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ORIGINAL RESEARCH

Continuous cultures of phytoplankton

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Abstract The development of cultures of phytoplankton adapting throughout several days in an axenic, continuous-flow chemostat to yield a steady kinetic state of competing species is described mathematically. The adaptation of the growth rate to the chemostat environment inhibits integration of the equation of conservation of phytoplankton populations, though eventually when a steady state is reached the growth rate becomes equal to the rate of flow through the chemostat. Representation of species growth rates by a Verhuls formulation utilising experimentally determinable intra- and interspecies interaction constants permits the rapid prediction of the adaptation and alteration in the populations of competing phytoplankton species with changes in the chemostat environment. Illustrations of the behaviour of two and three competing species are extended to consideration of the stabilities of cultures of many competing species. Stable steady states of phytoplankton in a continuous-flow chemostat comprise a classic thermodynamic system and consequently the utilisation of light energy by the cells varies inversely with their growth rate. It is probable that when growth is nutrient limited, intra-and interspecies interaction parameters diminish as the demands of consumption are more nearly matched by the ratios of the limiting nutrients.

Keywords Phytoplankton · Chemostat · Continuous-flow · Growth and adaptation · Steady state

Background

Phytoplankton, single-celled eukaryotes, the simplest of vegetation, harvest light to generate a very significant portion of global biomass. In recent decades, phytoplankton have been cultivated commercially to provide

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food for farmed fish and crustaceans. Cultures of genetically modified phytoplankton are being explored as a source of liquid fuel (See Wikipedia, Algal Fuel, 2013 and the many references therein). To be able to describe a sustainable world, one must be able to describe the growth of phytoplankton.

Most laboratory studies of phytoplankton have been of batch cultures in which the ages and consequently the properties of the phytoplankton cells change throughout the experiment. In a kinetic steady state, the characteristics of axenic (bacteria-free), continuous cultures of phytoplankton are constant and may be observed indefinitely. The changes in these characteristics, when the cells adapt to deliberate alterations in such environmental parameters as light intensity, nutrient supply, temperature, salinity, rate of flow and concentration of pollutants, can be studied readily (e.g. Droop 1968; Geider et al. 1996; Stramski et al. 2002; Okay et al. 2003).

The present paper describes and extends the theory of the growth of continuous cultures of phytoplankton in a chemostat. In particular, it examines the effects of changes in the rate of flow through the chemostat, the stability of kinetic steady states, the adaptation of phytoplankton cells to their environment and competition between different species.

The basic chemostat

The basic chemostat consists of a uniformly well-stirred vessel of volume V through which an aqueous liquid containing nutrient, often nitrogen-limited, at a concentration N_0 flows at a rate DV. The chemostat is seeded with phytoplankton to generate a continuous culture. The liquid emerging from the chemostat vessel (also at a rate DV) bears nutrient of concentration N and phytoplankton cells having a population P per unit volume, N and P being also the spatially uniform nutrient concentration and phytoplankton population within the vessel. The chemostat is bathed in light of a constant and uniform intensity, I, and is maintained at a constant temperature, T.

The behaviour of phytoplankton in a chemostat

In the standard experiment, populations of phytoplankton cells injected into the chemostat are monitored whilst the environmental chemostat parameters are carefully controlled. Monitoring is performed by frequent analysis of the nutrient and phytoplankton in the effluent from the chemostat. If the environmental parameters are held constant, the phytoplankton culture in the chemostat settles into a stable, kinetic steady state with a constant population maintained by equalisation of the rate of removal of cells in the effluent by the rate of growth of the cells within the chemostat. It takes from a few days to a month for such a steady state to develop. Variation of the environmental parameters produces a change in the steady-state population of phytoplankton. Phytoplankton cultures adapt to or are adapted by changes in their environment. Adaptation may take a few weeks though adaptation to changes in the intensity of the incident light takes but a few hours (Okay et al. 1994, 2003; Okay and Gaines 1996).

The rate of flow of aqueous liquid through the chemostat, DV, is an important environmental parameter. D is generally less than 3/day, values greater than this wash the cells out of the chemostat faster than they can grow. Cells survive in the chemostat $\sim 1/D$ days, that is, the cells in the chemostat are $\sim 1/D$ days old. Changes in D generate steady states of cultures of cells with changed ages and accordingly possibly having changed characteristics.

The mathematics of growth, competition and adaptation

Equation of conservation of phytoplankton cells in a chemostat

Let the population density of phytoplankton be P cells per unit volume at time t and let a fraction ϕ of these be live (participating in cell division). Then, the equation of conservation of live phytoplankton cells in the chemostat is

$$\frac{\mathrm{d}}{\mathrm{d}t}(P\phi) = -DP\phi + bP\phi - mP\phi = -(D + gP\phi) \tag{1a}$$

where b is the rate of cell division per live cell, m is the rate of mortality of live cells and g is the rate of growth per live cell.

The equation of conservation of dead cells in the chemostat is

$$\frac{\mathrm{d}}{\mathrm{d}t}(P(1-\phi)) = -DP(1-\phi) + mP\phi \tag{1b}$$

Adding (1a) and (1b)

$$\frac{\mathrm{d}P}{\mathrm{d}t} = -DP + bP\phi = -DP + b^*P \tag{1c}$$

where $b^* = b\phi$ = rate of cell division per cell whether live or dead.

In the steady state, S, when $\frac{\mathrm{d}}{\mathrm{d}t}(P\phi) = \frac{\mathrm{d}}{P}\mathrm{d}t = 0$, from (1a)

$$D = b_s - m_s = g_s \tag{2a}$$

and from (1c)

$$\phi_s = D/b_s = g_s/b_s \tag{2b}$$

Throughout these equations, it has been supposed that, the chemostat being axenic (free of bacteria), the rate of remineralisation of dead phytoplankton cells into chemicals (nutrient) can be neglected. We are aware of few laboratory studies of continuous cultures of phytoplankton in the presence of defined populations of bacteria (Mindl et al. 2005). Further such experiments would be of interest.

