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Modelling the interacting effects of nutrient uptake, light capture and temperature on phytoplankton growth

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*A model of phytoplankton growth developed by analogy with chemical kinetics (CR model) in Baird and Emsley (J. Plankton Res., **21**, 85–126, 1999) is explored further. The CR model parameterizes all biochemical reactions involved in phytoplankton growth by one parameter: the maximum growth rate. Phytoplankton growth rate is then calculated from an interaction of the maximum growth rate, and the physical limit to extracellular nutrient uptake rates and light capture. In this paper, the CR model was re-derived, with two corrections and a number of modifications to increase its generality. During derivation, the model's behaviour was compared with chemostat cultures at a variety of dilution rates, nutrient inputs and temperatures. Model output was then plotted against observations of a semi-continuous culture of Isochrysis galbana. Finally, the CR model was used to predict the growth rate of phytoplankton communities extracted from two temperate lakes under varying nutrient, light and temperature regimes. The CR model explained 37% of the variability of phytoplankton growth rate in cultures at environmental conditions similar to those of the lakes, compared with 25% explained by a non-linear best fit to 324 growth experiments. The following paper in this issue develops the CR model further, using it to predict stable carbon isotope fractionation.*

INTRODUCTION

In aquatic ecosystems, autotrophs often grow at suboptimal temperatures, nutrient concentrations and light conditions. Laboratory experiments have demonstrated an interaction between some of these limiting factors in phytoplankton (Rhee and Gotham, 1981), seagrass (Masini and Manning, 1997) and macroalgae (Kirk, 1994). Capturing the interaction of nutrient uptake rates, light capture and temperature dependence on autotrophic growth is, therefore, fundamental to developing predictive models of aquatic ecosystems [(Sterner and Grover, 1998) (SG98)].

Most growth models can be characterized by whether the model multiplies co-limiting factors (multiplicative) (Steele and Henderson, 1981; Taylor and Stephens, 1993) or uses only the most limiting factor (law of the minimum) (Legovic and Cruzado, 1997; Bormans and Webster, 1999), and whether an extracellular (Fasham *et al.*, 1990; Edwards and Brindley, 1996) or intracellular (Sharples and Tett, 1994) nutrient concentration is used. No single growth model has been preferred by the modelling or experimental community. The variety of growth models partly reflects the different uses for phytoplankton growth models. For example, models based on extracellular concentrations are preferred for ecosystem-scale models with computational constraints (Fasham *et al.*, 1990), while intracellular models are preferred by experimentalists who wish to test their understanding of underlying processes (Geider *et al.*, 1998). Nonetheless, as pointed out by Denman and Gargett, the range of phytoplankton growth models contrasts with the universal agreement over the governing equations of many physical systems, such as fluid motion (Denman and Gargett, 1995). Furthermore, the governing equations of physical systems typically have tightly constrained parameter values. The use of a common set of equations with tightly constrained parameter values has underpinned the development of predictive models of physical phenomena such as weather and ocean circulation (Kerr, 1998).

It is unlikely that a rigorous derivation of a single set of equations describing the observed range of phytoplankton

growth behaviours will be found in the near future. Nonetheless, a set of equations that takes advantage of easily quantified physical laws, and specifically designed to approximate phytoplankton growth behaviour for a range of potentially limiting factors (such as nutrients, light and temperature), may capture a broader range of *in situ* growth behaviour than empirical models based on laboratory experiments or field data alone.

The phytoplankton growth model developed by Baird and Emsley [(Baird and Emsley, 1999) (BE99)] considers growth to be an interaction of rates, quantified like a chemical reaction (CR model). The biochemical reactions involved in phytoplankton growth are parameterized by one parameter: the maximum growth rate. Phytoplankton growth rate is then determined from an interaction of the maximum growth rate, and the maximum rates of nutrient uptake and light capture. An upper limit on uptake rates of nutrients and light capture can be calculated by geometric considerations, such as shape and size. By considering growth as an interaction of maximum rates, the CR model takes advantage of those processes which are described by well-known physical laws, and uses parameter values which are constrained by geometric properties of the phytoplankton cells.

In this paper, we will assess the ability of the CR model to capture a range of phytoplankton growth behaviour with a minimum of calibration to experimental or field data. First, the CR model is re-derived, with two corrections and a number of modifications to increase its generality. The CR model is then assessed against the observations from a number of continuous cultures under varying nutrient and temperature regimes, and a semi-continuous culture of *Isochrysis galbana*. Finally, the CR model is used to predict the *in situ* growth rate of phytoplankton assemblages extracted from two temperate lakes to demonstrate its applicability to environmental systems, ease of use and predictive capabilities. In the second paper (Baird *et al.*, 2001), the CR model is developed further to predict stable isotope fractionation.

DERIVATION OF THE CR GROWTH MODEL

In this section, the CR growth model is re-derived. During the derivation, the behaviour of the CR model is compared to laboratory cultures with fixed growth rates in order to assess the assumption of physical limits and the performance of empirical approximations.

The chemical reaction

A growth model based on an analogy with chemical kinetics, considering nutrient uptake, light capture and algal growth to be interacting rates, was first developed in BE99. The conversion of stored intracellular reserves of nitrogen (N), carbon (C) and photons (energy) into structural organic matter of a set C:N ratio is modelled as a balanced chemical reaction:

$$C_{m_C}N_{m_N}I_{m_I} + m_C C + m_N N + m_I I \xrightarrow{k_p} 2C_{m_C}N_{m_N}I_{m_I} \qquad (1)$$

where C and N are the chemical symbols for carbon and nitrogen, and I represents photons, m_C, m_N and m_I are the stoichiometry coefficients quantifying the moles of carbon and nitrogen atoms and photons (energy), respectively, required to make another phytoplankton cell (mol cell^{-1}), and $C_{m_C}N_{m_N}I_{m_I}$ is the elemental composition of the structural material making up a single phytoplankton cell. Structural material is defined here as the composition of the cell excluding all reserves. The stoichiometry coefficients in equation (1) could be thought of as Droop's (Droop, 1983) minimum or subsistence quota. k_p is a rate constant (s^{-1}) quantifying the maximum rate at which the cell can convert internally stored nutrients into structural material, and is a measure of the maximum growth rate of the cell. Note that this reaction is different from equation (29) of BE99. Here, we present the reaction as 100% efficient (to keep things simple), and the products and reactants are represented as symbols (Correction 1; embarrassingly, BE99 used concentrations instead of using the chemical symbols in the balanced chemical reaction). BE99 also outlines the methodology for adding further nutrient requirements, such as phosphate.

