Title No. 3 10 900 420 3

ISSN 0006-8055

Typesetting Arthur Collignon GmbH, Berlin
Printing Mercedes-Druck, Berlin
Binding Buchbinderei Spiller, Berlin

Editorial Assistant and Technical Coordinator Cornelia Wolff v. d. Sahl

Printed in Germany

W DE G

Walter de Gruyter & Co., Genthiner Straße 13, D-1000 Berlin 30, West Germany, ☎ (030) 26005-124, Telex 184 027
Walter de Gruyter, Inc., 200 Saw Mill River Road, Hawthorne, N.Y. 10532, U.S.A., ☎ (914) 747-0110, Telex 646 677

WALTER DE GRUYTER · BERLIN · NEW YORK

Botanica Marina
Vol. XXVI, pp. 99–112, 1983

25 Years Botanica Marina

25 Years of Algal Growth Kinetics
A Personal View

M. R. Droop

Scottish Marine Biological Association, Dunstaffnage Marine Research Laboratory, P.O.Box 3, Oban, Argyll, Scotland

(Received August 17, 1982)

„Der liebe Gott läßt die Bäume
nicht in den Himmel wachsen"

The past 25 years have witnessed a growing interest in the relation of microalgal growth to the nutrient supply, with a view perhaps more to quantifying the role microalgae play in nature than elucidating the underlying biochemistry of the algal cell. I intend in this short essay for the *Botanica Marina* silver jubilee (Festschrift) to indulge in a personal, and in part retrospective, view of this field, and shall be concerned principally with the Cell Quota model of algal growth control.

From Malthus to Monod

The idea of exponential population growth owes its origin to Malthus. Under conditions of unlimited resource and space, a population will increase at a rate proportional to its size,

$$\frac{dx}{dt} = \mu x, \qquad (1)$$

the proportionality coefficient μ, the specific growth rate, being characteristic of the population and dependent on conditions prevailing.

However, as Malthus' critics pointed out, the fact that resource and space are usually limited and generally become depleted greatly limits the power of the exponential law to account wholly for observed population increases. It is obvious that in an isolated system, for instance a flask culture of an alga or bacterium, the total amount of resource must set a limit to the amount of biomass that can be produced. Moreover, the only way biomass can be limited is by reducing the rate of increase to zero. Thus in such a system the specific growth rate will appear as some function of the biomass. Consequently, most post-Malthusian thought on populations was concerned empirically with the nature of that function. The earliest, simplest and indeed most successful and still in many circumstances the most useful, hypothesis was that of the mathematician Verhulst (1845), the so-called logistic law, in which growth rate is a (negative) linear function of biomass and by inference a linear function of the resource concentration:

$$\mu = \mu_m (1 - \frac{x}{K_x}), \qquad (2)$$

μ_m being the maximum specific growth rate, x biomass and K_x a constant with the dimensions of biomass, representing the maximum biomass the region can support.

Most attempts to derive a law of growth from chemical or physiological principles were largely unsuccessful, though they did attempt to relate growth to the concentration of the resource. The breakthrough came with Teissier (1932) in the recognition of the asymptotic nature of the dependence. Teissier proposed an exponential relation of the form

$$\frac{d\mu}{ds} = \frac{\mu_m - \mu}{K_s} \qquad (3)$$

(s being the resource concentration and K_s a constant with the dimensions of a concentration). His student,

0060-8055/83/0026-0099$02.00

Monod (1942), suggested the simpler hyperbolic relation

$$\frac{\mu}{s} = \frac{\mu_m - \mu}{K_s}. \quad (4)$$

The practical difference between these last two formulations is small (the exponential saturates rather more quickly than the hyperbolic). However, the ease of handling the rectangular hyperbola and its attractive, though spurious, analogy with enzyme kinetics and the mass-action laws has led to the widespread use in microbiology of Monod's equation, more usually written

$$\mu = \frac{\mu_m s}{K_s + s}. \quad (5)$$

Both Teissier and Monod postulated the rates of biomass increase and substrate utilization to be in constant proportion,

$$\frac{dx}{dt} = -Y \cdot \frac{ds}{dt}. \quad (6)$$

Y is known as the yield coefficient. Implicit in the above is the assumption of constant cell composition. There is also the implication that a constant proportion of the resource is converted to biomass, but Monod (1942) forsaw that the requirement for maintenance would involve the modification of the simple law.

The assumption of constant chemical composition was justified when the chemical in question was carbon, since the major part of biomass, other than water, is carbon. There is no *a priori* reason why other cell components should be maintained in constant proportion under all circumstances, and indeed Herbert (1961) pointed out that they were not. The yield coefficient of equation (6) could be expected to vary, for instance, with growth rate. This, however, would not affect the steady-state operation of Monod's equation.

A case in point in the algal field was the luxury uptake of phosphorus, first observed by Ketchum (1939) and later studied in detail with the marine diatom *Phaeodactylum tricornutum* by Kuenzler and Ketchum (1962). When cells of this alga were placed in fresh medium virtually all the phosphorus was observed to be taken up by the cells from the new medium before even the first cell division had taken place. Thereafter it was apportioned among the progeny of the subsequent cell divisions, which continued until cell phosphorus had dropped sufficiently to arrest cell division. It is apparent that cell division during the life of the culture must have occurred without direct reference to the external concentration of the substrate. Luxury consumption of nutrients other than phosphorus has since proved to be the rule. Indeed Eppley and Strickland (1968) concluded that growth rate is more closely related to the cellular content than to the external concentration, and that variation in the cellular content of nitrogen and phosphorus observed in phytoplankton represents the concentrations necessary to maintain given rates of growth imposed by other factors.

The luxury uptake observed by Ketchum however, indicates that uptake and growth may generally be so loosely coupled that virtually no predictable relation can exist between external substrate concentration and growth rate, that is except under steady-state conditions, e.g., continuous culture, which, if achieved, necessarily imply effective coupling between all parts of the cell machinery.

The Cell Quota nutrient model

Cell quota could be defined as the quantity of substrate required to produce a given biomass, in other words, as a coefficient of demand and the reciprocal (in the absence of excretion) of the yield coefficient. However, the definition as weight of internal nutrient per unit biomass (Droop 1968) encourages the concept of an internal nutrient 'pool' upon which growth might be supposed to depend. Caperon (1968) expressed the same idea.

I was operating chemostats in an attempt to establish the physiological level of requirement for vitamin B_{12}, that is, to measure the parameters of the Monod relation with vitamin-limited *Monochrysis lutheri* using ^{57}Co labelled vitamin (Droop 1968). Instead of the expected rectangular hyperbola the curve of growth rate on measured external vitamin concentration took the form of a letter C. It was evident that some factor was obscuring the Monod relation, which could not therefore be used. (I shall return to this point later). The relation between growth rate and vitamin B_{12} cell quota (Q), on the other hand, did have the form of a simple rectangular hyperbola but one with an intercept on the abscissa (Fig. 1).

$$\frac{\mu}{\mu_m} = 1 - \frac{k_Q}{Q} \quad (7)$$

The parameter k_Q, the value of the intercept, is interpreted as the minimum quota necessary for life, and may conveniently be termed the 'subsistence quota'.

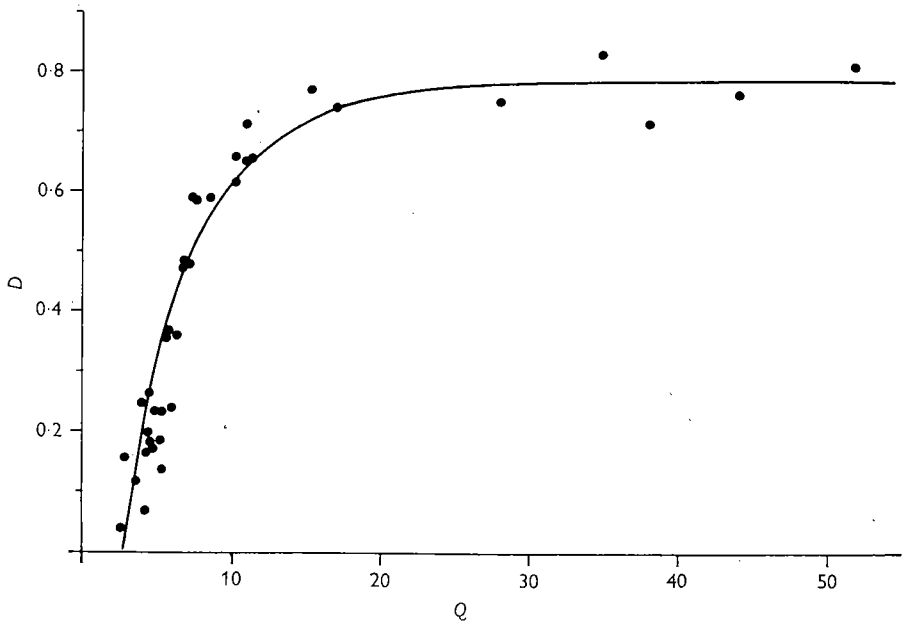

Fig. 1. *Monochrysis lutheri* in a vitamin B_{12}-limited chemostat: Relation between dilution rate and cell quota. (Reproduced from Droop (1968) with permission from the Journal of the Marine Biological Association, U.K.)

