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"A Model of Phytoplankton Growth on Multiple Nutrients"

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A model of phytoplankton growth on multiple nutrients based on the Michaelis–Menten–Monod uptake, Droop's growth and Liebig's law

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Abstract

A model of phytoplankton population growing on more than one potentially limiting nutrient is formulated and investigated. The model is based on the Michaelis–Menten–Monod uptake function for each nutrient, the Droop's function for growth of phytoplankton and Liebig's law for growth on different nutrients. The model is analyzed in a simple set up of phytoplankton culture reactor. Conditions are specified for which steady phytoplankton existence state is stable. Since growth depends on internal nutrient content, the limiting nutrient may be recognized as the one having the smallest content in phytoplankton relative to the subsistence quota. According to the model, in steady state during equal limitation by several nutrients, the Redfield ratio is equal to the ratio of subsistence quotas and to the ratio of uptake rates. Contrary to wide spread use, the ratio of nutrients in water is not the Redfield ratio but a function of the growth rate. In oligotrophic waters, however, nutrients are in another ratio that may be used as an analog to the Redfield ratio in phytoplankton. The model may be used as a submodel of larger ecosystem models.

Keywords: Phytoplankton model; Michaelis–Menten–Monod uptake; Droop growth; Liebig law; Redfield ratio

1. Introduction

In this paper we propose and analyze a model of phytoplankton dynamics which includes Michaelis–Menten–Monod uptake of nutrients, Droop's growth and the Liebig's law of the minimum.

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To our knowledge the first ecosystem model that used the Droop variable nutrient quota and multiple iiting nutrients was the model by Lehman et al. (1975). They calculated growth according to ıltiplicative limitation by nutrients. The formulation follows from Baule's principle (Baule, 1917) and ntradicts the Liebig law. De Groot (1983) has shown that multiplicative limitation gives the worst fit to ta.

Later phytoplankton growth models using multiple limiting nutrients either forced constant stoichiome- and therefore, omitting Droop's growth or have used multiplicative formulation.Inclusion of ichaelis Menten–Monod uptake, Droop's growth and Liebig's law seems to be the minimum model to ılistically capture response of phytoplankton to highly variable nutrient additions in coastal waters. As special case, the model enables one to compute important consequences of the Redfield ratio in ytoplankton and ratio of nutrients in water.

The model can be readily implemented as a sub-model in more general marine and freshwater system models that include phytoplankton. In addition, we hope that analytical results presented here l prove useful when numerical ecosystem models are analyzed and parameters estimated from ytoplankton culture experiments.

The model

Consider the concentration of n nutrients $N_1,\ldots, N_n$ potentially limiting growth of phytoplankton in a rfectly mixed phytoplankton culture reactor. Phytoplankton is characterized by $n+1$ variables: nutrient ncentration in phytoplankton $PN_n,\ldots, PN_n$ and cell density (X). The conceptual model is shown in Fig. he equations are:

$$_i/\mathrm{d}t = D(N_{0i} - N_i) - v_iX \tag{1}$$

$$N_i/\mathrm{d}t = v_iX - DPN_i \quad i = 1,\ldots, n \tag{2}$$

$$/\mathrm{d}t = (g - D)X \tag{3}$$

ere:

$$= V_iN_i/(N_i + k_i) \tag{4}$$

he Michaelis–Menten–Monod uptake function;

$$= \mu\left[1 - \max_i(q_i/Q_i)\right] \tag{5}$$

Droop's growth function coupled with Liebig's law;and

$$= PN_i/X \tag{6}$$

he ith nutrient quota in the phytoplankton.

Parameter $D(1/T)$ is the flushing rate of the reactor; $N_{0i}[M(N_i)/L^3]$ is the concentration of the ith rient in the inflow; V_i $[M(N_i)/(L^3$ cell $T)]$ and $k_i[M(N_i)/L^3]$ are maximum uptake rate and a f-saturation constant of phytoplankton for the uptake of the ith nutrient. The parameter $\mu(1/T)$ stands the maximum division rate and $q_i[M(N_i)/\text{cell}]$ is the nutrient quota found in alive but nondividing ytoplankton cells. This quota is called the subsistence quota. Dimensions denoted in square brackets : T is time, M is mass and L is volume.

Eqs. (1)–(6) represent a system of $2n+1$ nonlinear differential equations with a nonalgebraic term in . (3). All the parameters are constants and greater than zero.

In the above model it is assumed that the uptake of existing nutrients is independent of each other and that incorporation paths into the phytoplankton are separate. The concept includes the assumption that phytoplankton cells have at least n different types of uptake sites.