The concentration of phytoplankton, and stored reserves of carbon, nitrogen and energy are given by $P \equiv [C_{m_C}N_{m_N}I_{m_I}]$, $R_C \equiv [C]$, $R_N \equiv [N]$ and $R_I \equiv [I]$, respectively. R_C, R_N and R_I represent the stored reserves of carbon, nitrogen and energy inside the cell, but not including the structural material (mol cell^{-1}), and P is measured in cells m^{-3}. The minimum amount of an element, say nitrogen, that a cell can contain is m_N [minimum cell quota or subsistence quota of Droop (Droop, 1983)]. The total amount of nitrogen within a cell [cell quota, Q, of Droop (Droop, 1983)] is given by $m_N + R_N$, and in a 1 m^3 volume of water with P cells by $(m_N + R_N)P$. The C:N ratio of the cell is variable, and is given by $m_C + R_C : m_N + R_N$. The structural component of the cell, however, has a fixed stoichiometry, given by $m_C:m_N$. Note that we are using R to represent the reserves of an element within a cell, as opposed to Q in BE99 (which turned out to be confusing since Droop defined Q as total quota). The energy reserves simply represents photons that have been captured, and the energy from which can be used at a later time to construct structural material [a more process-based description might store energy as fixed carbon (see Discussion)].

The mass balance

For each nutrient, a mass balance can be written. At steady state ($dR/dt = 0$), for say nitrogen and carbon, the rate of uptake is equal to the consumption rate of nutrients:

$$\text{uptake of } N = \mu(m_N + R_N); \text{ uptake of } C = \mu(m_C + R_C) \quad (2)$$

where μ is the actual growth rate of the cells (s^{-1}). Note that internal nutrient is lost to production of the structural material of additional phytoplankton cells (μm) and to division of internal nutrient amongst offspring (μR). Division amongst offspring was not included by BE99 (Correction 2), and the effect of this on the results of BE99 can be found in Baird (Baird, 1999). Now we have derived the chemical reaction and mass balance, it is necessary to determine the rate at which the chemical reaction proceeds. The rate of reaction will be a function of the maximum growth rate, and the internal reserves of energy and nutrients.

The relationship between growth and internal reserves

The most commonly used relationship between internal reserves of nutrients and growth rate was first proposed by Droop (Droop, 1968). Droop's model, using the definitions of m and R above, is given by:

$$\mu = \mu^{\max}\left(\frac{R}{m + R}\right) \quad (3)$$

Equation (3) is found to have a good fit between the observed growth rate (μ) and the total nutrient per cell ($m + R$) for a number of combinations of phytoplankton species and nutrients (Droop, 1983). However, it cannot work for nutrients in which $m \cong R^{\max}$, the maximum cellular reserve, such as carbon or photons: if $m = R^{\max}$, the growth rate determined using equation (3) can never exceed half the maximum rate.

In BE99, the change in phytoplankton concentration due to growth is given by the product of the concentration of all the reactants in equation (1) multiplied by a rate constant, k_p. Changing the notation to represent nutrient reserves by R:

$$\left(\frac{dP}{dt}\right)_{\text{growth}} = k_p R_C R_N R_I P \quad (4)$$

The growth rate of the phytoplankton cell, μ (s^{-1}), is therefore given by:

$$\mu = k_p R_C R_N R_I \quad (5)$$

The value of k_p can be calculated from the maximum growth rate of the phytoplankton cell, $\mu^{\max}$, divided by the maximum internal reserves of carbon ($R_C^{\max}$), nitrogen ($R_N^{\max}$) and energy ($R_I^{\max}$):

$$k_p = \frac{\mu^{\max}}{R_C^{\max} R_N^{\max} R_I^{\max}} \quad (6)$$

Rearranging, the growth rate becomes:

$$\mu = \mu^{\max}\frac{R_C}{R_C^{\max}}\frac{R_N}{R_N^{\max}}\frac{R_I}{R_I^{\max}} \quad (7)$$

Equation (7) provides a good fit of growth rate when $m \cong R^{\max}$, such as for carbon (see the comparison with laboratory experiments below). However, if $R^{\max} > m$, equation (7) tends to underestimate the growth rate. To improve the fit of the relationship between R and μ in the CR model, the first-order reaction of equation (7) can be changed to a power law:

$$\mu = \mu^{\max}\left(\frac{R_C}{R_C^{\max}}\right)^{p_C}\left(\frac{R_N}{R_N^{\max}}\right)^{p_N}\left(\frac{R_I}{R_I^{\max}}\right)^{p_I} \quad (8)$$

where p_C, p_N and p_I are the exponents for R_C, R_N and R_I.

Comparison with laboratory experiments

We chose carbon-, nitrate-, phosphate- and vitamin B_{12}-limited chemostat cultures to compare the observed relationship between the total cellular nutrient, $m + R$, and the growth rate, μ, with that predicted by the Droop model [equation (3)] and the CR model [equation (8)]. For growth limited by just one nutrient, we assume that for the other nutrients $(R/R^{\max})^p$ approaches one. So, for growth limited by one nutrient only, analogous to the Droop model [equation (3)], equation (8) becomes:

$$\mu = \mu^{\max}\left(\frac{R}{R^{\max}}\right)^{p} \quad (9)$$

The Droop model performs well when $m \ll R^{\max}$, but fails for carbon and nitrate (Figure 1). To apply the CR model, an exponent p must be specified. Typically, the exponents of chemical reactions fitted to power laws are obtained from experiments. We looked for a relationship for p that was a function of parameters we already knew, so as not to have to empirically fit a new parameter. We found $p = m/R^{\max}$ to work over a range of elements from $R^{\max} \approx m$ (carbon) to $R^{\max} > m$ (nitrate, phosphate, vitamin B_{12}) (Figure 1). The CR model, with $p = m/R^{\max}$, provides a better generic description of the relationship between growth rate and internal reserves over a range of nutrients than the Droop model.

Single or multiple nutrient-limited growth?