It is interesting to note that the reciprocal form of the equation, i.e., in terms of yield coefficients ($1/Q$, $1/k_Q$ or Y, Y_m) has the same form as the logistic equation. The Verhulst independent variable could be defined as biomass per unit total resource, while that in equation (7) is in effect biomass per unit internal resource.

In the decade and a half since 1968 equation (7) has been applied sucessfully to an increasing number of cases with different organisms and nutrients (Table I), but there have been instances in which the equation was not applicable, notably nitrogen with ammonium-limited growth of *Monochrysis* and *Dunaliella* (Caperon and Meyer 1972, Laws and Caperon 1976). Nevertheless, the Cell Quota concept appears to be a useful one. Jones *et al.* (1978) have made an encouraging test of the applicability of the relation to P-limited natural phytoplankton.

It is possible in some cases to interpret the subsistence quota as an unreactive or structural component, which when subtracted from the cell quota leaves an active pool to which growth rate relates in a Michaelis fashion,

$$\frac{\mu}{\mu_m'} = \frac{Q - Q_0}{k_Q + Q - Q_0}. \qquad (8)$$

This is logically attractive as it makes use of a familiar relation, but it is hypothesis and there are many other ways of achieving a threshold, even in the field of enzyme kinetics. The main argument against this elaboration is that the 3-parameter model has not

Table I. Reports of algae showing a hyperbolic relation between specific growth rate and cell quota.

Organism	Nutrient	Reference
Thalassiosira fluviatilis	P	Fuhs 1969
T. pseudonana	P	Fuhs 1969
Scenedesmus sp.	P	Rhee 1973
Monochrysis lutheri	P	Droop 1974
Monochrysis lutheri	P	Goldman 1977
Monochrysis lutheri	P	Terry 1980
Oscillatoria rubescens	P	Feuillade and Feuillade 1975
Asterionella formosa	P	Tilman and Kilham 1976
Cyclotella meneghiniana	P	Tilman and Kilham 1976
Chlorella pyrenoidosa	P	Nyholm 1976
Chlorella pyrenoidosa	P	Senft 1978
Selenastrum capricornicum	P	Nyholm 1977
Chlorella vulgaris	P	Panikov and Pirt 1978
Anabaena wisconsinense	P	Senft 1978
A. flos-aquae	P	Gotham and Rhee (personal communication)
Microcystis sp.	P	Gotham and Rhee (personal communication)
Oscillatoria agardhii	P	Ahlgren 1980
Isochrysis galbana	NO_3	Caperon 1968
Chlorella pyrenoidosa	NO_3	Williams 1971
Dunaliella tertiolecta	NO_3	Caperon and Meyer 1972
Dunaliella tertiolecta	NO_3	Bienfang 1975
Monochrysis lutheri	NO_3	Bienfang 1975
Monochrysis lutheri	NO_3	Terry 1980
Thalassiosira pseudonana	NO_3	Caperon and Meyer 1972
Coccochloris stagnina	NO_3	Caperon and Meyer 1972
Oscillatoria rubescens	NO_3	Feuillade and Feuillade 1975
Oscillatoria agardhii	NO_3	Ahlgren 1980
Chaetoceras gracilis	NH_4	Thomas and Dodson 1972
Chlorella vulgaris	Urea	Panikov and Pirt 1978
Thalassiosira pseudonana	Si	Paasche 1973
Asterionella formosa	Si	Tilman and Kilham 1976
Cyclotella meneghiniana	Si	Tilman and Kilham 1976
Monochrysis lutheri	Vit.B_{12}	Droop 1968, 1974
Skeletonema costatum	Vit.B_{12}	Droop 1970
Monochrysis lutheri	Fe	Droop 1970

been shown to produce a better fit than the 2-parameter model, nor have k_Q and Q_0 been shown to differ significantly. Indeed equation (5) reduces to (7) when the two are equal. On grounds of economy and because it makes no assumptions, the 2-parameter model is to be preferred, at any rate until such time as it proves inadequate.

The fact that the Cell Quota equation (in whichever form) generally works indicates that whatever the active pool is, its size is proportional to the whole (less the subsistence quota). Rhee (1973) has shown that in *Scenedesmus* the rate-limiting pool is related to polyphosphate (in P-limited cells) and to the intracellular free amino acids (in N-limited cells), though Maske (1982) was able to find no evidence of the latter in *Skeletonema*. Attempts at dissection can lead to conceptual difficulties if taken literally. For example Davis *et al.* (1978) found that in silicon-limited *Skeletonema*, the active pool appeared to be associated with the wall silicon and not the soluble cell fraction.

When the product forms a major part of the biomass, as in the case of carbon, one is less likely, as Goldman and McCarthy (1978) point out, to find the rate limiting pool proportional to the whole. Caperon's work (Caperon 1968) suggests that nitrogen would not be included in this category, but my colleague M. Turner (personal communication), working with glycine-limited *Monochrysis*, found no variation in Keldahl cell N with growth rate. The most obvious symptom of the inapplicability of the Cell Quota model is the enlargement of μ'_m (Goldman and McCarthy 1978).

Monod and the Cell Quota models compared

The cell quota is maintained by nutrient uptake on the one hand and on the other dissipated by cell multiplication, the change being the sum of the two processes

$$\frac{dQ}{dt} = u - \mu Q \,. \qquad (9)$$

The steady-state rate of uptake is therefore μQ.

Uptake is usually assumed to depend on substrate concentration in a Michaelis fashion,

$$u = \frac{u_m\, s}{k_s + s} \,, \qquad (10)$$

and there is much evidence in support of this (for references, see Rhee, 1980).

If it is conceded that the Monod equation [equation (5)] and the Cell Quota [equation (7)] and uptake [equation (10)] equations are all true of the steady state, in other words the two models are strictly equivalent, the following relations between the various parameters necessarily hold (Droop 1973, Burmaster 1979)

$$\frac{k_Q}{u_m} = \frac{1}{\mu_m} - \frac{1}{\mu'_m} = \frac{K_s}{\mu_m\, k_s} \qquad (11)$$

μ'_m of the Cell Quota equation is thus seen to be greater than the Monod μ_m. The difference is important and the two parameters should not be confused.

Fig. 2 is a diagram comparing the two models in the steady state. Away from the steady state $u \neq \mu Q$, dissipation and uptake need no longer be equal, equation (11) no longer holds and the two models

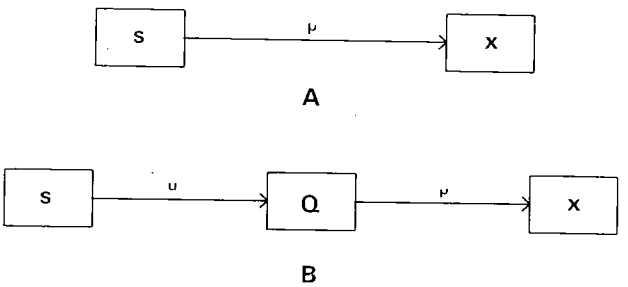

Fig. 2. Diagrammatic representation of Monod (A) and Cell Quota (B) models

are no longer equivalent. Monod responds to a step change in external substrate by a step change in specific growth rate, whereas in the Cell Quota model there is an exponential change in growth rate corresponding with an exponential change in the cell quota following a step change in the rate of uptake (Fig. 3). Monod is thus seen to be generally applicable to the steady state while the extra compartment in the Cell Quota model has lent it the potential also to handle transients. It can be shown that the time constant of the cell quota change is $1/\mu'_m$ (and is of the order of 24 hr for vitamin B_{12} or phosphorus in *Monochrysis*). Similarly the time constant for substrate change is

$$\frac{k_s + 1}{u_m\, x}$$

The act of placing an inoculum, for instance, in fresh culture medium, effects a step increase in substrate concentration and, unless the latter time constant is much shorter than $1/\mu'_m$, an imbalance will occur and give rise to the phenomenon of luxury consumption referred to earlier. The outcome of course depends

on the values s and x, the new substrate concentration and population size, which influence the time constant.