Equation for ϕ:

$$\text{From (1a)}, \qquad \frac{\mathrm{d}}{\mathrm{d}t}\ln(P\phi) = \frac{\mathrm{d}}{\mathrm{d}t}\ln P + \frac{\mathrm{d}}{\mathrm{d}t}\ln\phi = g - D \tag{3}$$

$$\text{From (1c)}, \qquad \frac{\mathrm{d}}{\mathrm{d}t}\ln P = -D + b\phi \tag{4}$$

$$\text{Subtracting (4) from (3)}, \qquad \frac{\mathrm{d}}{\mathrm{d}t}\ln\phi = g - b\phi \tag{5a}$$

In the steady state $\phi_s = g_s/b_s$ which is (2b). And (5a) gives

$$\frac{\mathrm{d}\phi}{\mathrm{dt}} = g\phi - b\phi^2 \tag{5b}$$

If one supposes that b, m and g are constants independent of time, then equations (5b) and subsequently (3) can be solved straightforwardly. But consider Eq. (3); if g is constant (3) has the simple solution $P\phi = P_o\phi_o \exp\{(g-D)t\}$, where P_o and ϕ_o are the population of phytoplankton and the fraction of live cells in the initial culture. If g, the constant rate of growth, is larger than D, one sees that $P\phi$, the population of live cells, becomes infinite. Conversely if g is smaller than D, the population of live cells becomes zero. Only if

$g = D$ $(= g_s)$ does one obtain a steady state—but it is a steady state in which the population of live cells remains constant, independent of time, at its arbitrary value in the initial culture (seed). None of this corresponds to experimental reality. One must conclude that the hypothesis that b, m and g are constant, independent of time is untrue. Equations (3) and (5b) cannot be integrated straightforwardly because cell growth and the rates of division and death of the phytoplankton cells adapt to the environmental conditions in the chemostat until eventually cell growth, g, becomes equal to D and a steady state is achieved. Presumably this is what happens in an 'environmental niche' in the field; the several species of phytoplankton that may be observed have each adapted to the same environmental conditions (Reynolds et al. 2001; Bayraktavoglu et al. 2003; Ballantyne et al. 2010; Chen and Liu 2010). Future studies of the adaptation of the rates of phytoplankton cell growth in a chemostat would be very worthwhile.

We proceed pragmatically. One finds, at least for *Phaeodactylum tricornutum* and *Dunaliella tertiolecta*Okay et al. (2003),

$$\frac{\mathrm{dP}}{\mathrm{dt}} = -DP + D_W P(1 - xP) = -DP + D_W P(1 - P/P_0) = f_1 \tag{6}$$

The rate of cell division of the phytoplankton cells, b^*P in Eq. (1c), has been expressed by a Verhuls' formulation $b^* = D_W(1 - P/P_0)$. D_W is the value of $D \sim (2$ to $3)$ that just 'washes out' all the phytoplankton cells from the chemostat and P_0 is the population density of the stable culture when D is zero.

Equation (6) has the solution $P = j/(k + Ke^{-jt})$, where $j = (D_W - D)$, $k = D_W/P_0$ and K provides P when $t = 0$.

Whereas Eq. (6) is certainly consistent with the dependence of steady-state population densities on D [Fig. 1 of Okay et al. (2003)], it is important to test it on a wider range of phytoplankton species. A further experimental problem is the determination of ϕ, the fraction of cells that are live. From Eq. (3), it follows that $b\phi = D + KPe^{-jt}$, whence in the steady state $b_s\phi_s = D$, Eq. (2b). It is well established that in the log phase of a batch culture when excess nutrient is present the population of phytoplankton cells grows at its maximum rate, exponentially with time. This suggests the hypothesis that in the chemostat $b_s = D_W$, the maximum rate of cell division, and this would be the rate of cell growth in the log phase of a batch culture with the corresponding light intensity, nutrient concentration and temperature. ϕ_s would be $D/D_W = (1 - P_S/P_0)$ and $m_s = D_W - D$. The hypothesis should be tested for both continuous and batch cultures.

In the steady state, the total rate of growth, and the total rate of removal of cells from the chemostat, is $D_W P_S(1 - P_S/P_0)$ which is $DP_0(1 - D/D_W)$. This parabolic function of D has a maximum value of $\frac{1}{4}D_W P_0$ when $D = \frac{1}{2}D_W$ and $P_S = \frac{1}{2}P_0$Okay et al. (2003). The age of the cells in the chemostat is $\sim 2/D_W$.

Equation of conservation of nutrient

Figure 1 of Okay et al. (2003) shows the steady-state concentrations of nitrogen-limited nutrient in the chemostat when *Phaeodactylum tricornutum* was cultured for a range of values of D. Similar results were obtained with cultures of *Dactylum tertiolecta* and apparently with *Thalassiosira pseudonana*Stramski et al. (2002) but a wide range of phytoplankton species should be investigated. Figure 1 of Okay et al. (2003) indicates two phases of consumption. In the steady state, when $0 < D < \frac{1}{2}D_W$ essentially all the nutrient was consumed by the culture as fast as the nutrient entered the chemostat but when $\frac{1}{2}D_W < D < D_W$ significant concentrations of nutrient remained unconsumed. When D exceeded $\frac{1}{2}D_W$, there were insufficient live cells present in the steady state to be able to consume the nutrient as fast as it entered the chemostat.

The equation for the conservation of nutrient in an axenic chemostat supposing that the remineralisation of phytoplankton cells may be ignored, is

$$\frac{\mathrm{dN}}{\mathrm{dt}} = D(N_0 - N) - AP\phi = f_2 \tag{7}$$

where N is the concentration of nutrient, N_0 its value when entering the chemostat and A is the rate of consumption per living cell.