The question of whether to use a multiplicative or law of the minimum formulation for combining limiting factors

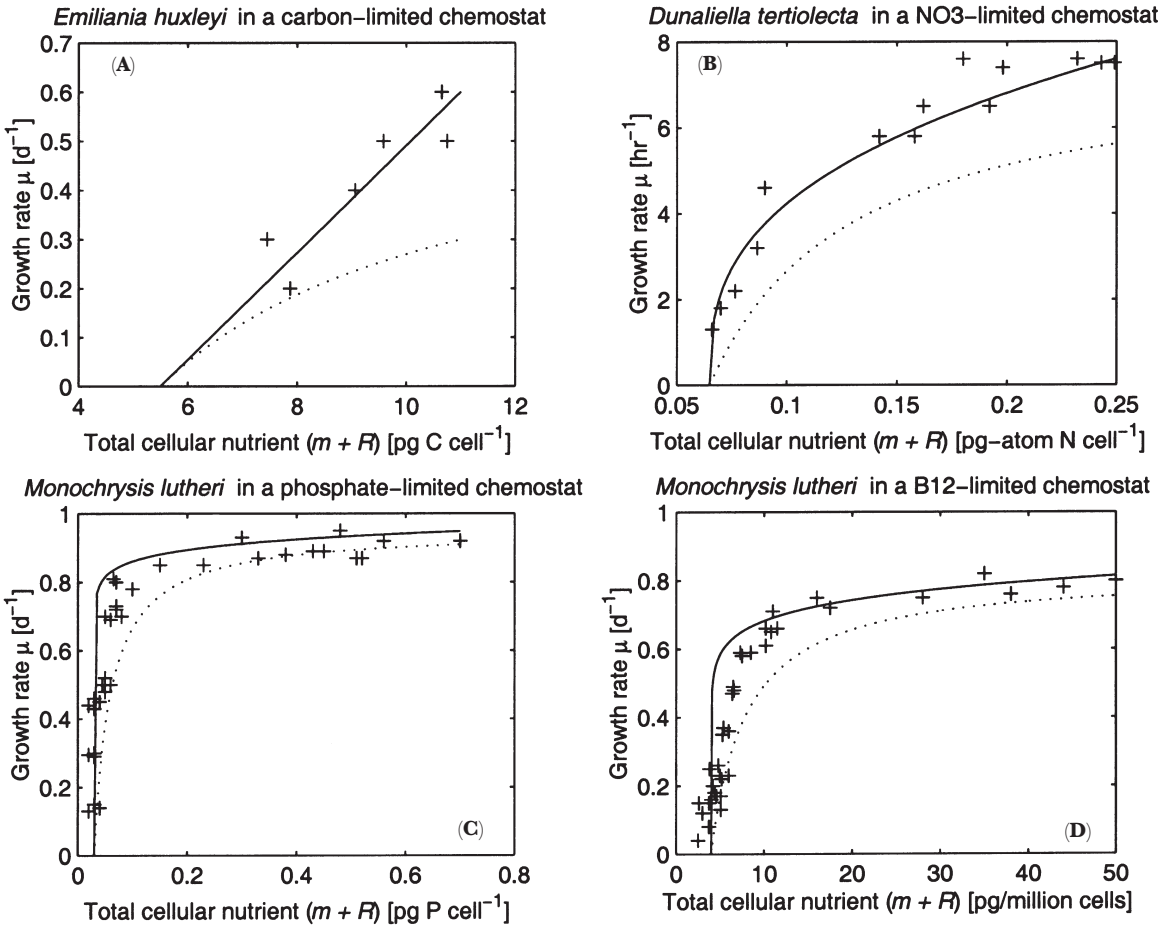

Fig. 1. The relationship between growth rate and internal nutrient reserves for four different nutrient-limited continuous cultures. Laboratory data (+) are compared with the Droop equation (. . .) and the power law (–) [equation 8] of the CR model. (**A**) Carbon: m = 5.5 pg C cell^{-1}, R^{max} = 5.5 pg C cell^{-1}, μ^{max} = 0.6 day^{-1} (Popp *et al.*, 1998). (**B**) Nitrate: m = 0.065 pg-atom N cell^{-1}, R^{max} = 0.185 pg-atom N cell^{-1}, μ^{max} = 7.6 h^{-1} (Bienfang, 1975). (**C**) Phosphate: m = 0.03 pg P cell^{-1}, R^{max} = 0.7 pg P cell^{-1}, μ^{max} = 0.95 day^{-1} (Goldman, 1979). (**D**) Vitamin B_{12}: m = 4 fM per 10^6 cells, R^{max} = 50 pg per 10^6 cells, μ^{max} = 0.82 day^{-1} (Droop, 1983). Note: We used different parameter values than the original authors to fit the Droop equation. The original authors determined m and μ^{max} from the best fit to the data over the whole range of internal nutrient reserves. We, instead, determined m and μ^{max} from the minimum quota and maximum growth rate observed (or an average of a few points at the limit). This significantly worsens the fit of D than in the original source. The original authors were empirically relating μ and R, and were therefore justified in their approach. The CR model, in contrast, is based on the definition of a constant m and R, and, as such, a different approach was used.

is unresolved, with proponents for both single limitation (Droop, 1983) and co-limitation (Davidson and Gurney, 1999). The main criticism of multiplicative models is that they underestimate growth rates. This is especially true of multiplicative models based on extracellular nutrient concentrations. However, Droop also demonstrated that the multiplicative formulation of the Droop model (an intracellular model) underestimated the growth rate in some continuous cultures by 30% (Droop, 1983). The CR model with a single-step reaction, as given here [equation (1)], is a multiplicative formulation [equation (8)]. We compared the CR model [equation (8)] to the same data that Droop used to argue for a law of the minimum formulation over a multiplicative formulation (Figure 2). A fit of the Droop law of the minimum and multiplicative formulations can be found in Droop (Droop, 1974). The CR model, in contrast to other multiplicative models, achieves a good fit to the Droop *Monochrysis lutheri* phosphorus–vitamin B_{12} experiments. While this does not resolve the issue of multiplicative versus law of the minimum for other growth formulations, it suggests that the CR model does