Mackereth (1953) was probably the first to stress the ecological importance of the internal nutrient (P) concentration in algae. Now in the light of the Cell Quota model one can state that the potential of any isolated body of water for supporting phytoplankton growth is proportional to the sum of internal and external concentrations (Droop 1973).

$$X_{potential} = \frac{s + Qx}{k_Q} \qquad (12)$$

This has some implication in the field of bioassay (Maestrini *et al.* 1983) for obviously for measurements made at the start of a bloom the external concentration is the more important, while for those made in the later stages the internal concentration and biomass dominate.

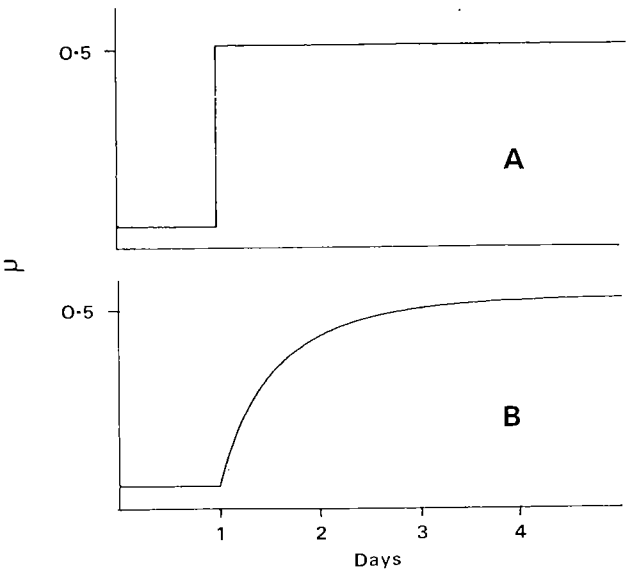

Fig. 3. Modelled response in growth rate to a step increase in external substrate concentration from 0.067 K_s to 1.33 K_s at day 1. (Computed from vitamin B_{12} parameters for *Monochrysis*). A: Monod. B: Cell Quota. (Reproduced from Droop (1977) with permission from the Journal of Protozoology)

Excess nutrients and double substrate limitation

It is convenient to define a limiting nutrient as the one in control of growth. With unstructured models such as Monod the criterion of limitation is simple: if the substrate concentration is without effect on growth rate the nutrient is not limiting. In the case of the more structured Cell Quota model, particularly in the application to transients, this criterion is unreliable because growth rate depends primarily on the internal concentration, which does not necessarily follow that in the external medium. A different criterion is needed.

It had been noticed (Droop 1973, 1974) that even when a nutrient (Vitamin B_{12}, iron, phosphorus) was clearly in excess in a chemostat the cell quota relation [equation (7)] held, only now the quotas in question (Q, k_Q) were very much greater. A corollary of this was that in steady-state culture the observed ratio between the cell quotas of the various nutrients was independent of the specific growth rate and only depended on the ratio between the input concentrations. This condition yields a straight line when the quotas of two nutrients are plotted against each other, whose slope depends upon the input ratio (Fig. 4). I coined the term 'luxury coefficient' (ϱ) for the factor by which the quota of an excess nutrient was enlarged, and later (Droop 1975) defined ϱ as the ratio between the standardized uptake rate of an excess nutrient to that of a limiting nutrient at the same growth rate

$$\varrho = \frac{u_N}{k_{Q_N}} \cdot \frac{k_{Q_L}}{u_L} = \frac{u_N^s}{u_L^s}. \qquad (13)$$

The superscript s denoting standardization relative to the respective subsistence quota and the subscripts N and L denoting respectively 'excess' and 'limiting'.

The value of ϱ was observed (in continuous culture with ^{32}P and ^{57}Co vitamin B_{12}) to depend on the ratio between the input concentrations, but since cells 'see' the ambient rather than input concentrations the dependence must be on the latter. It appeared to be a saturating function of this ratio

$$\varrho = \frac{\frac{s_N^s}{s_L^s} \cdot R_m}{(R_m - 1) + \frac{s_N^s}{s_L^s}} \qquad (14)$$

We can now define the controlling nutrient as that whose standardized quota is the least. ϱ is seen to take values between unity and R_m, being unity when the nutrient is in control.

A more general version of equation (7) can now be written

$$\frac{\mu}{\mu_m'} = 1 - \frac{\varrho k_Q}{Q}. \qquad (15)$$

These relations were observed with vitamin B_{12} and phosphorus in *Monochrysis* over an hundredfold range of relative input concentrations (Droop 1974). They are empirical and presumably a simplification of a complex state of affairs. Feuillade and Feuillade (1975) noted that in the N/P interaction in *Oscillatoria rubescens* the quota of the controlling nutrient controls that of the excess nutrient. Rhee's (1974) data on *Scenedesmus* and Ahlgren's (1980) on *Oscillatoria agardhii* indicate that nitrogen does not follow this pattern, for the N:P ratio varied with growth rate when nitrogen was greatly in excess. It has already been pointed out that the cell quota equation cannot be applied to total cell nitrogen and interpretation of nitrogen data will have to await the identification of the 'active' pool. Tilman and Kilham (1975), working with silicon and phosphorus in diatoms, also report ϱ varying with growth rates. The fact that most cell silicon is skeletal may account for this, so that again one awaits the identification of an active fraction.

The definition of the controlling nutrient adopted, namely that whose standardized cell quota is the least, assumes that control follows a threshold or non-interactive pattern. This concept originated with Liebig (1876) „Der Ertrag (des Bodens) ist von den *in minimo* in ihm enthaltenen Nährstoff abhängig", i.e. in respect of crop yields, but was later applied to rates of growth by Blackman (1905) and then by Lotka (1925) and employed by Ryder and Sinclair (1972) to model double substrate limitation in bacteria. The only interactive possibility that has been seriously considered was a multiplicative interaction first suggested by Baule (1918) and applied to primary production by Verduin (1964) and again by McGee *et al.* (1972). The multiplicative concept, though elegant, since it harbours no discontinuities, does present difficulties. For example, if

$$Y = F_1(x_1) = F_2(x_2) \ldots = F_i(x_i)$$

then clearly

$$Y \neq \Pi[F_1(x_1), F_2(x_2), \ldots F_i(x_i)].$$

In other words, if the growth rate of an organism can accurately be expressed in terms of the phosphate concentration by e.g., the Monod relation and also in terms of the nitrate concentration by the same relation, then it could not in general be expressed in terms of both substrates simultaneously by the product of the two functions.

Using the double label ^{32}P and ^{57}Co and arranging the chemostat input concentrations to give nearly equal limitation by phosphorus and vitamin B_{12}, it was not difficult to decide which hypothesis was most nearly correct for *Monochrysis* and that pair of nutrients. The answer was unequivocally the threshold hypothesis; the other underestimated the specific growth rate by some 30 per cent (Droop 1974). Figure 4 illustrates this. Two sets of data are shown, the one with the standardized ratio P:vitamin B_{12} of 0.46, and the other of 1.37.