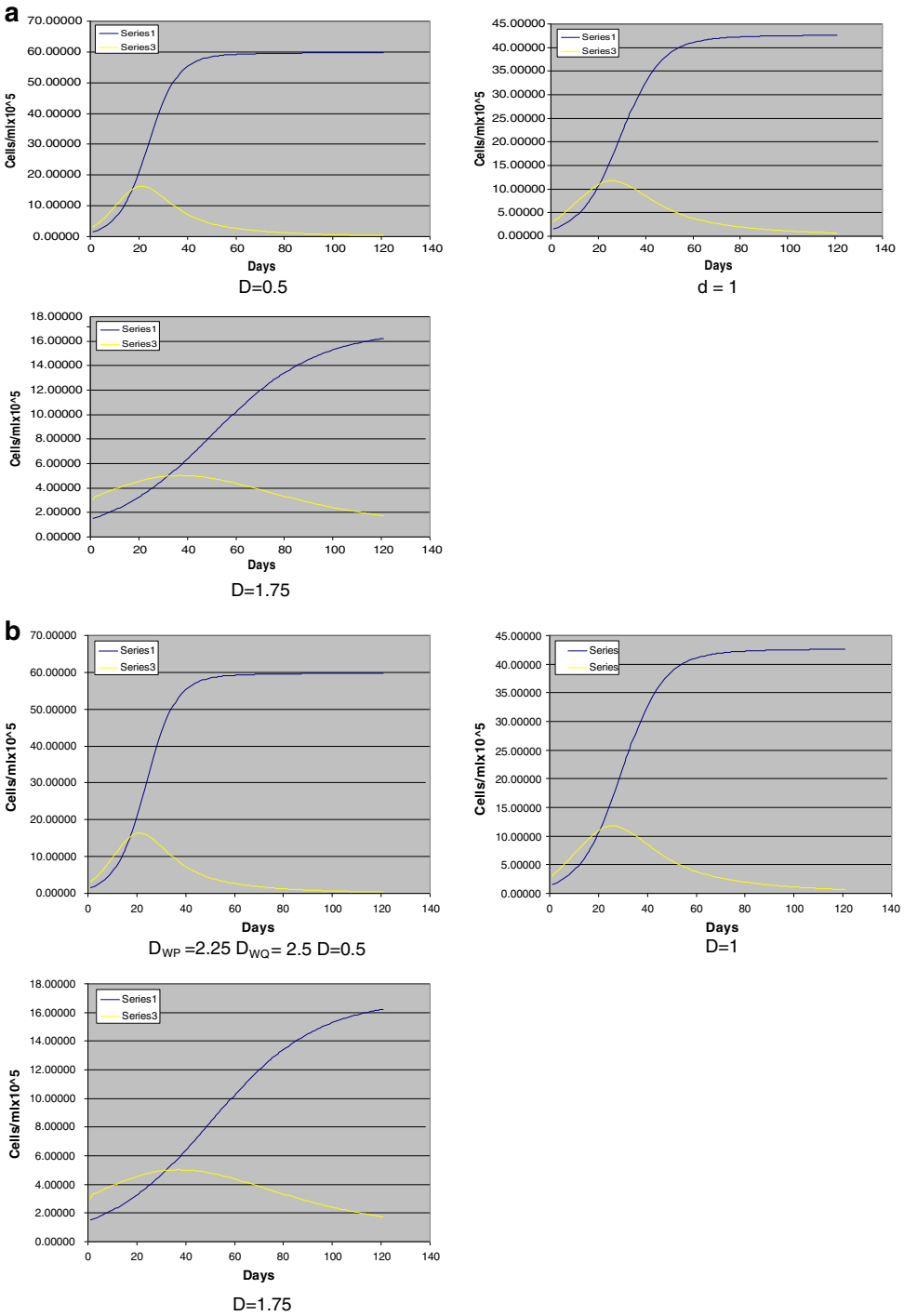


Fig. 1 **a** Generation of populations of two competing species. Variation with D. Units: *Horizontal axis*, days; *Vertical axis*, cells$\times 10^5$/ml. **b** Changes in generation of two species with changes in their Washout values, D_{WX}. Units: *Horizontal axis*, days; *Vertical axis*, cells$\times 10^5$/ml. **c** Changes in generation of two species with the intraspecies parameter, x_1. Units: *Horizontal axis*, days; *Vertical axis*, cells$\times 10^5$/ml. **d** Changes in generation of two species with interspecies parameter x_2. Units: *Horizontal axis*, days; *Vertical axis*, cells$\times 10^5$/ml. *Series 1*, population P; *Series 3*, population Q

In the kinetic steady state, $A_S = D(N_0 - N_S)/P_S\phi_s$ (or $D_W(N_0 - N_S)/P_S$ if ϕ_s may be taken to be D/D_W). $A_S\phi_s$ is $D(N_0 - N_S)/P_S)$ which is $(N_0 - N_S)/P_0(1/D - 1/D_W)$. When $D < \frac{1}{2}D_W$, N_S is small and A_S is the rate at which nutrient meets the cell walls; $A_s\phi_s$ increases with D approximately as $N_0/P_0(1/D - 1/D_W)$. When