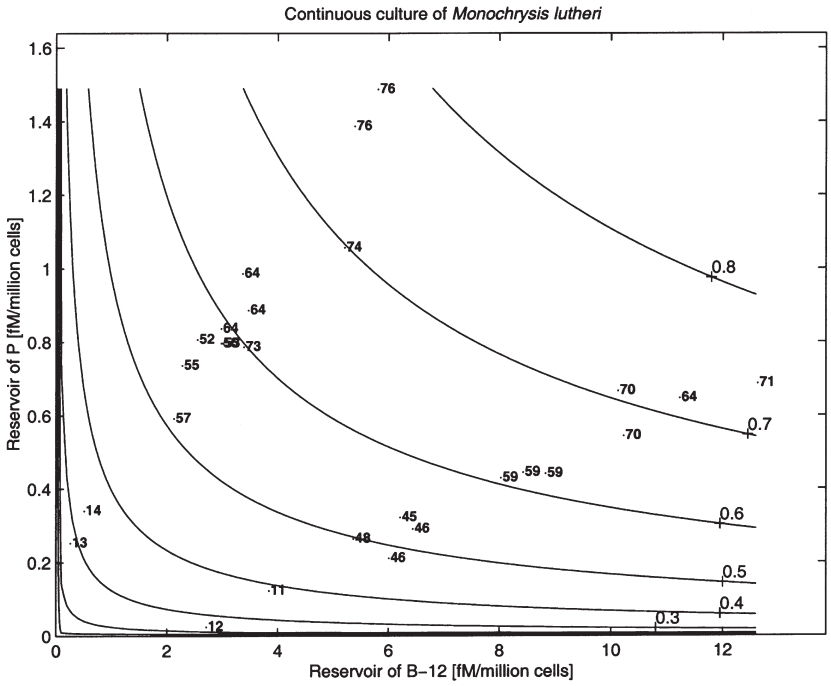

Fig. 2. The CR model [equation (8), with $p = m/R^{\max}$] used to predict the interaction of internal reserves of phosphorus and vitamin B_{12} in a continuous culture of *M. lutheri*. CR model parameters: $\mu^{\max} = 0.9$ day^{-1}, $m_P = 0.37 \times 10^{-3}$ mol cell^{-1}, $m_{B_{12}} = 2.4 \times 10^{-3}$ mol cell^{-1}, $R_P^{\min} = 1.49 \times 10^{-3}$ mol cell^{-1}, $R_{B_{12}}^{\min} = 12.6 \times 10^{-3}$ mol cell^{-1}. The contours represent the CR model predictions. Observations of growth rate, from Droop (Droop, 1974), are represented by a numerical value, whose position on the internal reserves axes is given by the decimal point.

not have the same problem of underestimating the growth rate.

The relationship between uptake and intracellular reserves

To model the uptake rates of nutrients and light, the rates are assumed to be determined by maximum uptake rate, k, multiplied by a function, $f(R)$, describing the dependence of nutrient uptake on the stored reserves of the nutrient. We have chosen to use maximum uptake rates based on geometric properties of the cells alone. This decision has been made because geometrically determined rates set an easily calculated physical limit, which cannot be exceeded. In the comparison with experiments section, we test this choice. However, we will first choose a functional form for $f(R)$.

In BE99, we used a linear dependence of the function, $f(R)$, on the stored internal reserves, R: $f(R) = \frac{R^{\max} - R}{R^{\max}}$. In this paper, we will represent $f(R)$ as:

$$f(R) = \left(\frac{R^{\max} - R}{R^{\max}} \right)^n \tag{10}$$

where n can vary between one (a linear dependence of uptake on the depletion of nutrients within the cell) and zero (no dependence of uptake on stored reservoirs). The overall uptake rate (in this case for carbon) per cell becomes:

$$\left(\frac{dQ}{dt} \right)_{\text{uptake}} = k_t \left(\frac{R_t^{\max} - R_t}{R_t^{\max}} \right)^n \tag{11}$$

and similarly for nitrogen and energy.

Comparison with laboratory experiments

First, we should assess whether diffusion limitation provides a good estimate of the maximum nutrient uptake rate. Many authors have suggested diffusion determination of maximum uptake rates (Munk and Riley, 1952; Pasciak and Gavis, 1975; Mierle, 1985; Wolf-Gladrow and Riebesell, 1997).

The maximum uptake rate, k in equation (11), is given by the initial slope of uptake versus nutrient concentrations at $R = 0$, multiplied by the nutrient concentration. The initial slope of phosphate uptake by six species of phytoplankton at $R = 0$ can be calculated from experiments detailed by Gotham and Rhee, who measured the maximum uptake, V, and half-saturation (of the reserve) of uptake, K, and also the cell volume (Gotham and Rhee, 1981). The initial slope of uptake versus R (α) can be approximated by $\alpha_{\text{measured}} = V/K$ (Healey, 1980). The

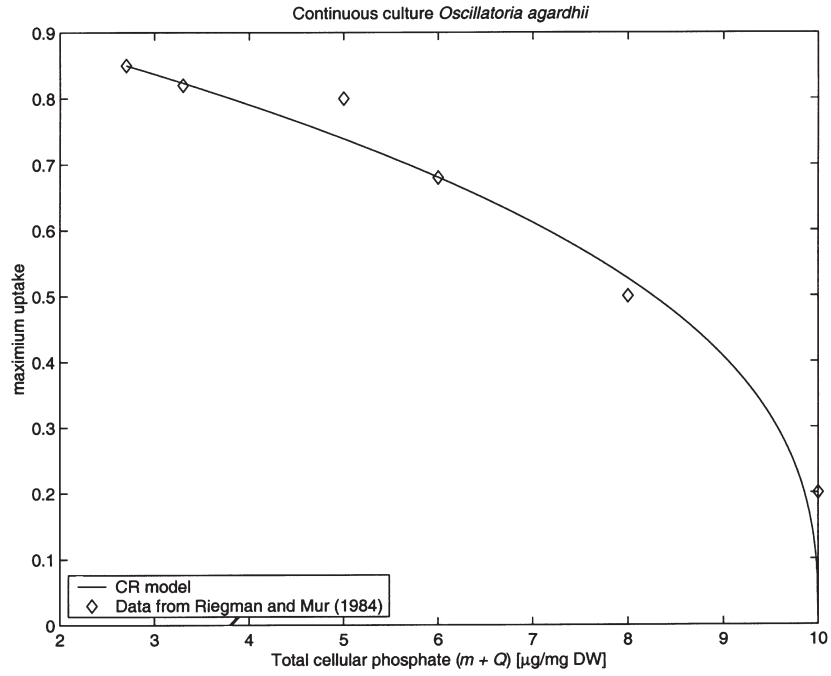

Fig. 3. The relationship between uptake rate and total cellular phosphate for a phosphate-limited chemostat culture of *O. agardhii*. Observations (◇) of Riegman and Mur (Riegman and Mur, 1984) versus the CR model [equation (11)] with $n = m/R^{max}$. Model parameters: $m = 2.7\ \mu g\ mg^{-1}$ DW, $R^{max} = 7.3\ \mu g\ mg^{-1}$ DW, $\mu^{max} = 0.85\ day^{-1}$.

initial slope of uptake versus nutrient concentration for the diffusion-limited uptake rate can be calculated from an equivalent spherical radius of the cell ($\alpha_{diffusion} = 4\pi rD$), where D is the molecular diffusivity of the nutrient species. If the diffusion limit is a good approximation of the maximum uptake rate, $\alpha_{diffusion}$ should be approximately equal to $\alpha_{measured}$.