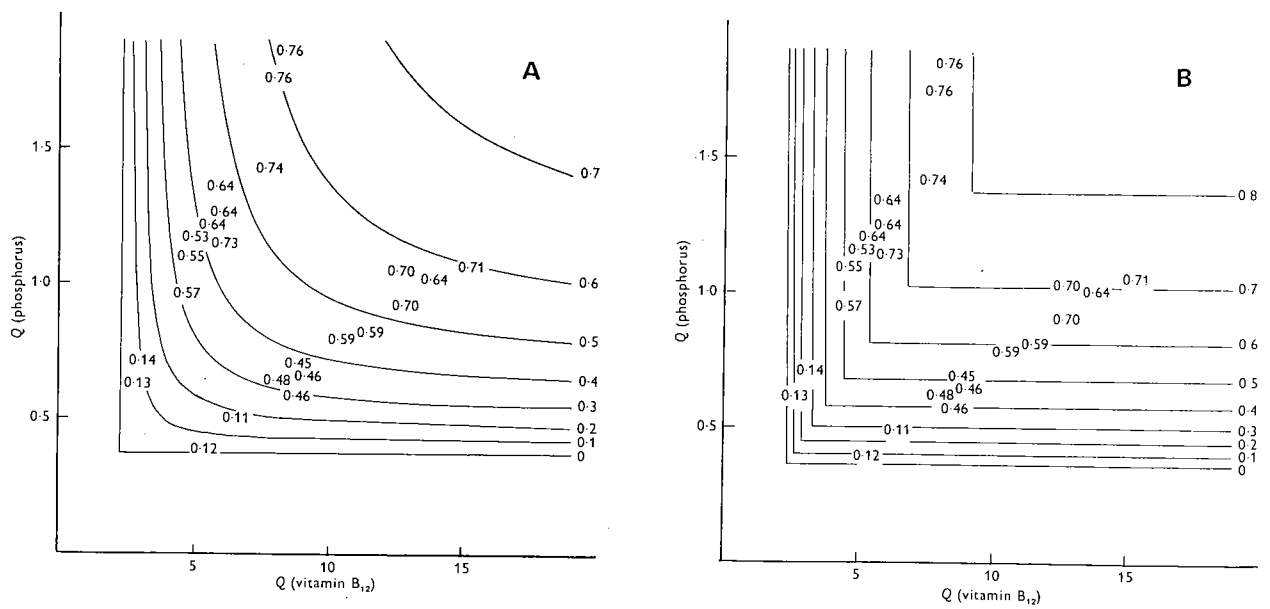

Fig. 4. *Monochrysis* in vitamin B_{12}-limited and P-limited chemostats plotted on Cell Quota coordinates. Experimental steady states indicated by position of decimal points; the figures referring to dilution rates. The contours computed according to A, the multiplicative and B, the threshold hypothesis. (Reproduced from Droop (1974) with permission from the Journal of the Marine Biological Association, U.K.)

It has also been shown by rather different approaches that the threshold pattern is followed by NO_3 and P in *Scenedesmus* (Rhee 1974) and by urea and P in *Chlorella* (Panikov 1979).

Bader (1982) has pointed out that it is unlikely that any two biochemical pathways leading to growth are completely independent and non-interactive. This, coupled with the greater mathematical elegance of interactive systems, has led him to advocate models based on the latter. I find it hard to agree; so long as interaction is so limited as to be unverifiable its inclusion is bound to be complex and arbitrary and indeed unnecessary. The multiplicative hypothesis has, however, found favour with ecologists often dealing with a multiplicity of factors, not all of which are nutritional, in natural situations (Rodhe 1978).

Further relations within the Cell Quota model

The maximum value a cell quota can take and the nutrient in question still be in control ($Q^s_{m_L}$) is obtained by putting μ_m (of the Monod equation) for μ in equation (7), and rearranging thus:

$$Q^s_{m_L} = \frac{\mu'_m}{\mu'_m - \mu_m}. \qquad (16)$$

The right hand side of this expression can be thought of as the standardized cell quota analogue of the factor limiting growth (e.g. light) when all nutrients are in excess. Any nutrient with a standardized quota in excess of this will not be in control of growth, though the converse does not necessarily hold. $\mu'_m/(\mu'_m - \mu_m)$ must be expected to be variable if it is conceded that factors other than the known nutrients (e.g., possible controlling nutrients omitted from consideration through ignorance, CO_2, light, temperature, inhibitors of systems not under consideration etc) may vary.

When a nutrient is in excess it follows from the fact that ϱ has a value greater than unity, dependent on the relative ambient concentrations of it and the controlling nutrient, that uptake (now u_N) must follow the Michaelis law but with parameters dependent upon the concentration of the controlling nutrient. The relative expressions may be derived by manipulation of equations (10), (13), (14), (15) and (16):

$$\frac{u^s_N}{u^s_{mN}} = \frac{s_N}{k_{s_N} + s_N} \qquad (17)$$

where

$$u^s_{m_N} = R_m\, u^s_{M_L} \qquad (18)$$

and

$$k^s_{s_N} = (R_m - 1)\, s^s_L \qquad (19)$$

Similarly, the maximum *steady-state* cell quota of an excess nutrient is

$$Q^s_{m_N} = R_m\, Q^s_{m_L} \qquad (20)$$

One can infer from the above that uptake of an excess nutrient should stop when the controlling nutrient becomes depleted. This surprising prediction was in fact fulfilled in the case of vitamin B_{12} and phosphorus when the model was tested both in chemostat and batch cultures of *Monochrysis*: the uptake of phosphorus stopped abruptly (with 6 µM P still in solution) when the available vitamin concentration approached zero (Droop 1974, 1975). This is an interaction not envisaged by any of the simple so-called interactive models (Bader 1982).

The parameter R_m, the maximum value ϱ can take, is in a sense a measure of the cell's capacity for 'luxury uptake' under steady-state conditions. In the case of phosphorus, the classic instance of 'luxury uptake' the steady-state R_m is quite small: the *Scenedesmus* data (Rhee 1974) suggest a value between 3 and 7 (when NO_3 is limiting) and my own data for *Monochrysis* (with vitamin B_{12} limiting) indicate values between 8 and 17. Rhee's data also suggest an R_m for NO_3 (with P limiting) of the same order. On the other hand, R_m for vitamin B_{12} in *Monochrysis* (with P or light limiting) is greater than 100. The capacity for luxury uptake of vitamin B_{12} is evidently at least an order greater than that of phosphorus (Droop 1974, 1975, Droop *et al.* 1982).

Nutrient Uptake

The common and generally successful use of Michaelis kinetics to describe nutrient uptake (for references see Rhee 1980) tends to obscure the difficulties associated with interpretation of the kinetic parameters derived from the measurements as usually performed.

The first problem arises from the fact that uptake is a more complex process than the passage across a single membrane and the technique used will determine which part of the total process is being examined. Even the passage across a single membrane probably involves two steps, absorption and transport. Presumably the dynamic steady-state rate of uptake as expressed by the product μQ refers to the complete

process; while on the other hand subjecting depleted cells to graded doses of a nutrient and following the time course of changes in cell and/or medium nutrient is more likely to result in absorption kinetics than those of the overall process. For example vitamin B_{12} absorption can be very rapid compared to the overall steady-state rate, but the capacity of the system is much smaller than that of the steady-state cell (Droop 1968, Bradbeer 1971). The opposite is the case with phosphorus; the capacity of the short-term system, in *Monochrysis* at least, is some 20 times that of the steady-state capacity (i.e., 200 times the subsistence quota, though Terry (1982) quotes 60 times). If transport is inhibited by the experimental treatment or if the transport mechanism is slow to respond to the changed conditions, the measured kinetic parameters in short-term experiments will not be those of the overall process and are therefore not appropriate to any of the growth models in common use. The use of steady-state batch cultures (from very small inocula) can overcome this problem (Droop 1968).

The uptake of phosphorus in the short term depends to a great extent on the amount already in the cells. Rhee (1973) has very elegantly described this phenomenon with the aid of the Briggs-Haldane enzyme product inhibition model

$$u = \frac{u_m}{Z\left(\frac{k_s}{s} + 1\right)} \qquad (21)$$

where

$$Z = 1 + \frac{Q - k_Q}{k_{i_q}} \qquad (22)$$

(k_{i_q} being an inhibition constant with the dimensions of a cell quota). The trouble starts when the attempt is made (Rhee *et al.* 1980) to combine this model with the steady-state formulation of the Monod and Cell Quota models. If equation (10) is replaced by Rhee's equation [equation (21)] one obtains the following in place of equation (11)

$$\frac{Zk_Q}{u_m} = \frac{1}{\mu_m} - \frac{1}{\mu'_m} = \frac{ZK_s}{\mu_m k_s}. \qquad (23)$$

This, however one looks at it, is incompatible with one or other of the growth equations, having regard for the meaning of the variable Z, since either μ_m and/or μ'_m, or both k_Q and/or u_m and K_s and/or k_s must vary with μ. Indeed it leads to a quadratic of μ in s, which when solved for zero s gives a finite value for μ. A chemostat would run quite happily with zero feed.