In the following we compute phytoplankton existence steady state, analyze its dependence on parameters, stability, determine the criterion for a nutrient to be the limiting one in steady state, determine parameters that can be obtained in steady state phytoplankton culture experiments, analyse consequences of the Redfield ratio and examine dynamic behavior.

3. Analysis of the model

3.1. Steady states

The equilibrium values of variables will be denoted by an asterisk. The trivial steady state ($N_i^* = 0$, $PN_i^* = 0$, $X^* = 0$; $i = 1,\ldots, n$) does not exist.

Phytoplankton extinction state

$$(N_i^* = N_{0i}, PN_i^* = 0, X^* = 0; \quad i = 1,\ldots, n) \tag{7}$$

exists but it will be unstable if certain conditions on parameters are satisfied (to be discussed below).

The phytoplankton existence steady state is characterized by:

$$\mathrm{d}N_i/\mathrm{d}t = \mathrm{d}PN_i/\mathrm{d}t = \mathrm{d}X/\mathrm{d}t = 0, \quad i = 1,\ldots, n$$

and

$$N_i^* > 0, PN_i^* > 0, X^* > 0 \quad \text{and} \quad Q^* > q \tag{8}$$

From Eqs. (1)–(3) the steady state equations are:

$$D(N_{0i} - N_i^*) = v_i^*X^* \tag{9}$$

$$v_i^*X^* = DPN_i^* \tag{10}$$

$$\mu\left[1 - \max_i(q_i/Q_i^*)\right] = D \tag{11}$$

Eqs. (9)–(11) form a system of $2+1$ nonlinear equations of which Eq. (11) is nonalgebraic.

In order to find the solution Eq. (8), suppose that of all q_i/Q_i^*, $i = 1,\ldots, n$ there exists one ratio, which we denote by q_r/Q_r^*, such that:

$$\max_i(q_i/Q_i^*) = q_r/Q_r^* \tag{12}$$

From Eqs. (11) and (12):

$$Q_r^* = q_r\mu/(\mu - D) \tag{13}$$

From Eqs. (9) and (10):

$$PN_i^*v = N_{0i} - N_i^* \tag{14}$$

From Eq. (6) for $i = r$ and Eq. (13):

$$PN_r^* = Q_r^*X^* = q_rX^*/(1 - D/\mu) \tag{15}$$

Substitution of Eq. (15) into Eq. (10) for $i = r$ yields:

$$N_r^* = k_r/(V_r/DQ_r^* - 1) \qquad (16)$$

Substitution of Eq. (16) into Eq. (14) gives:

$$PN_r^* = N_{0r} - k_r/(V_r/DQ_r^* - 1) \qquad (17)$$

Substitution of Eq. (17) into Eq. (6) for $i = r$ results in:

$$X^* = N_{0r}/Q_r^* - Dk_r/(V_r - DQ_r^*) \qquad (18)$$

In order to determine N_i^* where $i \neq r$, we substitute X^* from Eq. (18) into Eq. (9) and solve the resulting quadratic equation:

$$N_i^{*2} - (N_{0i} - k_i - V_i X^*/D)N_i^* - N_{0i}k_i = 0 \qquad (19)$$

Since the last term is negative, the equation has always only one positive solution.

Using N_i^* one computes PN_i^* from the expression Eq. (14). Now using PN_i^* one computes Q_i^* from the expression Eq. (6).

Note that in the steady phytoplankton existence state, the sum of nutrient concentration in the reactor and concentration in phytoplankton is equal to the incoming concentration (expression Eq. (14)). This statement is a consequence of nutrient conservation Eqs. (1) and (2) and it is invariant to dilution rate.

3.2. *Dependence of the steady phytoplankton existence state on parameters*

3.2.1. *Dependence of equilibrium values related to the limiting nutrient*

From Eqs. (13) and (16)–(18) it follows that only parameters related to the limiting nutrient N_{0r}, V_r, k_r, q_r in addition to the maximum growth rate μ and dilution rate, D, determine equilibrium values Q_r^*, N_r^*, PN_r^* and X^*.

Invariance to parameters related to the nonlimiting nutrient is a consequence of Liebig's law.

Specifically, Q_r^* depends on q_r, μ and D only; N_r^* depends on V_r, k_r, D, μ and q_r but not on N_{0r}; PN_r^* and X^* depend on all of the above and on N_{0r}.