The six phytoplankton species in Gotham and Rhee (Gotham and Rhee, 1981) were (with the equivalent spherical radius in parentheses): *Anabaena flos-aquae* (1.9 µm), *Ankistrodesmus falcatus* (3.5 µm), *Asterionella formosa* (3.8 µm), *Fragilaria crotonensis* (3.9 µm), *Microcystis* sp. (7.1 µm) and *Scenedesmus* sp. (3.9 µm). The measured initial slope, $\alpha_{measured}$, can be compared with the calculated diffusion-limit initial slope, $\alpha_{diffusion}$ [given in square brackets]: 3.1 [2.8] × 10^{-10} m^3 $cell^{-1}$ s^{-1}, 1.4 [5.1] × 10^{-10} m^3 $cell^{-1}$ s^{-1}, 12.0 [5.6] × 10^{-10} m^3 $cell^{-1}$ s^{-1}, 0.53 [5.8] × 10^{-10} m^3 $cell^{-1}$ s^{-1}, 0.7 [1.8] × 10^{-10} m^3 $cell^{-1}$ s^{-1}, 1.3 [5.7] × 10^{-10} m^3 $cell^{-1}$ s^{-1} and, for *Oscillatoria arghardii* (Riegman and Mur, 1984), 5.8 [1.3] × 10^{-10} mol P $cell^{-1}$ s^{-1}. Given the uncertainty required in these calculations, the diffusion limit provided a reasonable estimate of the initial slope of the uptake versus extracellular concentration at $R = 0$.

Further evidence that the diffusion limit is important in phytoplankton nutrient uptake is provided by a review of literature experiments by Hein *et al.*, in which they evaluated the relationship between log α (in units of uptake per unit biomass) and log surface area:volume ratio (SA:V) for 26 different algal species using a linear regression, determining an exponent (with 95% confidence intervals in parentheses) of 1.16 (1.00, 1.37) and 1.01 (0.75, 1.35) for nitrate and ammonia, respectively (Hein *et al.*, 1995). Using a dependence of biomass to cell volume of $V^{0.758}$ (Hofmann *et al.*, 2000), diffusion-limited uptake would result in an exponent ≈1.274 [SA:V ≈ $1/r$, uptake/biomass ≈$r/r^{2.274}$ ≈ $(1/r)^{1.274}$, so uptake/biomass ≈$SA:V^{1.274}$] within the 95% confidence intervals of both nitrate and ammonia uptake. Furthermore, the regression of Hein *et al.* (Hein *et al.*, 1995), determined that 84 and 50% of the variability in α for nitrate and ammonia uptake, respectively, could be accounted for by the changing SA:V alone. As pointed out by Wolf-Gladrow and Riebesell, SA:V is, to a good approximation, proportional to the diffusion-limited uptake rate (Wolf-Gladrow and Riebesell, 1997).

To a first approximation, therefore, the diffusion limit appears to be a good initial estimate of the maximum uptake rate. The other maximum rate used, the absorption cross-section, is well-accepted as the initial slope of

the photosynthesis versus irradiance curve (Kirk, 1994) and, with an appropriate conversion factor, can be used as a measure of the initial slope of light capture versus growth. Now we must assess whether equation (11) captures the functional dependence of uptake on R. Figure 3 plots the observed maximum uptake rates of *O. agardhii* under steady-state growth versus total cellular phosphate. Equation (11) is drawn with $n = m/R^{max}$. Again, the exponent m/R^{max} appears to be useful in reducing the number of parameters required to apply the CR model to a particular nutrient.

The effect of temperature on nutrient reserves and growth rate

In the CR model, temperature affects phytoplankton growth by changing the rates of maximum growth rate and supply rate of nutrients and light, although it does so in an uneven manner. The flux of photons that collide with the photosynthetic apparatus of the cell, k_I, is independent of temperature [although it is known that the apparatus itself may change with temperature (Raven and Geider, 1988)]. The maximum nutrient uptake rate, k_N for nitrate, is a function of the molecular diffusivity, D, of the nutrient species. According to the Stokes–Einstein equation (Atkins, 1994), D is a linear function of temperature, with an ≈ 0.87 change over 10°C (Li and Gregory, 1974). The maximum growth rate, like many chemical reactions, is often modelled using the Arrhenius equation (Raven and Geider, 1988), which is an exponential function of temperature. A Q_{10} value of ≈ 2.0 (representing a 2-fold increase in growth rate with a 10°C increase in temperature) is common (Raven and Geider, 1988).

In a chemostat, the effects of temperature on the rate of conversion of stored nutrient into organic matter [equation (1)] are isolated from the effects of temperature on nutrient uptake rates. From equation (9), we can solve for the internal concentration:

$$R = \left(\frac{\mu}{\mu^{max}}\right)^{1/2} R^{max} \qquad (12)$$

where μ^{max} is temperature dependent and μ is set by the dilution rate.

Comparison with laboratory experiments

The CR model [which solves equation (12)] is compared to a nitrogen-limited chemostat culture of *Scenedesmus* sp. (Rhee and Gotham, 1981) in Figure 4. The CR model appears to capture the interacting effects of temperature and total cellular nitrogen on growth rate. The changes in m and R^{max} with temperature correlated with cell volume (Rhee and Gotham, 1981), although we did not explicitly model changing cell volume with temperature here.

Effect of temperature on chemostat biomass

The temperature dependence of nutrient uptake has no impact on the relationship between growth rate and internal reserves of a nutrient, but it does affect the steady-state concentration of cells. Consider a nitrogen-limited chemostat at steady state at 20°C at dilution rates $\mathcal{D} = \mu_{20}R$. If the temperature is reduced by 10°C, initially the growth rate $\mu_{10}R$ will be less than the dilution rate, and the concentration of cells will decrease. The decreased number of cells will allow the extracellular concentration of nitrogen to increase, increasing uptake. Complicating this is the reduced maximum uptake rate of nitrogen due to slower molecular diffusivity at lower temperatures. The maximum uptake rate, k_N, can be given by $k_N = \psi DN$, where ψ is the diffusion shape factor (m), D is the molecular diffusivity and $\mathcal{N}$ is the extracellular concentration, and D is a function of temperature. The mass balance at steady state is given by:

$$\psi D\mathcal{N}(1 - R/R^{max})^{m/R^{max}} = \mu(m + R) \qquad (13)$$

Rearranging, the number of cells is given by:

$$\text{Number of cells} = \frac{\mathcal{N}_{source} - \dfrac{\mu(m + R)}{\psi D(1 - R/R^{max})^{m/R^{max}}}}{m + R} \qquad (14)$$

Unfortunately, in the experiments of Rhee and Gotham (Rhee and Gotham, 1981), almost all the nitrogen within the chemostat was held within the cells, so the number of cells reduces to $\mathcal{N}_{source}/(m + R)$, which does not really test equation (14). Nonetheless, equations (12) and (14) demonstrate the effect of temperature on the internal reserves (through μ^{max} only) and the steady-state population (through both μ^{max} and D).