The second problem arises from the fact that nutrient uptake in the short term depends on the physiological condition of the cells in addition to the product inhibition of phosphorus mentioned above. There is for example the observation that stationary phase vitamin B_{12}-limited cells of *Monochrysis* have lost *ca* 90 percent of their capacity to take up fresh supplies (Droop 1968) while Terry (1982) has recently observed that short-term NO_3 uptake in this organism is suppressed by high P concentrations by an amount depending on the preconditioning N:P supply ratio.

The third problem concerning the measurement of uptake and application to growth models concerns the implications of the coefficient of luxury discussed above (p. 103). The dynamic overall rate of uptake of such nutrients as vitamin B_{12}, phosphorus and iron, when in excess, is enlarged by an amount depending both on the degree of excess and on the uptake rate of the controlling nutrient. Therefore, unless the short term and overall systems are entirely unrelated, when therefore their parameters cannot be mutually applied, or unless it is certain that the nutrient whose uptake parameters are being measured by graded additions, not only is limiting, i.e., in control, at the start, but remains so after the various additions, one would not be sure whether the calculated parameters referred to conditions of luxury or limitation.

The problem with nutrient uptake, therefore, is not how to measure it, but how to relate the parameters to the dynamic state of the whole cell. In the steady state uptake and growth are fully coupled, $u = \mu Q$, but that is not to imply that growth is controlled by uptake. On the contrary, the converse may apply, as Rhee's (1973) model would suggest, for the phenomenon of luxury uptake, $u \neq \mu Q$ in the short term, indicates that the rate of uptake *per se* does not limit that of growth. On the other hand the short term kinetics are probably a vital factor in opportunistic competition between species, since in these circumstances they largely determine the share of the total that any species will get.

Some interfering factors

I have referred to the fact that the plot of steady-state specific growth rate of *Monochrysis* on measured external concentration of vitamin B_{12} has the form of a letter C and therefore cannot be fitted by Monod's rectangular hyperbola, which passes through the origin. Similar C-shaped curves have been published for other organisms and nutrients,

e.g., phosphorus (Müller 1972, Droop 1974, Goldman 1977) and silicon (Paasche 1973). Obviously when a model fails so dismally to describe events the associated parameters lose their meaning unless a satisfactory account can be taken of the deviation.

It is reasonable to assume that the enzyme balance in a cell changes with increasing rapidity as one approaches zero growth rate. Indeed, as Jannasch and Mateles (1974) point out, many bacteria have a stalling threshold, a finite lower limit to growth rate. When the effects of such changes are confined to regions near the origin a reasonable course is to ignore readings at the lowest substrate levels, but when the deviation reached well into the mid range of growth rates, as in the cases cited, this strategem will not suffice.

Two phenomena associated with vitamin B_{12} nutrition fully account for the observed deviations and provide a pointer to modelling the phenomenon in general, though not to general explanation. First, even moderately depleted *Monochrysis* cells excrete small amounts of vitamin (Droop 1968). If cells excrete nutrient at a constant relative rate there will be a point at which uptake and excretion are equal when zero net uptake would result from some finite nutrient concentration. Such excretion when incorporated into the steady-state version of the growth model (Droop 1968, Paasche 1973) provides an intercept on the substrate axis but does not cause the curve to be C-shaped. Excretion is most simply modelled empirically by subtracting the value of the intercept from the substrate so that s in equations (5) or (10) is replaced by $s - s_0$ (Droop 1968, 1974, 1975, Caperon and Meyers 1972).

Vitamin B_{12} was also known to be subject to sequestration by proteins excreted by algal cells. Therefore, if labelled vitamin is used as a tracer total, rather than free and available, vitamin in the external medium is measured. If one therefore assumes that the protein is excreted at a constant relative rate the amount of protein in the medium of a steady-state chemostat would be proportional to the biomass and inversely proportional to the specific growth rate, so that available substrate would be smaller than the total by a factor

$$\left(\frac{\tau}{K}\right) \cdot \frac{x}{\mu} + 1$$

($\frac{\tau}{K}$ being a composite constant incorporating the specific rate of protein release and the stability constant of the protein-vitamin complex). When the substrate (s) is increased by this factor the μ vs s curve takes on the observed C shape (Droop 1968) (Fig. 5).

The evidence for both excretion and protein binding of vitamin B_{12} is well established and does not rest on kinetic data. On the contrary, the observed hook-back should have been predicted from previous awareness of these phenomena.

The sequestration model can be used to simulate Paasche's data on silicon-limited growth of *Thalassiosira pseudonana* and also both my and Goldman's data from P-limited *Monochrysis* (Droop 1980). Both silicon and phosphorus are known to be excreted and it is conceivable, but not very likely, that there is P and Si binding similar to that of the vitamin. On the other hand, all the model requires is that the ratio between measured (i.e. total) and available nutrient should vary as x/μ as indeed would be the case if an unavailable form of a nutrient (e.g. a phosphate polimer) were excreted at a constant rate.

In discussing the hook-back both Müller (1972) and Goldman (1977) tentatively suggest enhanced death rate at low growth rates. This would model correctly provided the rate of nutrient release from the dead cells varied as the inverse of the dilution rate.

I suggest that until such time as the precise mechanisms are known there is some advantage to be had from using the secretion/sequestration model to model the hook-back, without however implying that either secretion or sequestration *per se* are actually involved.

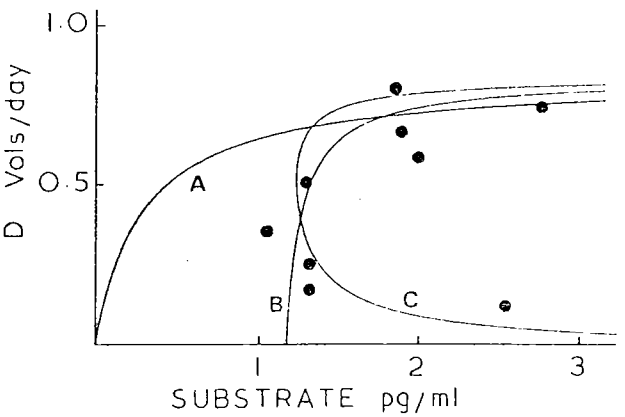

Fig. 5. Comparison of the Monod and Cell Quota models. Data from Droop (1968). *Monochrysis lutheri*, Vitamin B_{12} limiting. The curves: A, Monod model. B, with excretion. C, with excretion and sequestration. (Reproduced from Droop (1980) with permission)

The nutrient/light interaction

The precise relation between the rate of photosynthesis and incident irradiance is still a matter of debate [for reviews see Jassby and Platt (1976), Straškraba (1976), Lederman and Tett (1981)]. It

is generally agreed that it is a saturating function, but one that is virtually linear at low irradiances. Thus

$$P_G = F[I'] \quad (24)$$

I' being the specific rate of light energy absorption. F, the photosynthetic efficiency function decreases with increasing irradiance, but with low and moderate irradiance is virtually constant and maximal [the initial gradient of Talling (1979)]. Provided discussion is limited to this region the exact nature of the function F need not be specified.

There have recently been several attempts at modelling the light/nutrient interaction in algae, but none to my mind has squarely faced up to the problem. Energetics aspects have been most thoroughly studied by the Dutch school (e.g. Gons and Mur 1975, Van Liere and Mur 1979). Falkowski (1977) explored bisubstrate enzyme kinetics, but the model developed by Bannister (Bannister 1979, Laws and Bannister 1980), which clearly differentiates between the processes of energy absorption and growth, appeared to be the most promising.

The problem as I saw it was one primarily concerned with energy spillage under conditions of nutrient limitation. Since presumably

$$P_G = F[I'] = \mu + r \quad (25)$$

(r being the specific rate of dark respiration), when the growth rate is limited by nutrient depletion either r must increase or F or I' decrease. Moreover, my interest in the Cell Quota model prompted the question as to whether the nutrient/light interaction was multiplicative (interactive) or threshold like the nutrient/nutrient interaction, and more generally, whether it was possible to describe light limitation and luxury in terms compatible with the Cell Quota model.

An account of the investigation in my laboratory has recently been published (Droop *et al.* 1982) and can only be summarized in part and briefly here. First, we found that under vitamin B_{12} limitation the subsistence quota for vitamin B_{12} was not altered by changing the level of incident irradiance, whereas when light was limiting the subsistence quota for the vitamin was enlarged by a factor varying inversely with the incident irradiance. This indicates a threshold pattern of control and it would appear that the concept of the nutrient luxury coefficient (ϱ) can apply equally when light as when a nutrient is limiting.

To describe light limitation and excess in nutrient compatible terms requires definitions of such concepts as 'light luxury coefficient' and 'cell light quota' strictly analogous to the respective nutrient definitions. Thus, the former we can define as the ratio, for a particular rate of photosynthetic output, of the rate of light energy absorbed when in excess to that when limiting, namely I'_N/I'_L, which, since an unaltered output is specified, is F_L/F_N. Like the nutrient coefficient ϱ here will be equal to or greater than unity. Similarly, for the 'cell light quota' we can make use of the analogy between equation (25) and the steady-state version of equation (9). The cell light quota is therefore $1/F$, and should have the same value as $Q^s_{m_L}$ (equation (16)).

On the strength of this analogy it would be predicted that under nutrient limitation F should decrease with decreasing growth rate (i.e. increasing nutrient limitation) while under light limitation it should remain constant and maximal whatever the growth rate. Senft (1978) observed that in *Chlorella* and *Anabaena* under light-saturating conditions, photosynthetic rate was dependent on the cell quota of the limiting nutrient (phosphorus). That the predictions are fulfilled can be seen from Fig. 6, which shows photosynthetic output as a function of absorbed irradiance in *Monochrysis*. Two chemostat runs are represented, one with light-limiting (68 fM/ml vitamin B_{12}) and one with vitamin B_{12} limiting (6.8 fM/ml); dark respiration (r) being measured electrically (ΔO_2) in samples removed from the chemostats and I' obtained from incident irradiance, biomass, cell chlorophyll *a* and the application of the Beer/Lambert law. $1/F$ was also found to approximate $Q^s_{m_L}$ (4.414 as against 4.13).

The gross aspects of the pattern of nutrient control of growth as expressed by the Cell Quota model are thus seen to apply also to light and the same criterion of limitation can be employed throughout, namely that the limiting factor is that whose standardized cell quota is the smallest. The need for an arbitrary criterion of light limitation is removed and the way paved for ecological switching models of the kind developed by Tett (1981). In practice the criterion of light limitation would be provided by the value of Talling's (1979) inital gradient, i.e. F. A value less than the maximum (*ca* 0.23 for *Monochrysis*) indicates that light is not limiting.

Since the discussion has been based on the specific rate of light energy absorbed (I') and not on the power available, the conclusions are independent of any ability the cell may have to reduce the rate of absorption by, for instance, reducing cell chlorophyll

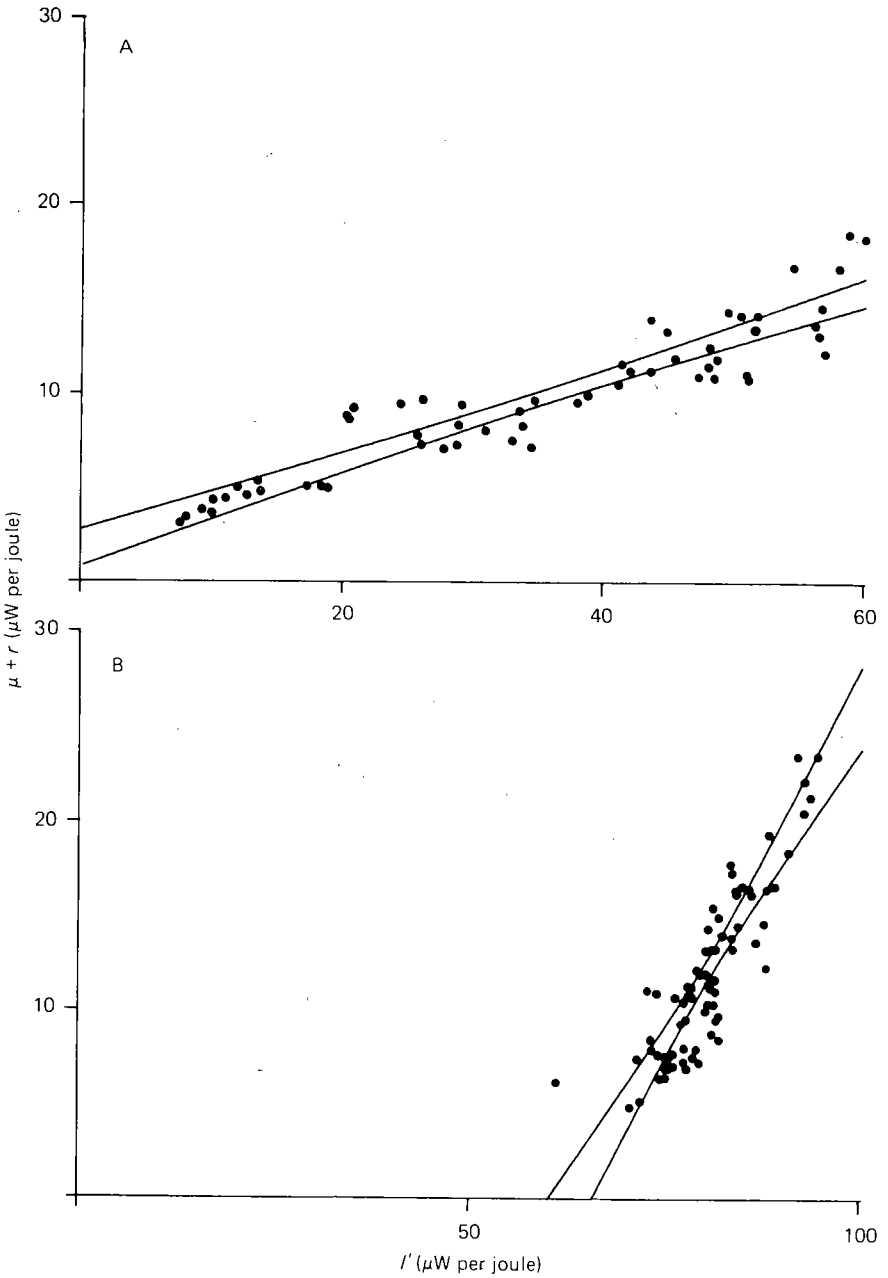

Fig. 6. *Monochrysis lutheri*. Photosynthetic output (μ + r) as a function of absorbed irradiance (I'). A, light-limited chemostat. B, vitamin B_{12}-limited chemostat. (Reproduced from Droop *et al.* (1982) with permission from the Journal of the Marine Biological Association, U.K.)

a under conditions of high incident irradiance. The energy flux of a photosynthesizing cell can be visualized as a chain with links, absorption, photosynthesis, growth, with losses possible at each. In the experiments with *Monochrysis* an 8-fold drop in overall efficiency between light and nutrient limitation at low growth rate was accompanied by a 4.5-fold drop in photosynthetic efficiency and a 1.4-fold drop in growth efficiency, whereas the drop in absorption efficiency due to reduced chlorophyll *a* was only 1.25-fold. The greater part of the energy loss therefore, was due to reduced photosynthetic efficiency while increased respiratory and absorption losses played a much smaller part. Bannister's model, for example, relies entirely on the last of these to model the nutrient-limited condition (Laws and Bannister 1980).

Concluding remarks

Within any cell the various nutrients follow distinct, though sometimes interconnected, pathways, a fact which would need to be taken into account in a highly structured model. However, the fact that the same

general kinetic pattern, namely the saturating function, can be observed whenever it is sought, suggests that it should be possible to apply a model uniformly throughout, provided the structuring is not too detailed.