3.2.2. *Dependence of equilibrium values related to the nonlimiting nutrients*

Since X^* depends on all six parameters and it is needed to compute N_i^*, PN_i^*, P_i^* and Q_i^*, $i \neq r$, these quilibrium values depend on all parameters related to the limiting nutrient and parameters N_{0i}, V_i, k_i elated to the ith nonlimiting nutrient. It is interesting to note that none of N_i^*, PN_i^*, P_i^* and Q_i^* depend on q_i. As we shall see later, this will exclude the possibility to determine q_i, $i \neq r$ in steady state phytoplankton culture experiments where only one nutrient is limiting growth.

.3. *Which nutrient is limiting growth?*

In order to compute the phytoplankton existence steady state, we had to assume that a certain, say rth utrient is limiting. Then, equilibrium values were computed based on parameters related to that nutrient. inally, all other equilibrium values that are components of the steady state were computed. Since the xpression Eq. (11) picks the limiting nutrient, we have no direct way of computing which nutrient is miting in the steady state.

In simulation experiments this problem is solved easily, although inexactly, by setting up one indicator ariable which points to the limiting nutrient, i.e. the variable for which $q_i/Q(t - \Delta t)_i$ is the maximum since at time t the variable Q is not known).

In steady state, one can use expressions from Eq. (13) to Eq. (19) and then expression Eqs. (14) and (6). irst one assumes in turn that each nutrient is limiting. Hence, one computes n potential existence steady ates of which only one is correct (assuming that only one nutrient is limiting).

We claim that the steady phytoplankton existence state will be the one with the smallest value of X^*. Since X^* is computed from expression Eq. (18) the limiting nutrient is known.

Proof:

Denote the n potential steady states by (^) and the true steady state by Eq. (8).

Let the smallest equilibrium value of X be denoted by $\hat{X}_r^*$ i.e. $\hat{X}_r^* = \min \hat{X}_i^*$. We claim that the rth nutrient is limiting:

$$\hat{X}_r^* = X_r^* = X^*$$

Suppose that we are given any pair $q_i/\hat{Q}_i^* \quad i \neq r$, and the pair q_r/Q_r^* where rth nutrient is limiting.

Our goal is to show that $X_r^* < \hat{X}_i^*$ for all $i \neq r$.According to expression Eq. (11):

$$q_i/Q_i^* < q_r/Q_r^* \quad \text{i.e.} \quad Q_i^* > q_i Q_r^*/q_r = q_i\mu/(\mu - D) = \hat{Q}_i^* \qquad (20)$$

From Eqs. (10) and (6):

$$\hat{N}_i^* = kD\hat{Q}_i^*/(V_i - \hat{Q}_i^*) < N_i^* \qquad (21)$$

From Eqs. (9) and (10):

$$\hat{P}N_i^* = N_{0i} - \hat{N}_i^* > PN_i^* \qquad (22)$$

Hence, from Eq. (6):

$$\hat{X}_i^* = \hat{P}N_i^*/\hat{Q}_i^* > X_r^* = X^* \qquad (23)$$

Since the above reasoning holds for all $i \neq r$, the claim is proven. As a corollary, in case m nutrients are limiting growth at the same time (where $n \geqslant m \geqslant 2$), then using the above procedure all of these nutrients will have the same X^* value. This value will be smaller than $\hat{X}_j^*$ for all j nutrients which are not limiting. Hence, the limiting nutrients can be identified.

In experiments using one phytoplankton culture it is of interest to compute in advance which nutrient will be limiting given that the above parameters are known.

The expression Eqs. (23) and (18) leads to the indicator variable:

$$I_i = \mu X_i^*/(\mu - D) = N0_i/q_i - k_i/[V_i(1/D - 1/\mu) - q_i] \qquad (24)$$

By computing the indicator variable for each potentially limiting nutrient, one determines the nutrient for which the indicator is the minimum. That nutrient is the limiting nutrient, i.e.

$$I_r = \min_i I_i \qquad (25)$$

When phytoplankton culture experiments are planned to determine parameters, one can still use the above expression by employing approximate values of parameters from the literature to get an estimate of whether one the limitation by an in advance specified nutrient will occur in steady state.

3.4. *Stability of the steady phytoplankton existence state*

In the steady phytoplankton existence state all equilibrium values must be positive: $N_i^* > 0$, $PN_i^* > 0$, $X^* > 0$ and $Q_i^* > q_i$.

From Eq. (12), to ensure that $Q_r^* > q_r$ we must have:

$$0 < D < \mu \qquad (26)$$

Then, according to Eq. (13) for all $i \neq r$, $Q_i^* > q_i$ is satisfied automatically.