ASSESSING THE CR GROWTH MODEL AT ENVIRONMENTALLY VARYING CONDITIONS

A semi-continuous laboratory culture

Continuous cultures are used to investigate the effect of setting the growth rate on internal nutrient reserves. Our aim, however, is to predict the growth rate under environmental conditions. As a step towards this end, the CR model is now assessed against a semi-continuous culture: dilution occurs like in a continuous culture, but the dilution rate varies periodically, allowing μ and R to become decoupled. This is more representative of a natural system than a continuous culture. The equations are the same as for a continuous culture, but with a time-varying dilution rate, $\mathcal{D}$. The experiments of Caperon (Caperon, 1968, 1969) (Figure 5) for a semi-continuous

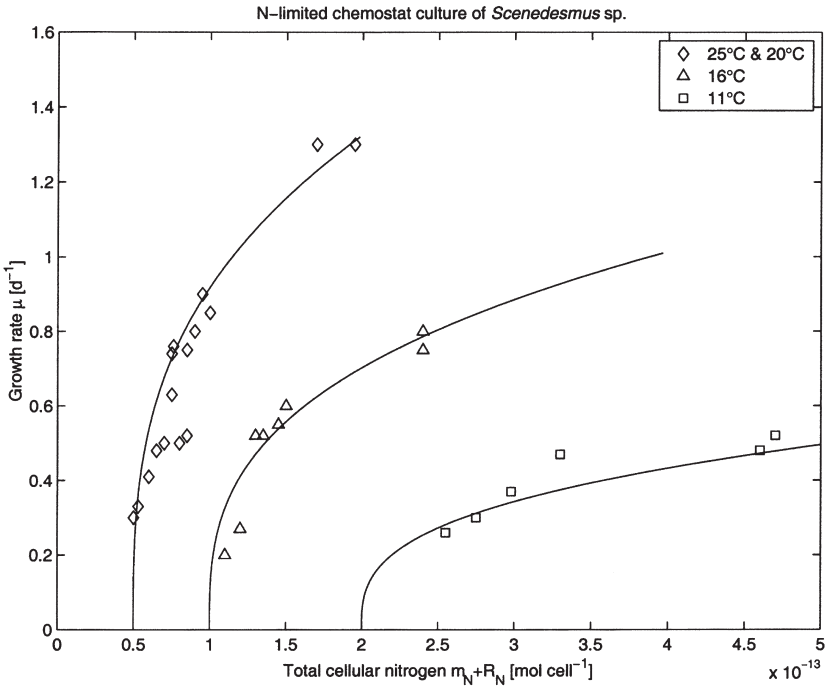

Fig. 4. Total cellular nitrogen versus growth rate at various temperatures in an N-limited chemostat culture of *Scenedesmus* sp. Data at 25 and 20°C (◇), 16°C (△) and 11°C (□) from Rhee and Gotham (Rhee and Gotham, 1981), model lines using equation (12) and $p = m/R^{max}$, and for 20, 16 and 11°C, m = 0.5, 1.0 and 2.0 × 10^{-13} and R^{max} = 1.5, 3.0 and 6.0 × 10^{-13} mol cell^{-1}, respectively.

culture were used to assess the model's predictive capability. Although Caperon used two sets of model parameters to fit the two populations, since they came from the same inocula, we used only one set of parameters, based on geometric properties of the cells and the maximum growth rate. It was considered that this would be more representative of attempting to model field populations.

Phytoplankton assemblages in temperate lakes

The growth rate experiments of SG98 can be used to assess the performance of the CR model at predicting phytoplankton growth in the field. SG98 measured the growth rate of the phytoplankton communities in 54 samples from two warm temperate reservoirs (Cedar Creek Lake and Eagle Mountain Lake, northern Texas, USA) at temperatures ranging from 6.4 to 30°C, nitrate concentrations between 2 and 248 μg N l^{-1}, and light levels between 50 and 360 μmol (photons) m^{-2} s^{-1}. A further 270 determinations of the growth rate of the natural phytoplankton assemblages were made under increased nutrient concentrations. Based on a total of 324 measurements of growth rate, SG98 fitted the growth rate to a rectangular hyperbolic equation:

$$\mu = \mu_T \left(\frac{N}{K + N} \right) \quad (15)$$

where T is the temperature (°C), and SG98 obtained μ_T = 0.0256 day^{-1} °C^{-1} and K = 66.0 μg N l^{-1} from a non-linear regression of the 324 growth experiments. The ability of equation (15) to predict the growth rate in the 54 controls (those without nutrient additions) was used to assess the model's ability (calibrated against 324 measurements) to predict *in situ* growth rate. Equation (15) could explain only 25% of the variability of *in situ* growth rates (r = 0.51, n = 54, $P < 0.01$).

The application of the CR growth model required a knowledge of the maximum growth rate (SG98 gives 0.77 day^{-1}) and the reference temperature at which this growth rate is achieved (SG98 uses T_{ref} = 30°C). We also assumed a Q_{10} of 2. To obtain the maximum nutrient uptake and light uptake rates, a cell radius is required. The dominant species reported from the samples of the two reservoirs was *Cyclotella* sp., including *C. atomus*, *C. comta* and *C. glomerata*, with diameters between 5 and 10 μm [J. Grover,