The degree of structuring, the number of 'pools' in this instance, that can profitably be included depends very much on the purpose of the model. The ability to treat all nutrients uniformly is an advantage of simpler models. It must be remembered that the transfer function between any two adjacent compartments (pools) contains at least two parameters, which, if the model is to be applied, must be measured, or at least estimated. The implications of this for an ecologist are horrific: at least two parameters per pool per nutrient per organism. The need for economy is obvious. At one extreme we have the very stimulating conceptual models of Button (Button 1978, Law *et al.* 1976), and at the other Monod. The Cell Quota model has the minimum structure necessary both for functioning in transient situations and for handling more than one nutrient and/or organism simultaneously.

The predictive, as opposed to the conceptual, function of a model is not necessarily improved by increasing the number of compartments, though this may prove necessary, as for instance in the present case if short-term uptake measurements are to be slotted in to the overall cell economy (Droop 1968). In general, if the time constant of a pool to be filled is much less than the frequency of the measurements being carried out the inclusion of that pool will not generally improve the predictive ability of the model, but will only add irrelevant detail at the expense of considerable labour, a fact worth considering when the application is to natural populations and the measurements made at sea.

The same considerations apply to the inclusion of oscillatory behaviour following sudden changes in conditions, although clearly any delays and overshoots associated with transients are very relevant to the fine structure of cell control as indeed are the substrate pools the Cell Quota model chooses to ignore. Any delay arising from accumulated time constants associated with a series of linked pools may cause instability, for oscillation may be expected in any control system when there is a phase change between receipt of information and response to it. Several workers have observed delays or damped oscillations in algal growth rate in response to step changes in substrate concentration (Caperon 1969, Williams 1967, Cunningham and Maas 1978, Nyholm 1978). They can be modelled fairly satisfactorily empirically by making μ in equation (7) respond at time t to Q at time $t - \tau$ (Caperon 1969, see also MacDonald 1982).

Although the effect of a single transient may be confined to the fine structure, the same cannot be said of periodic transients and indeed of periodic fluctuations in general, for entrainment can occur. Since the natural day and night cycle profoundly affects the ordering of metabolic processes in phototrophic organisms, equally profound short comings in any model based on constant illumination can be expected. Studies with continuous synchronous (cyclostat) cultures in which entrainment is effected by imposed light/dark cycles reveal the existence of 'phase loops' in such variables as substrate concentration, cell quota and biomass, whose period average in the cyclostat need not be the same as that of a simple chemostat running under the same (period-averaged) conditions. Moreover the existence of phase loops whose shape is species-specific admits of the possibility of coexistence of competing species when the simpler model would not (Frish and Gotham 1977, Rhee *et al.* 1980).

Finally, the personal view of growth kinetics I have outlined has omitted many important areas of interest; it is hardly more than a rough shadow of a portion of some general pattern of control. How true is that pattern to the envelope of the intimate biochemistry of the cell only time will tell.

References

Ahlgren, G. 1980. Effects on algal growth rates by multiple nutrient limitation. *Arch. Hydrobiol. 89*: 43–53.

Bader, F. G. 1982. Kinetics of double-substrate limited growth. *In*: (M. Bazin, ed.) *Microbial Population Dynamics*. CRC Press, Boca Raton, Florida, pp. 1–32.

Bannister, T. T. 1979. Quantitative description of steady-state, nutrient saturated algal growth including adaptation. *Limnol. Oceanogr. 24*: 76–96.

Baule, B. 1918. Zu Mitscherlichs Gesetz der physiologischen Beziehungen. *Landwirtsch. Jahrb. 51*: 363–385.

Bienfang, P. K. 1975. Steady-state analysis of nitrate-ammonium assimilation by phytoplankton. *Limnol. Oceanogr. 20*: 402–411.

Blackman, F. F. 1905. Optima and limiting factors. *Ann. Bot. 19*: 281–295.

Bradbeer, C. 1971. Transport of vitamin B_{12} in *Ochromonas mathamensis*. *Arch. Biochem. Biophys. 144*: 184–192.

Button, D. K. 1978. On the theory of control of microbial growth kinetics by limiting nutrient concentrations. *Deep-Sea Res. 25*: 1163–1177.

Burmaster, D. 1979. The continuous culture of phytoplankton: mathematical equivalence among three steady-state models. *Am. Nat. 113*: 123–134.

Caperon, J. 1968. Population growth response of *Isochrysis galbana* to variable nitrate environment. *Ecology 49*: 866–872.

Caperon, J. 1969. Time lag in population growth response of *Isochrysis galbana* to a variable nitrate environment. *Ecology 50*: 188–192.

Caperon, J. and J. Meyer. 1972. Nitrogen-limited growth of marine phytoplankton. I. Changes in population characteristics with steady-state growth rate. *Deep Sea Res. 19*: 601–618.

Cunningham, A. and P. Maas. 1978. Time lag and nutrient storage in the transient growth response of *Chlamydomonas reinhardii* in nitrogen-limited batch and continuous culture. *J. gen. Microbiol. 104*: 227–231.

Davis, C. O., N. F. Breitner and P. J. Harrison. 1978. Continuous culture of marine diatoms under silicon limitation. 3. A model of silicon-limited diatom growth. *Limnol. Oceanogr. 23*: 41–52.

Droop, M. R. 1968. Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. *J. Mar. Biol. Ass. U.K. 48*: 689–733.

Droop, M. R. 1970. Vitamin B_{12} and marine ecology. V. Continuous culture as an approach to nutritional kinetics. *Helgoländer Wiss. Meeresunters. 20*: 629–636.

Droop, M. R. 1973. Some thoughts on nutrient limitation in algae. *J. Phycol. 9*: 264–272.

Droop, M. R. 1974. The nutrient status of algal cells in continuous culture. *J. Mar. Biol. Ass. U.K. 54*: 825–855.

Droop, M. R. 1975. The nutrient status of algal cells in batch culture. *J. Mar. Biol. Ass. U.K. 55*: 541–555.

Droop, M. R. 1977. An approach to quantitative nutrition of phytoplankton. *J. Protozool. 24*: 528–532.

Droop, M. R. 1980. Aspects of the Cell Quota concept. *In*: (B. Sikyta, Z. Fencl and V. Poláček, eds.) *Continuous Cultivation of Microorganisms*. Czechoslovak Academy of Sciences, Prague. pp. 279–290.

Droop, M. R., M. J. Mickelson, J. M. Scott and M. F. Turner. 1982. Light and nutrient status of algal cells. *J. Mar. Biol. Ass. U.K. 62*: 403–434.

Eppley, R. W. and J. D. H. Strickland. 1968. Kinetics of phytoplankton growth. *In*: (M. R. Droop and E. J. Ferguson Wood, eds.) *Advances in Microbiology of the Sea*, Vol. 1. Academic Press, London and New York. pp. 23–62.

Falkowski, P. G. 1977. A theoretical description of nitrate uptake kinetics in marine phytoplankton based on bisubstrate kinetics. *J. Theor. Biol. 64*: 375–379.

Feuillade, J. B. and M. G. Feuillade. 1975. Etude des besoins en azote et en phosphore *d'Oscillatoria rubescens* D.C. à l'aide de cultures en chemostats. *Verh. Internat. Verein Limnol. 19*: 2698–2708.

Frish, H. L. and I. J. Gotham. 1977. On periodic algal cyclostat populations. *J. Theor. Biol. 66*: 665–678.

Fuhs, G. W. 1969. Phosphorus content and rate of growth in the diatom *Cyclotella nana* and *Thalassiosira fluviatilis*. *J. Phycol. 5*: 312–321.

Goldman, J. C. 1977. Steady-state growth of phytoplankton in continuous culture: comparison of internal and external nutrient equations *J. Phycol. 13*: 251–258.

Goldman, J. C. and J. J. McCarthy. 1978. Steady-state growth and ammonium uptake of a fast growing marine diatom. *Limnol. Oceanogr. 23*: 695–703.

Gons, H. J. and L. R. Mur. 1975. An energy balance for algal populations in light-limiting conditions. *Verh. Internat. Verein. Limnol. 19*: 2729–2733.

Herbert, D. 1961. The chemical composition of microorganisms as a function of their environment. *Symp. Soc. Gen. Microbiol. 11*: 391–416.