The condition Eq. (26) is obviously a necessary condition for the existence of the nonextinction steady state, because it may happen that cells are washed out with the quota $Q_r^* > q_r$.

nsuring that $N_r^* > 0$ (from Eq. (16)), D must be even lower than given by Eq. (26):

$$1/(q_r/V_r + 1/\mu) \tag{27}$$

m the condition $N_r^* < N_{0r}$ Eq. (16) or equivalently $X^* > 0$ Eq. (17):

$$D < 1/[q(k_r/N_{0r} + 1)/V_r + 1/\mu] = D_c \tag{28}$$

ce the condition Eq. (28) is more stringent than Eq. (27), it determines a range of admissible dilution es. In other words, if $0 < D < D_c$ the steady phytoplankton value $X^* > 0$ exists.

DeAngelis (1992) has shown that a single nutrient model, similar to the above when $n = 1$, has a stable ady phytoplankton existence state.

n the case of $n = 1$, the characteristic equation for the linearized system around the existence steady e is:

$$\begin{vmatrix} -D - VkX^*/(k+N^*)^2 - \lambda & 0 & -DQ^* \\ VkX^*/(k+N^*)^2 & -D-\lambda & DQ^* \\ 0 & \mu q/Q^{*2} & -\mu q/Q^* - \lambda \end{vmatrix} = 0 \tag{29}$$

ere the use has been made of $VN^*/(k + N^*) = DQ^*$ and $\mu(1 - q/Q^*) = D$.

Eq. (29) is the following cubic equation:

$$+ (A + \mu)\lambda + A\mu q/Q^*](D + \lambda) = 0 \tag{30}$$

ere $A = VkX^*/(k + N^*)^2$

One solution of Eq. (30) is $\lambda = -D$, while the other two are either negative (for $\mu < D + 1$) or have gative real parts (for very large μ). This means that the steady phytoplankton existence state is locally ymptotically stable.

The only other existing steady state is the extinction state which can be shown to be unstable if ndition Eq. (28) is satisfied. Hence, the phytoplankton existence steady state is stable for all initial ites: $N(t = 0) > 0$, $PN(t = 0) > 0$, $Q(t = 0) > q$ and $X(t = 0) > 0$.

In order to consider n nutrients first rearrange the nutrients in such a way that the limiting nutrient comes the nth nutrient. The characteristic equation is:

$$\begin{vmatrix} L_1 & & & P_1 \\ & L_2 & 0 & P_2 \\ & & \ddots & \vdots \\ & 0 & & P_{n-1} \\ & & & L_n \end{vmatrix} = 0 \tag{31}$$

here matrices located on the diagonal, L_i, $i = 1,\dots, n-1$, are 2×2 matrices of the following form:

$$_i = \begin{bmatrix} -D - V_ik_iX^*/(k_i + N_i^*)^2 - \lambda & 0 \\ V_ik_iX^*/(k_i + N_i^*)^2 & -D - \lambda \end{bmatrix} \tag{32}$$

he last column is made up of entries $P_i = [-DQ_i^*, DQ_i^*]^T$, $i = 1,\dots, n-1$. Finally, the last matrix is lentical to the matrix whose determinant appears in Eq. (29) except that the index of V, k, N^* and Q^* n.

The rest of the entries are zero.

Solutions to the characteristic Eq. (31) are a union of solutions of characteristic equations $\det(L_1) = 0,\dots, \det(L_n) = 0$. Since solutions to the first $n - 1$ equations are negative and solutions to the last equation have negative real parts, we conclude that the steady state is locally asymptotically stable. On the other side, the stability of steady phytoplankton existence state implies the instability of the extinction state. Hence, if the condition Eq. (28) is satisfied, the steady phytoplankton existence state is stable for all initial conditions $N_i(t = 0) > 0$, $PN_i(t = 0) > 0$, $Q_i(t = 0) > q_i$ and $X(t = 0) > 0$, $i = 1,\dots, n$.

3.5. Determination of parameters

Parameters in phytoplankton continuous culture experiments are determined by varying dilution rate D, and recording values of variables at the steady phytoplankton existence state. In this exercise N_{0i} and D are known parameters while μ and V_i, K_i, q_i, $i = 1,\dots, n$ are unknowns.

Which variables ought to be monitored and what parameters can one determine?

From relationship Eq. (14) it follows that either N_i^* or PN_i^* must be monitored. In addition from Eq. (15) it follows that X^* must be monitored.