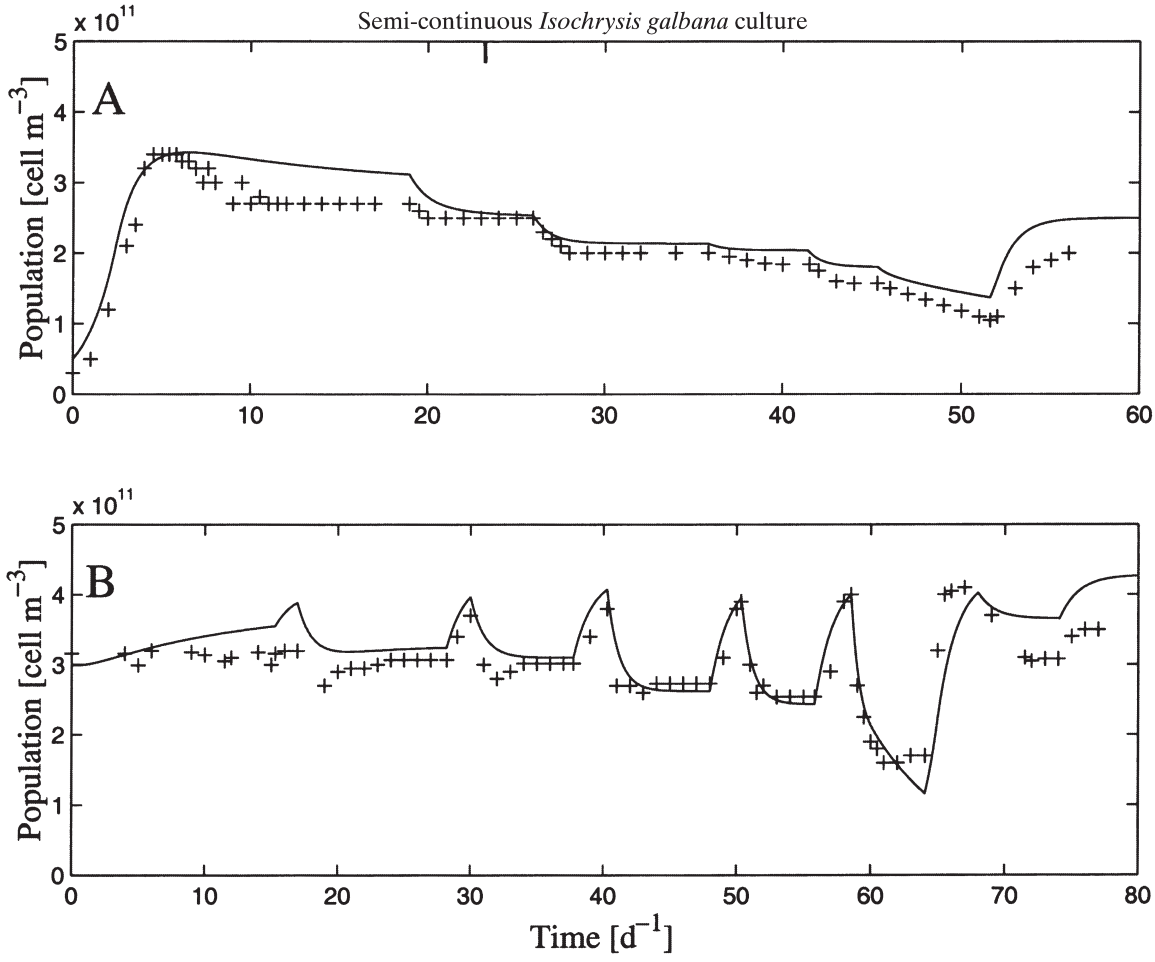

Fig. 5. Comparison of laboratory data (+) of *I. galbana* grown in semi-continuous culture conditions (Caperon, 1968, 1969) with CR model output (–), assuming unlimiting carbon and light ($R_C = R_C^{max}$, $q = q^{max}$). *Isochrysis galbana* was assumed to be a prolate ellipsoid (radii = 3.5 × 1.9 × 1.9 μm, $\mu^{max} = 0.7$ day^{-1}, $R_N^{max} = 2.55 \times 10^{-4}$ mol N cell^{-1}), and $k_N = 2.7236 \times 10^{-14}$ m^3 s^{-1} (BE99). (**A**) $N_{in} = 8.65 \times 10^{-3}$ mol m^{-3}, and initial conditions of $R_N = 2.55 \times 10^{-4}$ mol N cell^{-1}, $N = 8.65 \times 10^{-3}$ mol m^{-3} and $P = 50 \times 10^9$ cells m^{-3}. (**B**) $N_{in} = 8.65 \times 10^{-3}$ mol m^{-3}, and initial conditions of $R_N = 0.255 \times 10^{-4}$ mol N cell^{-1}, $N = 10.9 \times 10^{-3}$ mol m^{-3} and $P = 300 \times 10^9$ cells m^{-3}.

personal communication; (Grover *et al.*, 1999)]. The maximum nutrient uptake rate, assuming diffusion limitation of uptake, is then given by $k_N = 4\pi r D_N N$ mol s^{-1}, while the maximum light uptake rate, assuming that all light reaching the cell is absorbed, is given by $k_I = \pi r^2 I$ mol (photons) s^{-1}. It is assumed that the cell requires $m_C = 9.14 \times 10^3 V$ (BE99) atoms of carbon, where V = cell volume (m^3), and that the ratio of cell requirements of carbon, nitrogen and photons comes from the Redfield ratio (C:N:photons = 106:16:848) (Kirk, 1994). In the absence of experimental data to suggest otherwise, we assumed that the exponents of equations (8) and (11) were one. No attempt was made to tune μ^{max}, T_{ref}, Q_{10}, m_C, n or p to fit the experimental results of SG98.

To use the CR model to predict *in situ* growth rate, we assumed that the cells were growing at steady state (i.e. uptake of a nutrient is equal to consumption for growth, and therefore the internal reserves of a nutrient are constant). The growth rate is determined by solving two non-linear simultaneous equations

$$k_N \left(\frac{R_N^{max} - R_N}{R_N^{max}} \right) = \mu^{max} \left(\frac{R_N}{R_N^{max}} \right) \left(\frac{R_I}{R_I^{max}} \right) m_C + R_N \quad (16)$$

$$k_I \left(\frac{R_I^{max} - R_I}{R_I^{max}} \right) = \mu^{max} \left(\frac{R_N}{R_N^{max}} \right) \left(\frac{R_I}{R_I^{max}} \right) m_C + R_I \quad (17)$$

to determine R_N and R_I. The growth rate can then be calculated using equation (8). The non-linear simultaneous equations were solved using MATLAB software, by applying Newton's method for solving systems of non-linear equations, truncating the Taylor series approximation to one term, and using Gaussian elimination to solve the intermediate linear simultaneous equations until successive approximations were within 10^{-9} (Hoffman, 1992).

Using a radius of 3 μm, the CR model explained 37% of the variability in the growth rate of the controls ($r = 0.61$, $n = 50$). At radii from 1, 2, . . ., 10 μm, the r values were 0.15, 0.61, 0.61, 0.59, 0.57, 0.56, 0.56, 0.50, 0.53 and 0.52, respectively. To improve on the prediction of phytoplankton growth of *in situ* growth based on 324 determinations of growth rate in the laboratory, using the CR model, required being able to estimate the radii of the dominant species ± a factor of 2. Using $r = 3$ μm, the CR model also improved the prediction of the growth rate of all the samples ($r = 0.45$ versus 0.40, $n = 324$), although it produced a poorer prediction of those samples that SG98 identified as being N limited ($r = 0.75$, versus 0.83, $n = 162$).

DISCUSSION

There are a large number of models of phytoplankton growth, ranging from very simple models based on extracellular nutrients (Steele and Henderson, 1981) to those that consider many of the complex biochemical reactions involved in autotrophic growth (Flynn *et al.*, 1997; Geider *et al.*, 1998). So how is the CR model different? The primary consideration during the construction of the CR model has been its ability to predict the *in situ* growth rate of phytoplankton communities. To achieve this goal, the CR model takes advantage of those processes that can be well constrained by physical laws (the maximum uptake rates of nutrients as limited by diffusion and maximum light absorption).