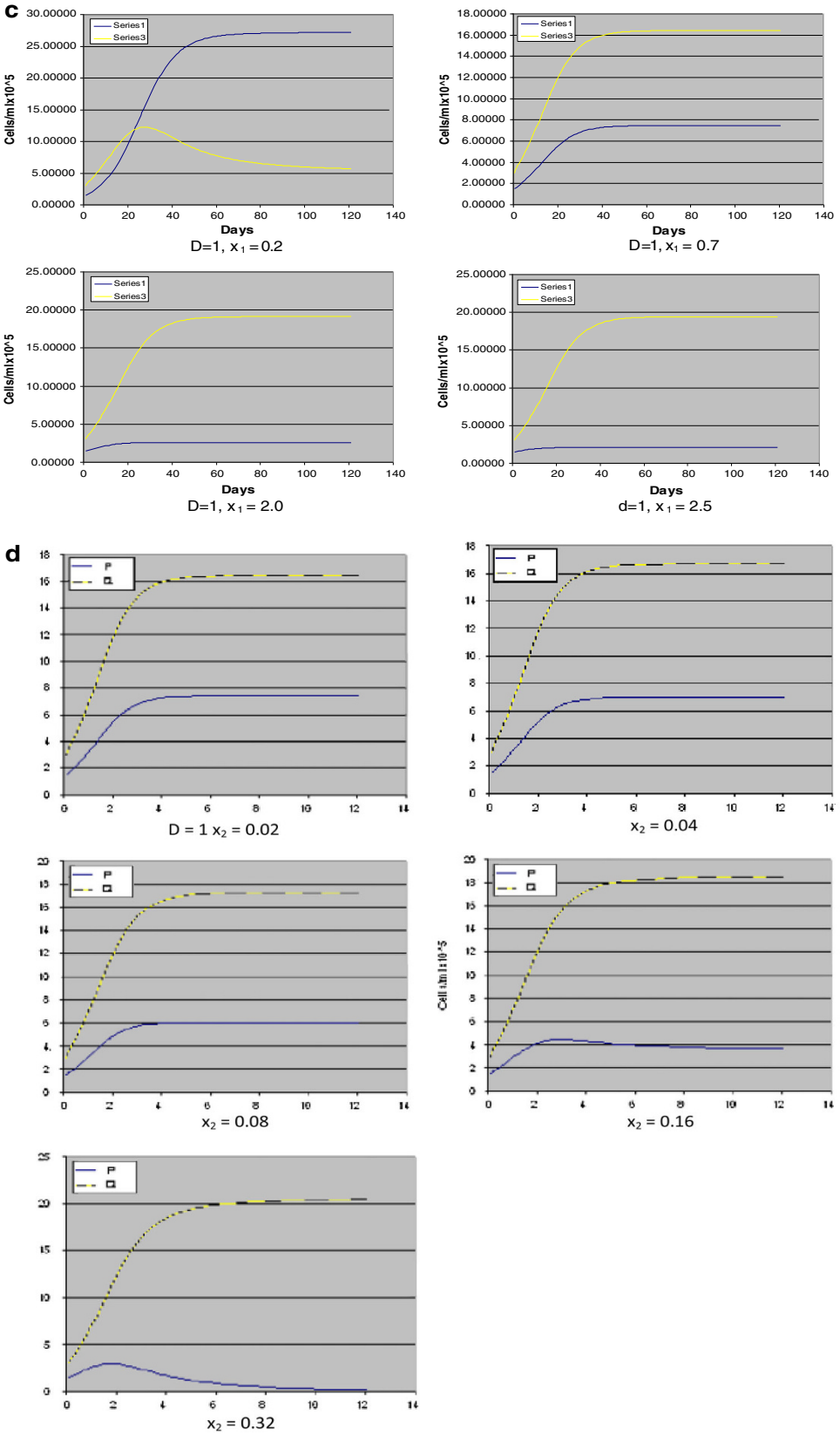


Fig. 1 continued

$D > \frac{1}{2}D_W$, N_S becomes significant; presumably $AP\phi$ is given by a Monod formulation but nevertheless the rate of consumption, $A_S\phi_s$, continues to increase as D increases.

The stability of the steady state

The steady state is stable if the roots, λ, of the community matrix

$$\begin{vmatrix} df_1/dP - \lambda & df_1/dN \\ df_2/dP & df_2/dN - \lambda \end{vmatrix} = 0$$

evaluated at the steady state are real and negative. f_1 and f_2 are defined by Eqs. (6) and (7), respectively. df_1/dP evaluated at the steady state is readily found to be $-(D_W - D)$. df_1/dN is zero.

The roots, λ, are therefore negative providing df_2/dN (ie. $-D - \frac{d}{dN}(AP\phi)$) evaluated at the steady state is always negative. This is true in all cases known to us but needs experimental confirmation for a larger number of phytoplankton species.

Two competing species

Equations (6) and (7) apply to single species of phytoplankton though they might be applied to continuous cultures of mixed species using the average properties of the culture. The equations have the merit that they are readily extended to embrace multiple species. Consider two axenic species, P and Q, competing in a continuous flow chemostat.

Extending Eq. (6), we have

$$\frac{\mathrm{dP}}{\mathrm{dt}} = -DP + D_{WP}P(1 - x_1P - x_2Q) \tag{6a}$$

$$\text{and} \qquad \frac{\mathrm{dQ}}{\mathrm{dt}} = -DQ + D_{WQ}Q(1 - y_2P - y_1Q) \tag{6b}$$

while the natural extension of Eq. (7) is

$$\frac{\mathrm{dN}}{\mathrm{dt}} = D(N_0 - N)A_PP - A_QQ = f_3 \tag{7a}$$

The interaction parameters between cells, x_1, y_1, x_2 and y_2 are given by $x_1 = 1/P_0$, $y_1 = 1/Q_0$, $x_2 = (P_0 - P_0^*)/P_0Q_0^*$, $y_2 = (Q_0 - Q_0^*)/P_0^*Q_0$, where $P = P_0$ when $D = Q = 0$ (i.e. one species, P, present) and $Q = Q_0$ when $D = P = 0$ (i.e. one species, Q, present). P_0^* and Q_0^* are the steady-state values of P and Q when $D = 0$ and both species are present. These interaction parameters are, therefore, deducible from observations of stable continuous cultures when $D = 0$; they depend on N_0 but not on N.

In a stable, steady state $P_S = \alpha y_1 - \beta x_2)/(x_1y_1 - x_2y_2)$ and $Q_S = (\beta x_1 - \alpha y_2)/(x_1y_1 - x_2y_2)$, where $\alpha = (1 - D/D_{WP})$ and $\beta = (1 - D/D_{WQ})$ (Okay et al. 2005).

The steady state is stable providing, as always, that the roots of the community matrix are real and negative when evaluated at the steady state. In fact, the roots of the community matrix are real and negative if and only if df_3/dN is negative and if $x_1y_1 > x_2y_2$.That is provided the interactions between cells of the same species are greater than the interactions between cells of different species (Okay et al. 2005). Volterra investigated a symmetrical case in which, one of the roots of the community matrix being zero, P_S and Q_S varied cyclically.

Whereas the steady state is stable if $x_1y_1 > x_2y_2$, it is theoretically possible for P_S or Q_S to be negative. The conditions for P_S and Q_S to be positive are $y_1/x_2 > \beta/\alpha > y_2/x_1$. In practice, during the development of the steady state should the population of either species die out it remains zero and does not become negative, the

population of the other species then achieves the steady state appropriate for a single species. We shall show examples of this in the section on spread sheets.

We now consider the possible behaviours of stable steady states as D is varied. There are three cases:

Case 1, $1 < y_2/x_1 < y_1/x_2$. In this case, if $D_{WP} > D_{WQ}$ then there is no steady state for any D. If $D_{WP} < D_{WQ}$ then there is a steady state for $D_1 < D < D_2$ where $D_1 = (y_2 - x_1)/(\frac{y_2}{D_{WP}} - \frac{x_1}{D_{WQ}})$ and $D_2 = (x_2 - y_1)/(\frac{x_2}{D_{WQ}} - \frac{y_1}{D_{WP}})$. As D increases from D_1 to D_2, P decreases to 0 at D_2 while Q increases from 0 at D_1. The growth rate DP attains its maximum at D_1 or $\frac{1}{2}D_2$, whichever is larger. DQ attains its maximum at D_2.

Case 1', $y_2/x_1 < y_1/x_2 < 1$. This is the same as case 1, with P and Q interchanged.

Case 2, $y_2/x_1 < 1 < y_1/x_2$. In this case, a stable state exists for $0 < D < D_3$ where $D_3 = D_1$ (as defined in Case 1 above) if $D_{WP} > D_{W_Q}$ while $D_3 = D_2$ if $D_{WP} < D_{W_Q}$. If $D_{WP} > D_{W_Q}$ then as D increases from 0 to D_3, Q decreases to 0 at D_3, and DQ attains its maximum at $D = \frac{1}{2}D_3$, while P either increases or decreases, according to whether $x_2D_{WP} - y_1D_{WQ}$ is positive or negative. If this quantity is positive, DP attains its maximum at D_3, while if it is negative the maximum is attained at D_3 or $\frac{1}{2}D_2$, whichever is smaller.

If $D_{WP} < D_{W_Q}$, the results are the same with P and Q interchanged.