From Eqs. (9) and (10):

$$1/N_i^* = (V_i/Q_i^*)(1/D) - 1/k_i \tag{33}$$

Since Q_i^* changes with a change in D, $1/N_i^*$ will not be a linear function of $1/D$. This holds for all $i \neq r$.

For the limiting nutrient, however, from Eq. (16):

$$1/N_r^* = a(1/D) - b \tag{34}$$

where

$$a = V_r/(q_rk_r) \tag{35}$$

and

$$b = a/\mu + 1/k_r \tag{36}$$

In other words, by plotting $1/N_r^*$ vs. $1/D$ one obtains a straight line from which two combinations of parameters are extracted.

The qualitative difference between Eq. (33) (which is not linear in D) and Eq. (34) (which is linear in D), may serve as a basis for identification of the limiting nutrient when parameters are not known. From:

$$1/Q_r^* = -(1/q_r\mu)(1/D) + 1/q_r \tag{37}$$

one determines q_r and μ.

Substitution to Eqs. (35) and (36) yields V_r and k_r.

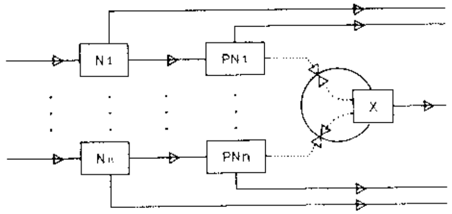

Fig. 1. Conceptual diagram of the model.

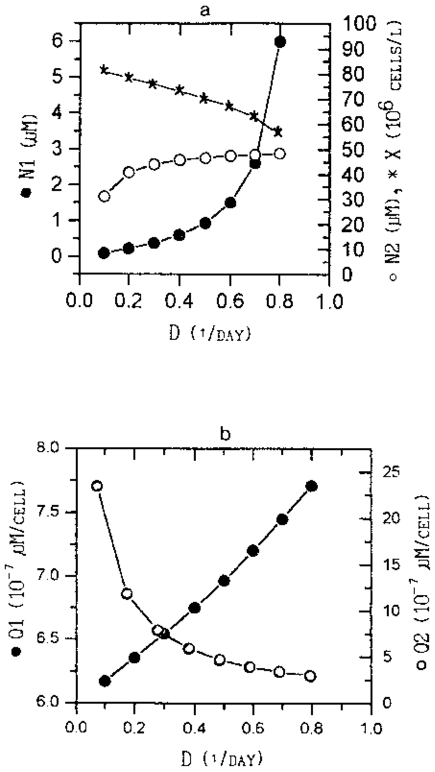

Fig. 2. (a) Concentration of nutrients and phytoplankton cells in steady state versus dilution rate. (b) Phytoplankton quota of nutrient N_1 (Q_1) and nutrient N_2 (Q_2) at the steady state vs. dilution rate.

Since through expression Eq. (12)) the dependence of equilibrium values on q_i is ruled out, q_i (where $i \neq r$) can not be determined as long as rth nutrient is limiting.

In order to determine V_i and k_i one uses the expression Eq. (33) but plots $1/N_i^*$ vs. $1/DQ_i^*$ and since this plot is linear the parameters are easily extracted.

In conclusion, to determine V_i, k_i, $i = 1,\ldots, n$, q_r and μ it is necessary to monitor N_i^* or PN_i^*, $i = 1,\ldots, n$ and X^* as a functionof D. However, q_i where $i \neq r$ can not be determined.

3.6. *Special cases*

3.6.1. *Equal nutrient limitation and the Redfield ratio*

Suppose that present nutrients are all equally limiting phytoplankton growth.

The Redfield ratio (Redfield, 1958) is defined as:

$$R = PN_i^*/PN_j^* \tag{38}$$

From Eqs. (15) and (13) it follows:

$$R = Q_i^*/Q_j^* = q_i/q_j \tag{39}$$

The Redfield ratio is equal to the ratio of steady state nutrient quotas in phytoplankton and to the ratio of subsistence quotas.Furthermore, from Eqs. (10) and (38):

$$R = v_i^*/v_j^* \tag{40}$$

The Redfield ratio is also equal to the ratio of uptake rates of limiting nutrients in the steady state.

Finally, using the expression Eq. (16) to compute N_i $i = 1,\ldots, n$ the ratio of nutrients in water is:

$$N_i^*/N_j^* = k_iq_i(V_j - DQ_j^*)/k_jq_j(V_i - DQ_i^*) = k_i(V_jz/q_j - 1)/[k_j(V_iz/q_i - 1)] \tag{41}$$

where $z = 1/D - 1/\mu$.