The CR model eliminates the need to model biochemical processes explicitly, by representing the sum of all biochemical reactions as a maximum growth rate. The maximum growth rate interacts with the maximum uptake rates of nutrients and light capture. One consequence of not explicitly modelling biochemical reactions is that the CR model has an internal reserve of energy (photons), which is unrealistic. In contrast, Geider *et al.* provide a more process-based understanding of the biochemical reactions, and particularly the use of photons within a cell (Geider *et al.*, 1998). In their model, light drives photosynthesis, which, when balanced by respiration, changes the store of carbon within the cell; so the energy is effectively stored as carbon. Geider *et al.* also consider the adaptation of cell functions (such as higher concentrations of pigments under low light) (Geider *et al.*, 1998). However, to achieve this level of representation of biochemical processes, they required 10 parameters. If they had chosen to explicitly represent other arguably as important biochemical processes, such as multiple pathways for nutrient assimilation (Flynn *et al.*, 1997), the number of parameters would have increased further. The large number of species-specific parameters makes it difficult to use such complex models for phytoplankton communities in natural water bodies. In contrast, the application of the CR model to the two temperate lakes, after assuming $T_{ref} = 30$°C (the annual maximum temperature), $Q_{10} = 2.0$, and that the exponents of p and n were 1, required the determination of only two parameters: the maximum growth rate and radii of the dominant algal species.

The data of SG98 illustrate a common problem associated with using experimental data to determine *in situ* phytoplankton growth rates as a function of environmental conditions. To obtain a function of the growth rate over a variety of nitrate concentrations, SG98 only added nitrate to the samples (as opposed to removing it). As a result, the SG98 relationship is based on growth experiments with an average nitrate concentration of 397 μg N l^{-1}. A total of 203 of the 324 experiments were conducted at concentrations greater than the fitted half-saturation constant. For the experiments that SG98 determined were nitrate limited ($n = 162$), the SG98 relationship [equation (15)] performed better than the CR model ($r = 0.83$ versus 0.75). The average concentration of the nitrate-limited experiments was 340 μg N l^{-1}, and the assumption of equation (15) of single-nutrient limitation was met. For the experiments conducted under *in situ* conditions, the CR model outperformed the SG98 equation ($r = 0.61$ versus 0.51). The average concentration of nitrate during *in situ* experiments was 83 μg N l^{-1}. Sterner, musing over his results, states that 'it is perhaps sobering that a detailed study on rate kinetics such as this can do no better than explain 25% of the variation in algal community growth'. The lack of success of the SG98 model at predicting *in situ* growth rate is because the relationship was determined by elevating the nutrient concentration of samples. In doing so, the SG98 relationship is biased towards elevated nutrient concentrations, and is not a good predictor of *in situ* growth rate.

In the CR model, the growth rate is determined from the interaction of maximum growth rates and maximum uptake rates, which have been determined from physical laws. The approximations of the CR models are best fitted under extreme limitation by one of the rates. For example, under low nutrient conditions, the growth rate predicted by the CR model will approach the diffusive uptake rate of that nutrient. Given that the parameters involved in determining the diffusive uptake limit (i.e. molecular diffusivity and cell dimensions) are well constrained, the CR model should perform well at low extracellular nutrient concentrations. In contrast, the SG98 relationship determines the growth rate at low nutrient concentrations, based on a non-linear fit to predominantly high nutrient growth experiments. The CR model also captures much of the interaction of temperature with nutrients and light. It is common for growth models to have an exponential temperature dependence under all conditions. The CR model has a variable temperature dependence: a result of

temperature affecting the rates of uptake, light capture and biochemical reactions differently. At high nutrient and light levels, growth is an exponential function of temperature. At high light levels and low nutrient levels, growth is a linear function of temperature, and at high nutrient and low light conditions, growth is temperature independent. The improved representation of the changing temperature dependence of growth under varying environmental conditions is an important part of the CR model approach.

Despite an improvement over the SG98 relationship, 63% of the variability in SG98 controls (the unmanipulated samples) remained unexplained by the CR model. Some of this may be put down to the approximations of maximum nutrient uptake (such as a spherical cell) and maximum light absorption (all photons hitting a portion of the cell are absorbed). A more detailed consideration of cell shape and pigment concentrations (Baird and Emsley, 1999) may improve the CR model's fit to the data. Another component of this variability may be accounted for by including more detailed representation of biochemical processes, as discussed earlier. We suspect, however, that the greatest source of uncertainty is associated with the changing populations of different species. In order to account for 90% of the biomass of all samples throughout the year, Grover *et al.* needed to identify 33 and 27 algal taxa in Cedar Creek Lake and Eagle Mountain Lake, respectively (Grover *et al.* 1999). If species diversity is the major source of variability, fine tuning the calculation of maximum nutrient uptake and light absorption rates, or including detailed biochemical processes, may not improve the model predictions.

Why might a model based on the interaction of physical limits and a maximum growth rate provide a predictive capability in a biological system? To speculate, in a competitive environment, cells (at least fast-growing cells) with fast internal biochemical reactions will tend to outcompete those with slower internal reactions. Under this evolutionary pressure, it is easy to imagine the rate of the biochemical reactions tending to increase. Given a constant physical limit, such as diffusion to the cell wall, it is likely that the physical process will become the rate-limiting step to growth.

In this paper, the first of a two-part series, we revisited the CR model of BE99, making two corrections (representing nutrients as chemical symbols rather than concentration and including the sharing of internal reserves of parents with offspring during growth), and suggested modified forms of the growth and uptake versus internal reserves to explain laboratory experiments better. We showed that the application of the CR model required knowledge of the maximum growth rate of a cell, μ^{max}, the temperature at which it achieves this maximum growth rate, T_{ref}, the amount of an element in the structural material of a cell, m, and the radius of the cell. Even for a mixed algal community, with knowledge of these parameters for the dominant species, the CR model provided a better estimate of *in situ* algal growth, as measured in samples extracted from two temperate lakes, than a model based on 324 experimentally determined points.

As pointed out by SG98, the laboratory culture of unmanipulated samples provides only an estimate of *in situ* growth rate. Direct measurement of *in situ* growth rate is problematic: any changes in phytoplankton population size in the lake due to growth are hard to measure with changes in population due to other processes such as grazing, sinking, diseases, programmed cell death, etc. In the second paper in this series, the CR model is used to derive the rate of stable isotope fractionation during growth. Stable isotope fractionation is a function of environmental conditions and growth rate, but not the mode of death. It should, therefore, be possible to use stable isotope fractionation to assess the performance of the CR model at predicting *in situ* growth rate without using laboratory experiments as a measure of field growth rates, and at the same time get an estimate of *in situ* growth rate without sampling live cells.

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