Three competing species

Equation (6) is readily extended to embrace three competing species:

$$\begin{aligned} \frac{\mathrm{dP}}{\mathrm{dt}} &= -D_P + D_{WP}P(1 - x_1P - y_1Q - z_1R) \\ \frac{\mathrm{dQ}}{\mathrm{dt}} &= -D_Q + D_{WQ}Q(1 - z_2P - x_2Q - y_2R) \\ \frac{\mathrm{dR}}{\mathrm{dt}} &= -D_R + D_{WR}R(1 - y_3P - z_3Q - x_3R) \end{aligned} \tag{6c}$$

We apologise that it is convenient to change slightly the nomenclature of the parameters x, y, z of the interactions between cells.

One also has

$$\frac{\mathrm{dN}}{\mathrm{dt}} = D(N_0 - N) - A_PP - A_QQ - A_RR = f_4 \tag{7b}$$

(6c) yields the population densities in the steady state when $\mathrm{dP}/\mathrm{dt} = \mathrm{dQ}/\mathrm{dt} = \mathrm{dR}/\mathrm{dt} = 0$,

$$\begin{aligned} P_S &= [(x_2x_3 - y_2z_3)\alpha + (z_1z_3 - x_3y_1)\beta + (y_1y_2 - x_2z_1)]/\text{denominator}, \\ Q_S &= [(y_2y_3 - x_3z_2)\alpha + (x_1x_3 - y_3z_1)\beta + (z_1z_2 - x_1y_2)\gamma]/\text{denominator}, \\ R_S &= [(z_2z_3 - x_2y_3)\alpha + (y_1y_3 - x_1z_3)\beta + (x_1x_2 - y_1z_2)\gamma]/\text{denominator}, \end{aligned} \tag{8}$$

where denominator$= (x_1x_2x_3 + y_1y_2y_3 + z_1z_2z_3 - x_1y_2z_3 - x_2y_3z_1 - x_3y_1z_2)$, α and β have already been defined and γ is $(1 - D/D_{WR})$.

One will see shortly that for the steady state to be stable the denominator has to be positive. Consequently for P_S, Q_S and R_S to be greater than zero, the respective numerators have to exceed zero. Should one of the species has a negative population in the steady state, then, of course, when its population becomes zero it dies out and one has a two species system in the chemostat (Van den Driessche and Zeeman 1998).

Evaluation of the Community Matrix at the steady state yields

$$\left(\frac{df_4}{dN} - \lambda\right)(\lambda^3 + C_1\lambda^2 + C_2\lambda + C_3) = 0 \tag{9}$$

The steady state is stable only if the values of the roots, λ, are all negative, which implies that df_4/dN should be negative and that the three coefficients, C, should all be positive. There is a need for more experimental evidence that df_4/dN evaluated at the steady state is negative.

We have $C_1 = x_1 D_{WP} P_S + x_2 D_{WQ} Q_S + x_3 D_{WR} R_S$, which is obviously positive if each individual species is stable. And

$$C_2 = (x_1 x_2 - y_1 z_2) D_{WP} D_{WQ} P_S Q_S + (x_1 x_3 - z_1 y_3) D_{WP} D_{WR} P_S R_S + (x_2 x_3 - y_2 z_3) D_{WQ} D_{WR} Q_S R_S$$

This is obviously positive if the parentheses together with P_S, Q_S and R_S are all positive. Inspection shows that the condition for the parentheses to be positive is just the condition for each of the three combinations of two species to have stable steady states. Thus, for P and Q to have a stable steady state, $x_1 x_2$ must be greater than $y_1 z_2$. In other words for C_2 to be positive, it is sufficient, but not necessary, for each of the three combinations of two species to be stable. Next

$$C_3 = (x_1 x_2 x_3 + y_1 y_2 y_3 + z_1 z_2 z_3 - x_1 y_2 z_3 - x_2 y_3 z_1 - x_3 y_1 z_2) \times D_{WP} D_{WQ} D_{WR} P_S Q_S R_S$$

This can be written as

$$C_3 = \left[x_1 x_2 x_3 \left(1 - \frac{1}{a} - \frac{1}{b} - \frac{1}{c} \right) + y_1 y_2 y_3 + z_1 z_2 z_3 \right] \times D_{WP} D_{WQ} D_{WR} P_S Q_S R_S$$

where $a = x_1 x_2 / y_1 z_2$, $b = x_1 x_3 / z_1 y_3$ and $c = x_2 x_3 / y_2 z_3$.

From the discussion of C_2, it is clear that if each of the three combinations of two species is stable, a, b and c are each greater than unity. Should a, b and c be greater than 3, and if P_S, Q_S and R_S exceed zero, then obviously C_3 is positive but this may not be true if a, b and c lie in the range 1–3. It is the positivity of C_3 that determines whether the culture of all three species together is stable. Generally, the condition for the stability of three species is more stringent than that for the stability of pairs of species. Nevertheless, it is possible to find values of the parameters that provide a stable steady state with three species of phytoplankton in which all three pairs of two of the species are not stable. An example will be described in the section, Spread Sheet Algebra.

Systems mathematically equivalent to (6c) have been much studied (e.g. May and Leonard 1975; Zeeman et al. 1993; Van den Driessche and Zeeman 1998). It is mathematically possible for systems of three species to develop periodicity (cyclicity). Future experimentation is necessary to establish whether this occurs in reality in a chemostat.

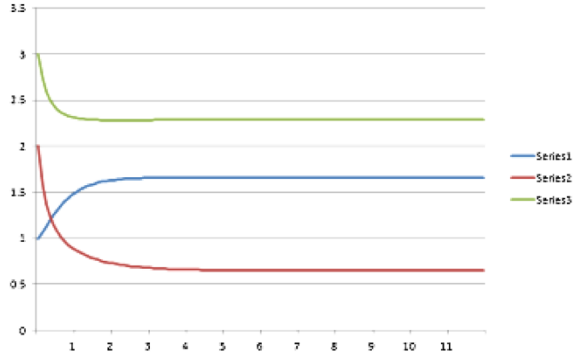
There is a vast body of research on competition models in general. We mention the following papers which refer specifically to phytoplankton: (Martines et al. 2009; Shatwell et al. 2013; Blottière et al. 2014; Record et al. 2014; Mutshinda et al. 2013).