Using the above results one can connect Redfield ratio to parameters measured in the phytoplankton culture experiments and point to an unfounded extension that is widely used.

Redfield postulated a ratio of nutrients in phytoplankton which will allow it to grow optimally. The implication was that the ratio is independent of the growth rate. Indeed, in the steady phytoplankton existence state this ratio is constant and given by Eqs. (38) and (39) or Eq. (40). The importance of determining subsistence quotas is apparent.

Over the years, biological and chemical oceanographers have extended the use of Redfield ratio based on the following conjecture:"Since the Redfield ratio holds for phytoplankton then in the steady state the same ratio must hold between concentrations in water".

According to this conjecture by computing the ratio of nutrients in water one would know which nutrient is limiting. Use of this indicator to determine the limiting nutrient is wide spread.

From the standpoint of the present model the conjecture is false on two accounts.

First it is evident from the expression Eq. (41) that the ratio of nutrients in water during equal limitation is a function of the growth rate, g, which in the phytoplankton reactor is equal to D.

Only in the region of very small uptake rates ($v_i^* = DQ_i^*$) in comparison to the maximum uptake rates (V_i), i.e.

$$V_i \gg DQ_i^* \quad \text{and} \quad V_j \gg DQ_j^*$$

the ratio N_i^*/N_j^* may be approximated by a constant.

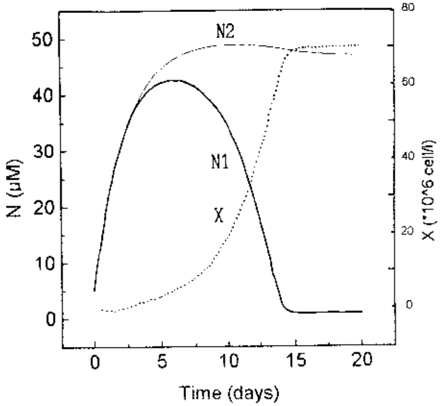

Fig. 3. Dynamics of the model with two nutrients during N_1 limitation.

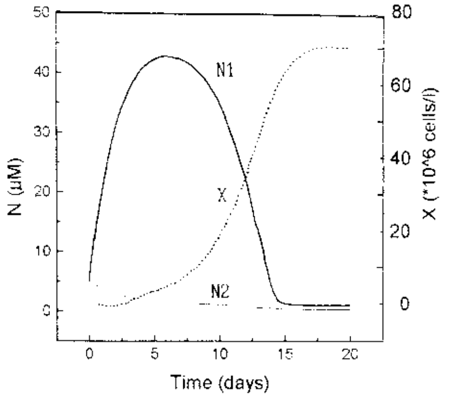

Fig. 4. Dynamics of the model from N_1 limitation to equal limitation by N_1 and N_2.

econd, even in the above case, the ratio is not equal to the Redfield ratio but to:

$$N_j^* \cong Rk_iV_j/k_jV_i \tag{42}$$

re R is the Redfield ratio. Since in practice k_iV_j/k_jV_i may be different from 1, it is unfounded to expect the ratio of concentrations of limiting nutrients will be equal to R, except by chance.

2. *Equal subsistence quotas and equal nutrient concentrations in the inflow*

uppose that the phytoplankton population has the same subsistence quota q for all nutrients: $q_i = q$, l,..., n.Then

$$\min_i PN_i^* = PN_r^*$$

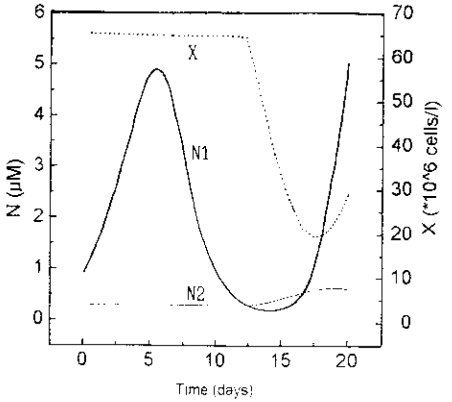

Fig. 5. Dynamics of the model during the first cycle of change in N_{0i} and starting from equal limitation.

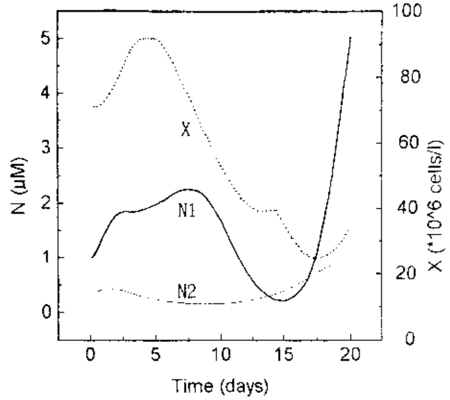

Fig. 6. Dynamics of the model during the first cycle of a change in N_{01} and N_{02} and starting from limitation by N_1.

From Eq. (11) we conclude that whichever nutrient quota Q_i^* is the smallest, that nutrient will be limiting growth. But from Eq. (6) in the steady state, $Q_i^* = PN_i^*/X^*$, so the smallest PN will determine the nutrient which is limiting growth.

If in addition all N_{0i} are equal: $N_{0i} = N_0$ for $i = 1,..., n$, then from Eq. (14):

$$\max_i N_i^* = N_r^*$$

i.e. the nutrient with the largest equilibrium value N_i^* is limiting growth.

3.7. *Example: two potentially limiting nutrients*

Let us take familiar nutrients: nitrogen and phosphorus (denoted here as N_1 and N_2, respectively). The following parameters (when available) are taken for *Thalassiosira pseudonana*. All the parameters are extracted from Jørgensen, 1979:$\mu = 3.6$ 1/day; $k_{N1} = 1$ μM; $k_{N2} = 0.7$ μM; $V_{N1} = 7.2\ 10^{-7}$ μM/(cell day) (for Dunaliella tertiolecta for NO$_3$ uptake); $V_{N2} = 2.4\ 10^{-8}$ μM/(cell day) (for *Asterionella formosa*). $q_{N1} = 6\ 10^{-7}$ μM/cell; $q_{N2} = 1.2\ 10^{-8}$ μM/cell (for *Thalassiosira fluviatilis*).

Assume further that $N_{01} = 50$ μM, $N_{02} = 50$ μM and $D = 0.5$.

Computing the indicator variables I_1 and I_2 using the expression Eq. (24), one finds that in the steady state the nutrient N_i^* will be limiting.

Expressions Eqs. (13)–(19) give:$N_1^* = 0.94$ μM, $N_2^* = 46.6$ μM, $PN_1^* = 49.06$ μM, $PN_2^* = 3.32$ μM, $X^* = 70 \times 10^6$ cell/l

From the condition Eq. (28) one finds the range of D for which $X^* > 0$: $0 < D < 0.84$ (1/day).

Obviously, if D is increased beyond 0.84/day, the phytoplankton would be washed out from the reactor.

By varying D in the range from 0.1 to 0.8 1/day one obtains a series of steady phytoplankton existence states. Graphs of N_i^* ($i = 1, 2$) and X vs. D, and Q_i^* vs. D are shown in Fig. 2(a, b), respectively. This is a typical behavior that one may expect in phytoplankton reactor experiments.

Furthermore, I_1 and I_2 indicate that N_1 nutrient is limiting throughout the range of change in D. Although both N_1^* and N_2^* are increasing with an increase in D, a qualitative difference is seen. In culture experiments where phytoplankton uptake and growth requirements for each nutrient are unknown, the above qualitative differences will determine which nutrient is limiting.

By increasing D, Q_1^* increases while Q_2 decreases, this means that one is moving from N_1 toward N_2 limitation.

In this example, as D increases, N_1^*/N_2^* increases too. This happens to agree with the classical expectation.

Obviously, if limitation by both nutrients happens to be equal, a change in D may select only one of the two nutrients to be limiting growth.

3.8. *Dynamic behavior*

In order to analyze dynamic behavior quantitatively we must turn to specific examples.

Consider two potentially limiting nutrients.

Using parameters from the above example let us discuss results of three simulation experiments.

3.8.1. *Limitation by N_i while N_{0i}, N_{02} and D are constant*

Let us start with initial nutrients concentration $N_i(t=0) = 0.2$ μM, $i = 1, 2$ and a small number of cells $X(t=0) = 1000$ cells/l.

Concentration of nutrients in the in flowing water is $N_{0i} = 5$ μM, $i = 1, 2$ and $D = 0.5$. The dynamic behavior is shown in the Fig. 3. It is seen that there is an overshoot in both N_1 and N_2 followed by an asymptotic tendency to the steady state. Indeed, a computation of characteristic roots of the determinant Eq. (31) with $n = 2$ yields:

$$\lambda_1 = -0.5, \quad \lambda_2 = -13.93, \quad \lambda_3 = -0.5005, \quad \lambda_4 = -0.23, \quad \lambda_5 = -5.03$$

which means that the phytoplankton existence steady state is a stable node.

3.8.2. *From N_1 limitation, into equal N_1 and N_2 limitation*

By setting $N_{01} = 50$ μM and using the expression Eq. (24) one can compute N_0 such that equal limitation by N_1 and N_2 will be insured in the steady state. In our case $N_{02} \cong 1.27$. The evolution from N_1 limitation at $t = 0$, i.e. $N_1(t=0) = 5$, $N_2(t=0) = 5$ toward equal limitation by both nutrients in the steady state is shown in Fig. 4. Here as well as in the previous case, the steady phytoplankton existence state is a stable node. Although the concentration of N_{02} is about 40 times smaller than in the previous case, the steady state concentration of phytoplankton cells is the same. As the expression Eq. (18) shows, this is because the concentration of cells is independent of parameters related to the non limiting nutrient.

The steady state with approximately equal limitation when $N_{01} = 50\mu$M and $D = 0.5$ is:

$$N_1^* \cong 0.94, \quad N_2^* \cong 0.29, \quad PN_1^* \cong 49.06, \quad PN_2^* \cong 0.98, \quad X_1^* \cong 70 \times 10^6 \tag{43}$$

3.8.3. *From equal limitation to N_2 limitation and back to equal limitation: N_{0l} is periodic, N_{02} and D are constants*

Let us start the simulation from the steady state of the previous case. We change N_{01} into $N_{01} = 50 + 40 \sin(2\pi/20)$ and follow the dynamics during the first cycle. At the start of the simulation both nutrients are equally limiting. Then, according to the above expression, N_{01} is increased. We see from Fig. 5 that N_{01} increases while both N_{02} (which is now limiting) and X stay constant. After $t = 12.5$ days, N_1 has decreased below equal limitation and hence becomes limiting. As a result X decreases as it would be expected. Subsequently, N_1 increases due to increase of N_{01}. However, the extent of increase of N_1 also depends on X which has decreased. As N_{01} increases toward equal limitation and if subsequently N_{01} stays constant, the system would tend to the initial steady state. Instead, if N_{01} continues to cycle the mean value of $\bar{N}_1$, $\bar{N}_2$ and $\bar{X}$ over one cycle will not be the same as the steady state Eq. (43) since the system is nonlinear.

3.8.4. *From N_1 to N_2 and back to N_1 limitation: N_{01} and N_{02} are periodic, D is constant*

The difference from the previous case is that here $N_{02} = 1.27 + 0.6 \cos(2\pi/20)$.

The simulation starts with initial conditions from the previous case. At the start of the simulation N_{02} is higher while N_{01} increases as in the previous case. We see that during the interval $t = 0$–5 both N_{01} and N_{02} are higher than in the previous case and therefore X increases (Fig. 6). Limitation by N_1 extends from the start of the simulation until 2.5 days when limitation by N_2 begins. N_2 limitation continues until approximately $t = 14$ day when N_1 limitation resumes. Initially, although N_1 is limiting, it is increasing together with X^* due to increase of N_{01}. Although it is quite clear what is happening here, such behavior of N_1, N_2 and X in nature can not be explained by constant stoichiometry where uptake is equal to growth. Note that between $t = 10$ and $t = 14$, while N_2 is limiting, its concentration is increasing. On the other side both N_1 and X are decreasing. When this behavior occurs in nature, using a classical constant stoichiometry model, a typical misjudgment would be made that N_1 is limiting phytoplankton growth.

4. Conclusion

A model has been presented based on three well established principles in phytoplankton growth on multiple nutrients. Based on parameter values, the phytoplankton existence steady state has been calculated. It has been shown that the steady state is stable to perturbations in initial conditions. Number of cells in steady state does not depend on parameters related to nonlimiting nutrient.

The Redfield ratio in this model is equal to ratio of uptake rates and to the ratio of subsistence quotas. However, the ratio of nutrients in water is not equal to the Redfield ratio and it is a function of the growth rate.

In order to determine the limiting nutrient in a dynamic state there is no alternative but to compute q_i/Q_i, $i = 1, \ldots, n$.

The limiting nutrient N_r is the one with the property

$$q_r/Q_r = \max_{i=1}^{n}(q_i/Q_i)$$

Based on parameters of the model, an indicator variable is proposed which allows one to determine in advance which nutrient will be limiting in the steady state.

A further consequence is that in phytoplankton culture experiments in steady state, subsistence quotas of nonlimiting nutrients can not be determined.

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