Jannasch, H. W. and R. I. Mateles. 1974. Experimental bacterial ecology studied in continuous culture. *In*: (A. H. Rose and J. F. Wilkinson, eds.) *Advances in Microbial Ecology*, Vol. 11. Academic Press, London and New York. pp. 165–212.

Jassby, A. D. and T. Platt. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr. 21*: 540–547.

Jones, K. J., P. Tett, A. C. Wallis and B. J. B. Wood. 1978. Investigation of a nutrient-growth model using a continuous culture of natural phytoplankton. *J. Mar. Biol. Ass., U.K. 58*: 923–941.

Ketchum, B. H. 1939. The absorption of phosphate and nitrate by illuminated cultures of *Nitzschia closterium*. *Am. J. Bot. 26*: 399–407.

Kuenzler, E. J. and B. H. Ketchum. 1962. Rate of phosphorus uptake by *Phaeodactylum tricornutum*. *Biol. Bull. Mar. Biol. Lab., Woods Hole 123*: 134–145.

Law, R. A. T., B. R. Robertson, S. S. Dunker and D. K. Button. 1976. On deriving kinetic information from continuous culture. Some systematic difficulties, analysis methodology and general concepts. *Microbial Ecol. 2*: 261–283.

Laws, E. A.and T. T. Bannister. 1980. Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture with implications for phytoplankton growth in the ocean. *Limnol. Oceanogr. 25*: 457–473.

Laws, E. A. and J. Caperon. 1976. Carbon and nitrogen metabolism of *Monochrysis lutheri*: measurement of growth rate dependent respiration rates. *Mar. Biol. 36*: 85–97.

Lederman, T. C. and P. Tett. 1981. Problems in modelling the photosynthesis-light relationship for phytoplankton. *Bot. Mar. 24*: 125–134.

Liebig, J. 1840. *Die Chemie in ihrer Anwendung auf Agricultur und Physiologie*. 4th ed., 1847. Taylor and Walton, London.

van Liere, L. and L. R. Mur. 1979. Growth kintics of *Oscillatoria agardhii* Gomont in continuous culture, limited in its growth by the light energy supply. *J. Gen. Microbiol. 115*: 153–160.

Lotka, A. J. 1925. *Elements of Physical Biology*. Dover ed. 1956. Dover Publications, New York.

MacDonald, N. 1982. Time delays in chemostat models. *In*: (M. Bazin, ed.) *Microbial Population Dynamics*. CRC Press, Boca Raton, Florida. pp. 33–53.

Mackereth, F. J. 1953. Phosphorus utilization by *Asterionella formosa* Hass. *J. Exp. Bot. 4*: 296–313.

Magee, R. D., J. F. Drake, A. G. Frederickson and H. M. Tsuchiya. 1972. Studies in intermicrobial symbosis. *Saccharomyces cerevisiae* and *Lactobacillus casei*. *Canad. J. Microbiol. 18*: 1733–1742.

Maestrini, S. Y., D. J. Bonin and M. R. Droop. 1983. Phytoplankton as indicators of sea waters quality. *In*: (L. E. Shubert, ed.) *Algae as Ecological Indicators* Academic Press, London and New York (in the Press).

Maske, H. 1982. Ammonium-limited continuous cultures of *Skeletonema costatum* in steady and transitional state: experimental results and model simulation. *J. Mar. Biol. Ass., U.K. 62*: 919–943.

Monod, J. 1942. *Recherches sur la Croissance des Cultures Bactériennes*. Herman, Paris.

Müller, H. 1972. Wachstum und Phosphat Bedarf von *Nitzschia actinastroides* (Lemm.) v. Goor in statischer und homokontinuierlichen Kultur unter Phosphat-limitierung. *Arch. Hydrol.* (Supp.) *38*: 399–484.

Nyholm, N. 1976. A mathematical model for microbial growth under limitation by conservative substrates. *Biotech. Bioeng. 18*: 1043–1056.

Nyholm, N. 1977. Kinetics of phosphate limited growth. *Biotech. Bioeng. 19*: 467–492.

Nyholm, N. 1978. Dynamics of phosphate limited algal growth: simulation of phosphate shocks. *J. Theor. Biol. 70*: 415–425.

Paasche, E. 1973. Silicon and the ecology of marine plankton diatoms. 1. *Thalassiosira pseudonana* (*Cyclotella nana*) grown in a chemostat with silicate as limiting nutrient. *Mar. Biol. 19*: 117–126.

Panikov, N. 1979. Steady-state growth kinetics of *Chlorella vulgaris* under double substrate (urea and phosphate) limitation. *J. Chem. Tech. Biotech. 29*: 442–450.

Panikov, N. and S. J. Pirt. 1978. The effects of co-operativity and growth yield variation on the kinetics of nitrogen or phosphate limited growth of *Chlorella* in a chemostat culture. *J. Gen. Microbiol. 108*: 295–303.

Rhee, G.-Y. 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. *J. Phycol. 9*: 495–506.

Rhee, G.-Y. 1974. Phosphate uptake under nitrate limitation by *Scenedesmus* sp. and its ecological implications. *J. Phycol. 10*: 470–475.

Rhee, G.-Y. 1980. Continuous culture in phytoplankton ecology. *In*: (M. R. Droop and H. W. Jannasch, eds) *Advances in Aquatic Microbiology* Vol. 2. Academic Press, London and New York. pp. 151–203.

Rhee, G.-Y., I. J. Gotham and S. W. Chisholm. 1980. Use of cyclostat cultures to study phytoplankton ecology. *In*: (P. H. Calcott, ed.) *Continuous Culture of Cells*. CRC Press, Boca Raton, Florida. pp. 000–000.

Rodhe, W. 1978. Algae in culture and nature. *Verh. Internat. Verein Limnol. 21*: 7–20.

Ryder, D. N. and C. G. Sinclair. 1972. Model for the growth of aerobic microorganisms under oxygen-limiting conditions. *Biotech. Bioeng. 14*: 787–798.

Senft, H. W. 1978. Dependence of light-saturated rates of algal photosynthesis on the intracellular concentrations of phosphorus. *Limnol. Oceanogr. 23*: 709–718.

Straškraba, M. 1976. Development of an analytical phytoplankton model with parameters empirically related to dominant controlling variables. *Abh. Akad. Wiss., DDR, 1974*: 33–65.

Talling, J. F. 1979. Factor interactions and implications for the prediction of lake metabolism. *Arch. Hydrobiol. 13*: 96–109.

Teissier, G. 1942. Croissance des populations bactériennes et quantité d'aliment disponible. *Revue Scient., Paris, 80*: 209–214.

Terry, K. L. 1980. Nitrogen and phosphorus requirements of *Pavlova lutheri* in continuous culture. *Bot. Mar. 23*: 757–764.

Terry, K. L. 1982. Nitrate and phosphate interactions in a marine prymnesiophyte. *J. Phycol. 18*: 79–86.

Tett, P. 1981. Modelling phytoplankton production at shelf-sea fronts. *Phil. Trans. R. Soc. Lond. A. 302*: 605–615.

Thomas, W. H. and A. N. Dodson. 1972. On nitrogen deficiency in tropical Pacific Oceanic phytoplankton. II: Photosynthetic and cellular characteristics of a chemostat-grown diatom. *Limnol. Oceanogr. 17*: 515–523.

Tilman, D. and S. S. Kilham. 1976. Phosphates and silicate growth and uptake kinetics of the diatoms *Asterionella formosa* and *Cyclotella meneghiniana* in batch and semi-continuous cultures. *J. Phycol. 12*: 375–383.

Verduin, J. 1964. Principles of primary productivity. *In*: (D. F. Jackson, ed.) *Algae and Man*. Plenum press, New York and London. pp. 221–238.

Verhulst, P. F. 1845. Recherches matématiques sur la loi d'accrossement de la population. *Mem. Acad. Roy. Belg. 18*: 1–38.

Williams, F. M. 1967. A model of cell growth dynamics. *J. Theor. Biol. 15*: 190–207.

Williams, F. M. 1971. Dynamics of microbial populations. *In*: (B. C. Patten, ed.). *Systems Analysis*. Academic Press, New York and London. pp. 197–267.