Many competing species

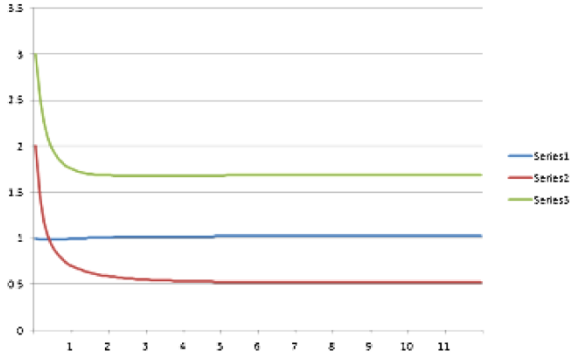
The formulation for three species can be extended to the general case of n competing species. The steady state for each species is given from the set of simultaneous linear equations in terms of the interaction parameters $x, y, z \ldots$. The interaction parameters may be deduced from observations of the steady state when $D = 0$ but this may not prove very illuminating. Evaluation of the community matrix at the steady state yields

$$\left(\lambda - \frac{df}{dN} \right) (\lambda^n + C_1 \lambda^{n-1} + C_2 \lambda^{n-2} + \cdots + C_m \lambda^{n-m} + \cdots + C_n) = 0$$

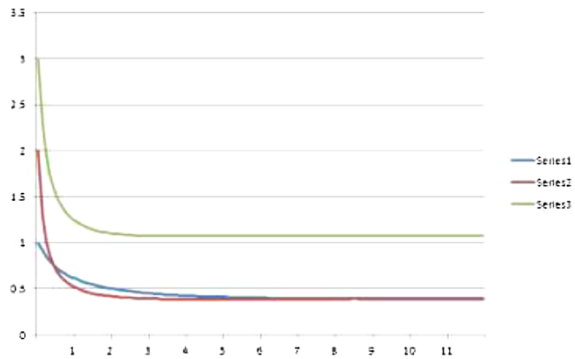
a



D = 0.25

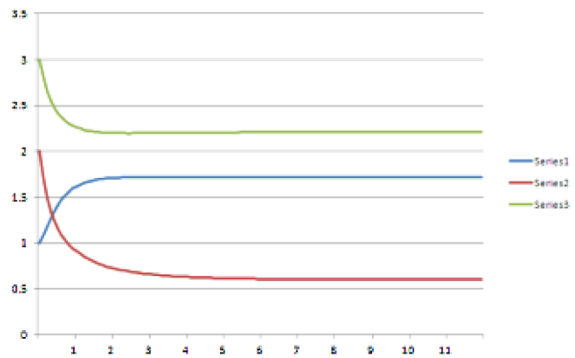


D = 1

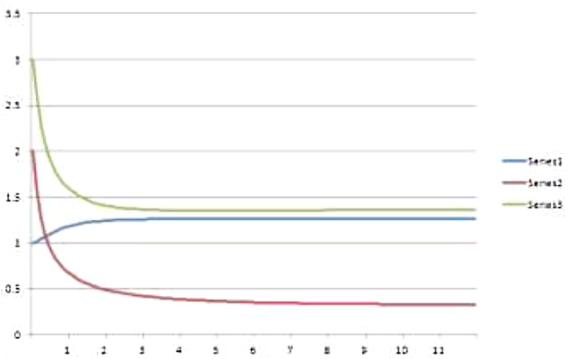


D = 1.75

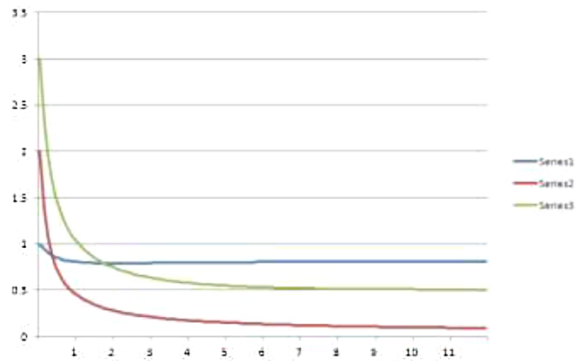
b



D = 0.25



D = 1



D = 1.75

◄ **Fig. 2** **a** Generation of populations of three competing species. Units: *Horizontal axis*, days; *Vertical axis*, cells$\times 10^5$/ml. **b** Three competing species; the effect of changes in washout values, D_{WX}. Units: *Horizontal axis*, days; *Vertical axis*, cells$\times 1^0 5$/ml. (ci): Three species; two pairs are unstable; $D_{WX} = 2.25 \times 10^{-5}$ days^{-1}. Units: *Horizontal axis*, days; *Vertical axis*, cells$\times 10^5$/ml. (cii): Three species; two pairs unstable; $D_{WX} = 3.0 \times 10^{-5}$ days^{-1}. Units: *Horizontal axis*, days; *Vertical axis*, cells$\times 10^5$/ml. **d** Three species; two pairs unstable; differing D_{WX}. Units: *Horizontal axis*, days; *Vertical axis*, cells$\times 10^5$/ml. (Note cells$\times 10^5$ = number of hundreds of thousands of cells). *Series 1*, population *P*; *Series 2*, population *Q*; *Series 3*, population *R*

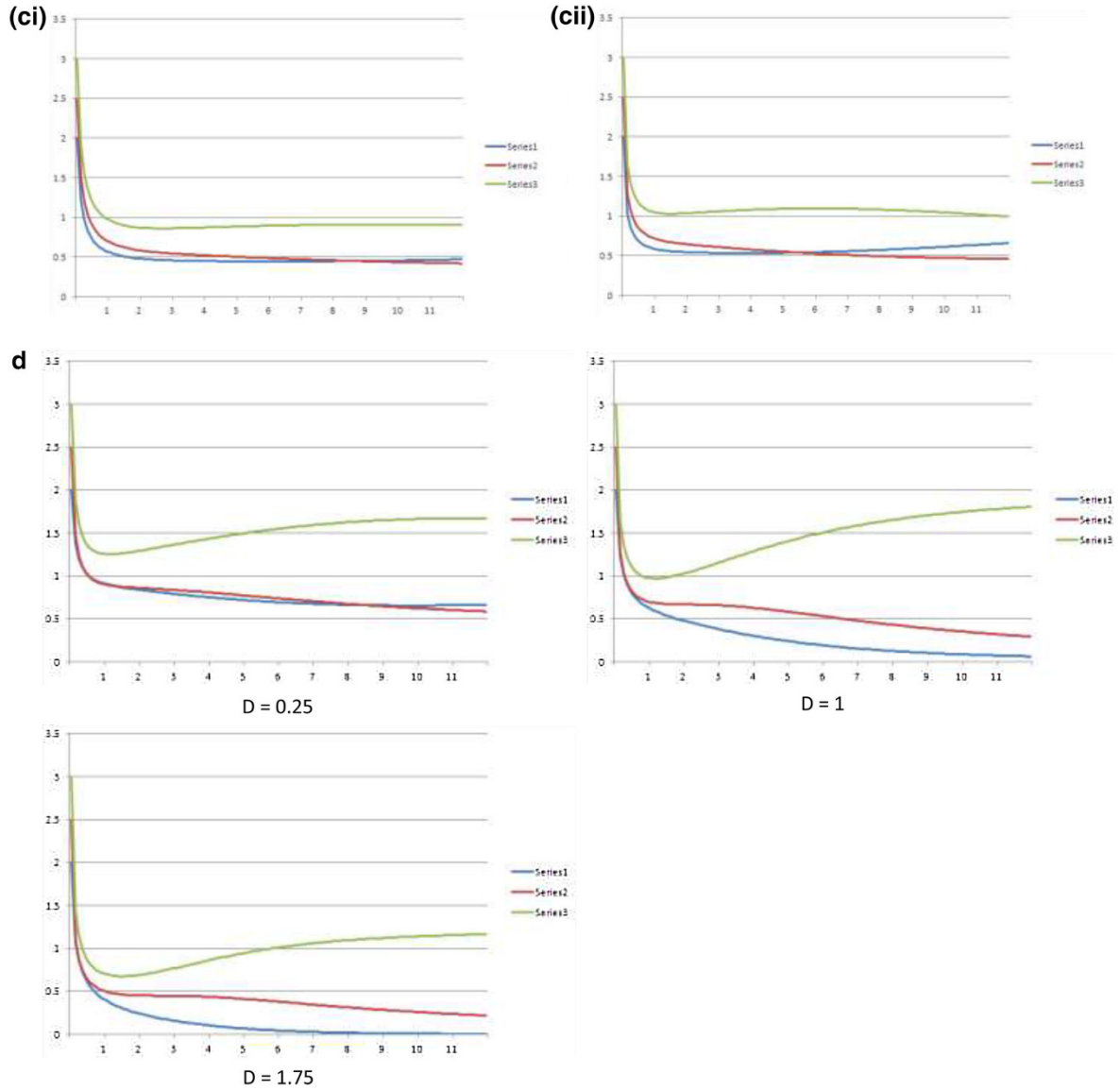


Fig. 2 continued

where n is the total number of species. The n species yield a stable steady state only if all the values of λ are real and negative. This means that, as usual, df/dN must be negative, all the coefficients C_m are positive and the steady-state populations of each species exceed zero. In fact, as with three competing species one finds C_1 to be positive providing each individual species is stable. More importantly, if all possible pairs of competing species are stable in the steady state, C_2 is positive and if all possible combinations of m species are stable in the steady state, C_m is positive. Stability of the steady state would appear consistent with the stability of all possible combinations of the competing species but the analysis of three competing species indicates that this will not be essential. The mathematical complexities of a system of n competing species are formidable; Vano et al. (2006) consider four competing species. The generalisation to n competing species may not be useful

until further work has established which of the mathematical complexities actually occur when n species of phytoplankton are continuously cultured in a chemostat. Since phytoplankton cultures adapt to their environment, it might be worthwhile to simulate a culture of n species in a chemostat as a system of only three competing species, two species having the most extreme values of the characterising parameters (properties) together with a third having the average properties of the remaining $(n-2)$ species. Redefining n competing species as a single species having average properties could be helpful—it should reveal that virtually all nutrient is consumed when D is low—but the average properties may vary significantly with D as the proportions of each species contributing to the steady state change.

The continuous flow chemostat model may be extended to well-defined gyres (eg. Gaines et al. 2006).

Spread sheet algebra

One wishes to integrate equations (6a), (6b) and (6c) numerically. Consider Eqs. (6a) and (6b).

Write $P_{i+1} = P_i + \frac{dP_i}{dt}\Delta\tau$ and $Q_{i+1} = Q_i + \frac{dQ_i}{dt}\Delta\tau$, where $\Delta\tau$ is a small interval of time, taken to be 0.1 days, and P_i and Q_i are the values of P and Q at time $i\Delta\tau$ (i is an integer from 0 to about 120, that is $i\Delta\tau$ becomes ~ 12 days).

Then, Eqs. (6a) and (6b) yield

$$P_{i+1} = P_i + D_{WP}P_i(1 - x_1P_i - x_2Q_i)\Delta\tau - DP_i\Delta\tau \tag{10a}$$

$$\text{and} \qquad Q_{i+1} = Q_i + D_{WQ}Q_i(1 - y_2Pi - y_1Q_i)\Delta\tau - DQ_i\Delta\tau \tag{10b}$$

With the use of a computer spread sheet, equations (10) provide graphs of P and Q as functions of time and these equations are readily extended to more than two competing species of phytoplankton. Figures 1 and 2 illustrate results obtained for two and three competing species of phytoplankton, respectively. They exemplify that whereas it may take several days for kinetic steady states to be obtained experimentally, once spread sheets have been set up, competition between species in a continuous flow chemostat may be modelled in a matter of seconds. Should further work show that the fundamental Verhuls Eq. (6) applies to a wide range of phytoplankton species, spread sheets may be used to model and very rapidly predict the effects of varying the environmental parameters regulating the chemostat system, much more rapidly than by direct experiment.

Figure 1a shows the change in populations of two competing species, the parameters being chosen to be similar to those observed in experiments with *Phaeodactylum tricornutum* and Dunaliella tertiolecta (Okay et al. 2005). The figures model how the populations of the two species change from their initial values of 1.5 and 3.0×104 cells per ml, respectively, to their stable steady-state populations. Table 1 summarises the steady-state populations for the complete range of flows.

Figure 1a shows clearly that as D increases the ratio of the populations of the two species changes markedly and it takes longer for growth to approach the steady state. Table 1 establishes that competition may cause marked changes in steady-state populations even between species that by themselves live stably in the chemostat and together form a stable steady state. In Table 1, $x_1y_1 > x_2y_2$ yet the population of species Q is markedly reduced and its proportion *increases* with D. In Fig. 1b, the parameters have the same values as in Fig. 1a, the Figures illustrate, with $D = 0.5$, 1.0 and 1.75 days^{-1}, the obvious use of spread sheets in rapidly predicting or demonstrating alterations in the generation of populations with species having different washout values, D_{WP} and D_{WQ}, regulating their maximum rates of growth. Spread sheets can also show the effects of changes in the populations of the initial seed.

Table 2 confirms that, with the given values of the other parameters, reasonable changes in D_{WP} and D_{WQ} make somewhat small differences to the stable steady state populations. Notice that although their community matrix shows the combination of both species to yield a stable steady state, nevertheless one species is often completely dominant.

One readily finds that any species with an increased intraspecies parameter, x_1, has a decreased population, P, and increases the population of Q. This is illustrated in Fig. 1c which shows the effect of changes in x_1 from

Table 1 Steady-state populations of two competing species of phytoplankton*

D	0.25	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.25
P_S	6.91	6.02	5.14	4.2	3.37	2.49	1.61	0.726	0
Q_S	–	–	–	0.104	0.286	0.467	0.649	0.831	1.04
P_S	6.84	5.99	5.13	4.28	3.42	2.57	1.71	0.855	0
Q_S	3.40	3.09	2.78	2.47	2.16	1.85	1.54	1.24	0.926

P_S = steady-state population of species 1 ($\times 10^5$ cells/ml); $D_{WP} = 2.25$ days^{-1}, x_1, y_1, x_2, y_2 being 0.13, 0.27, 0.02 and 0.15×10^{-5}, respectively. P_S are the corresponding steady -state populations were the species to be cultured by itself

Q_S = steady-state populations of species 2 $\times 10^5$ cells/ml): $D_{WQ} = 3.0$ days^{-1}, x_1, y_1, x_2, y_2 being 0.13, 0.27, 0.02 and 0.15×10^{-5}, respectively. Q_S are the corresponding steady-state populations were the species to be cultured by itself

– Calculations yield a negative population. The experimental population may be presumed zero

Table 2 The effect of D_{WP} and D_{WQ} on steady-state populations

D_{WP}, D_{WQ}	2, 3	2.25, 3	2.75, 3	2.25, 2	2.25, 2.5	2.25, 2.75
$P_SD = 0.5$	5.79	6.02	6.36	6.07	6.04	6.03
$P_SD = 1$	3.79	4.26	4.94	4.36	4.30	4.27
$P_SD = 1.75$	0.485	1.31	2.94	1.67	1.40	1.30
$Q_SD = 0.5$	–	–	–	–	–	–
$Q_SD = 1$	0.364	0.104	–	–	–	–
$Q_SD = 1.75$	1.10	0.649	–	–	0.177	0.434

Values of x_1, y_1, x_2 and y_2 and all units as in Table 1

– Calculations yield a negative population

0.2 to 2.5 when D is constant (unity). (In Fig. 1c, the washout values D_{WP} and D_{WQ} are 2.25 and 3.0 days^{-1} and y_1, y_2 and x_2 are 0.33, 0.15 and 0.02×10^{-5}, respectively). As x_1 increases, the second species, Q, becomes dominant. Increases in y_1 cause similar changes to changes in x_1 and it has not been considered necessary to illustrate them. Increase in interspecies Verhuls parameters produces significant changes in species populations. This is illustrated in Fig. 1d where D_{WP}, D_{WQ}, y_1 and y_2 have the same values as in Fig. 1c, D is again unity, x_1 is 0.7×10^{-5} and x_2 is varied from 0.02 to 0.16×10^{-5}. One finds that increase in x_2 increases the eventual stable population of Q and reduces the corresponding population of P.

Table 3 and Figure 2 illustrate the behaviour of three competing species in a continuous flow chemostat. Table 4 shows the parameters used in the modelling. The figures show the population of each of the three species changing from its initial value to its value in the final, kinetically stable state; the initial values have been selected to be of the order of ten times the initial values in Fig. 1 so, whereas Fig. 1 simulates the growth of species in the chemostat, Fig. 2 simulates the decrease of chemostat populations to their eventual steady states. The parameters in Table 3 and Fig. 2a, b; Table 4 has been selected such that all pairs of combinations of two species give stable steady states. Not unexpectedly, a few days are generally required before a steady state is reached. During this time, all three species adapt to the environmental conditions. In Table 3, two species have the same interaction parameters as in Fig. 1a, but the third species has a rather larger intraspecies interaction parameter, z_3. Comparison of Tables 2 and 3 shows that, the third species having the largest intraspecies parameter has the lowest steady-state populations, R_S. Introduction of the third species caused a small, significant change in the steady-state population, P_S, of the first species but a dramatic increase in the steady-state population, Q_S, of the second species. Figure 2a shows the third species with the same Verhuls interaction parameters as in Table 3 but now dominating two other species with larger intraspecies interactions (Table 4). As the spread sheet exploration of two competing species showed, increase in the interaction between cells within a species diminishes the species' population. Figure 2b illustrates the competition between three species having the same interaction parameters as in Fig. 2a but different washout values, D_{WX}; the effect of changing the washout values within the experimentally observed range produces small but significant changes, especially when the flow rates, D, through the chemostat approach the washout values.

Table 3 The effect of the introduction of a third species on steady-state populations*

D	0.25	0.5	1.0	1.75
P_S	6.29	5.48	3.86	1.43
Q_S	2.14	1.95	1.55	0.97
R_S	1.42	1.32	1.13	0.94

D in days^{-1}; P_S, Q_S, R_S steady-state populations $\times 10^5$ cells/ml; x_1, y_1, z_1, x_2, y_2, z_2, x_3, y_3, z_3 being 0.13, 0.02, 0.02, 0.27, 0.15, 0.02, 0.35,0.05 and 0.05 $\times$ 10^{-5}, respectively. D_{WP} is 2.25, D_{WQ} and D_{WR} are 3.0 days^{-1}

Table 4 Parameters used in Fig. 2

Figure	x_1	y_1	z_1	x_2	y_2	z_2	x_3	y_3	z_3	D_{WP}	D_{WQ}	D_{WR}
2a	0.5	0.02	0.02	0.5	0.15	0.15	0.35	0.05	0.05	2.25	3.0	3.0
2b	0.5	0.02	0.02	0.5	0.15	0.15	0.35	0.05	0.05	3.0	2.25	2.25
2c	0.357	0.286	0.286	0.25	0.333	0.333	0.333	0.444	0.111	2.25	2.25	2.25
2d	0.357	0.286	0.286	0.25	0.333	0.333	0.333	0.444	0.111	2.25	3.0	3.0

Figure 2c comprises 2 graphs, 2Ci with $D_{WX} = 2.25$ (as above) and 2Cii with $D_{WX} = 3.0$, Units: x, y, z $\times 10^{-5}$; D_{WX} (days^{-1})

Figure 2c, d shows a spread sheet exploration of competition between three species having interaction parameters such that, whereas all three species can exist together in a stable steady state (C3 is positive) this is not possible for two of the pairs ($x_1x_2 < y_1z_2$ and $x_1x_3 < z_1y_3$) (Table 4). Figure 2c illustrates two straightforward situations in which the washout values, D_{WX}, are identical for all three species; Fig. 2d explores the more complex but realistic situation when the species may differ in their washout values. Figure 2c shows, with $D = 1$, the three species coexisting and rapidly approaching a steady state after only a couple of days. As one expected from the values of the interaction parameters, the third species had the greatest population in the steady state. