. Nevertheless, at a value of photon irradiance of 1600 μmol m^{-2} s^{-1} at the water surface, the net density increase of a 10 μm colony was still greater than that of a 100 μm colony.

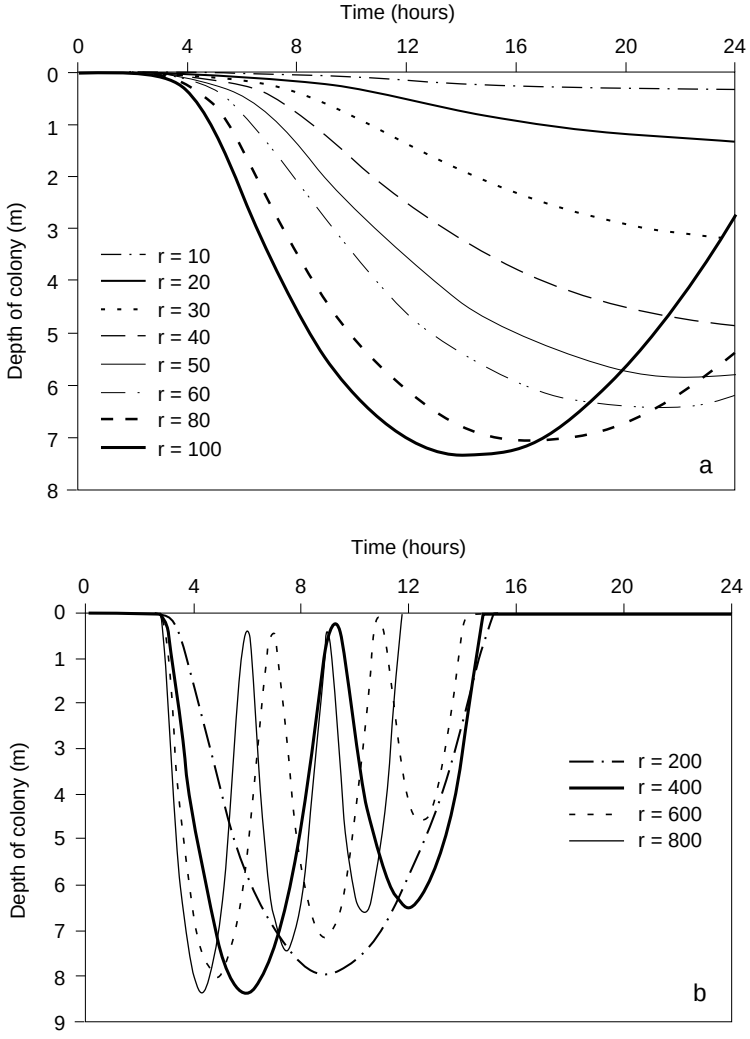

Figure 6. Simulation of movement of *Microcystis* colonies of various (a) small and (b) large radii (legends in μm). The photoperiod (12 h) started at t = 0. The maximum value of photon irradiance at the surface was 1000 μmol m^{-2} s^{-1}.

Discussion

There was good agreement between the calculated and measured values of the carbohydrate content relative to protein in the two continuous cultures of *Microcystis*. It can therefore be concluded that the method of determining the rate equations of density change, based on measurements of carbohydrate content during incubations at various constant values of photon irradiance, are appropriate for the vertical migration model.

The rates of density increase in *Microcystis* were inhibited at high values of photon irradiance. The irradiance-response curve used in this model for *Microcystis* therefore gave better results than the Michaelis-Menten equation used by Kromkamp & Walsby (1990) for *Oscillatoria*. The consequences of the inhibition of carbohydrate synthesis at high values of photon irradiance for the modelling of vertical migration were more important in simulations with small colonies. Due to inhibition at high values of photon irradiance at the surface, their vertical migration was shallower and their net increase in density during the photoperiod was lower than at low values. Photoinhibition had little effect on the larger colonies which could escape the high value of photon irradiance by their higher sinking velocity, following density increase in the morning.

The rates of density decrease did not show a linear change with the previously experienced photon

irradiance, another difference between our result and those of Kromkamp & Walsby (1990). A linear correlation was found, however, between the rate of density decrease and the density of the cells. A linear correlation of respiration and carbohydrate content of the cells has been described previously by Gibson (1975), Markager et al. (1992) and Watanabe & Kimura (1990). The use of the carbohydrate content of the cells produced a more accurate model of the density changes than the use of the light history. The carbohydrate content has a direct effect on carbohydrate metabolism, while the light history of the cells is less direct and therefore has a more complex relationship with the rates of carbohydrate consumption. Due to the non-linear nature of the relationship between the rates of density change and the values of photon irradiance, the light history of colonies that experience a fluctuating light regime on their way through the water column cannot be well described by the mean value of photon irradiance they experience. The results of the two continuous cultures receiving the same daily photon dose, but with the culture with $Z_m/Z_{eu} = 3.6$ receiving a much more fluctuating light regime, show that carbohydrate accumulation is not primarily dependent on the daily photon dose but rather on the time course of the values of photon irradiance during the photoperiod.

The patterns of colony movement of colonies with radii > 200 μm are comparable with the simulation done by Kromkamp & Walsby (1990) for colonies of *Oscillatoria*, but the maximum depth of the *Microcystis* colonies was higher and the duration of migration was somewhat longer. Comparison of the rates of density change (Figure 2) showed a higher rate of density increase in the light, and a somewhat lower rate of density decrease in the dark, for *Microcystis* compared to *Oscillatoria*, which can explain the greater migration depth of *Microcystis*. This lower rate of density decrease in the dark agrees with the higher dark growth yield (the amount of protein formed per carbohydrate consumed) of *Microcystis* (Konopka et al., 1987a) compared to *Oscillatoria* (Loogman, 1982) at a comparable growth rate.

Simulations of the migration of the largest colonies are less reliable, because they depend on the validity of the Stokes's equation, which holds precisely at Reynolds numbers (Re) of ≤ 0.1 (Reynolds, 1984). This holds for a colony radius of ≤ 108 μm. Simulations with smaller colonies (Figures 6a and 7) showed that the maximum depth of the migration increases with colony radius. This is in agreement with the suggestion of Reynolds & Walsby (1975) that large colonies will migrate to greater depths than small ones, due to higher flotation and sinking velocities. At radii > 200 μm hardly any difference between the maximum depth of the colonies was found. Kromkamp & Walsby (1990) simulated vertical migration of colonies with radii > 200 μm only and also found hardly any correlation between migration depth and colony radius.

It is interesting that the net increase in cell density during the photoperiod is predicted to be higher in small colonies than in large colonies (Figure 8). Even at inhibiting values of photon irradiance, the net increase is still higher than that of the larger colonies. These results suggest that there is no real advantage of a larger colony size in a stable water column besides possible resistance to grazing (Haney, 1987). However, exposure of small colonies to high values of photon irradiance over several hours might cause damage to the photosynthetic apparatus, a factor not considered in the model. This damage would require extra protein synthesis for repair (Vincent, 1990) at the cost of growth. Larger colonies that escape from high values of photon irradiance at the surface will therefore have an advantage over small colonies. Furthermore, a lower light absorption by large colonies (Robarts & Zohary, 1984) will result in less inhibition at high values of photon irradiance than in small colonies (Ibelings & Mur, 1992). The advantage of large size in a less stable water column is a faster return to the illuminated layer after a mixing episode, which will result in an increase in its daily photon dose in a mixed layer (Humphries & Lyne, 1988; Ibelings et al., 1991). In nutrient-limited systems, a large colony may have additional advantages from migration to deep, nutrient-rich layers.

Further validation of the model requires comparison with field observations. A rough comparison is possible with chlorophyll profiles from Lake Nieuwe Meer (Visser et al., 1996). In August and September 1990, when the phytoplankton in this lake was dominated by *Microcystis*, chlorophyll was concentrated in the upper 5–7 m. Simulation of vertical migration of *Microcystis* colonies of various sizes showed a maximum migration depth of 5.9 metres for colonies of 50 μm (= mean colony radius of *Microcystis* in Lake Nieuwe Meer in 1990); a shallower maximum migration depth was predicted for smaller colonies and a greater depth for larger colonies. A more detailed comparison of the simulations with field data would require some extension to the model, such as dependence of the rate equations on temperature and nutrient limitations. Since mixing of the water column plays an important role in the vertical distribution of phytoplankton in a

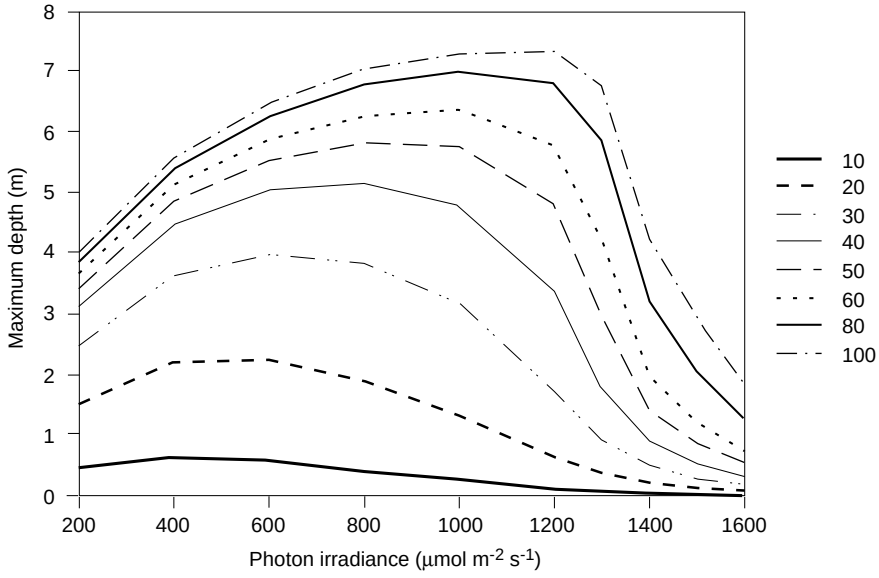

Figure 7. The influence of photon irradiance at the water surface on the maximum depth of migration by *Microcystis* colonies of various radii (legends in μm).

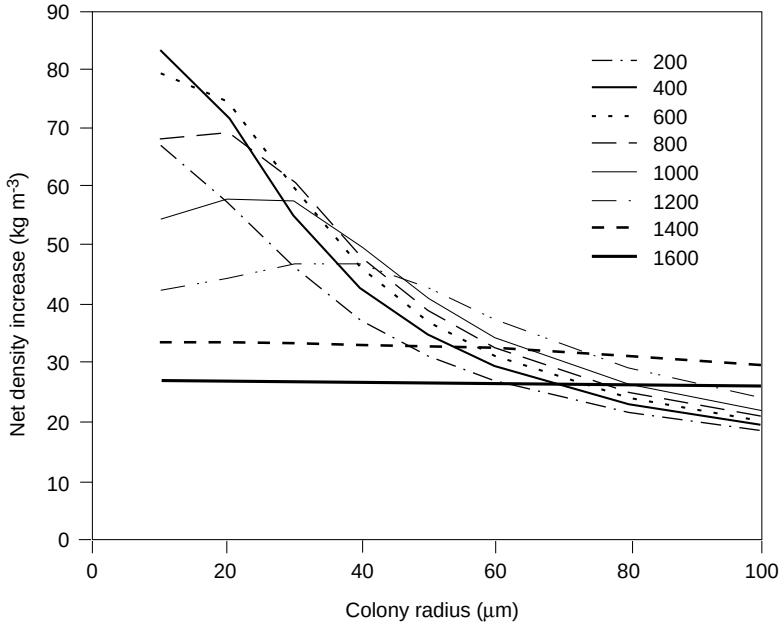

Figure 8. The influence of colony radius of *Microcystis* on the net increase in cell density (difference between cell density at the start and at the end of the light period) during the photoperiod at various values of photon irradiance (legends in μmol m^{-2} s^{-1}) on the surface.

lake, this should also be included. This has been done by Howard (1993) and Howard et al. (1996) in their vertical migration model. Additionally, these authors performed simultaneous simulations of more than one colony, which also facilitates comparison with vertical distribution patterns in the field.

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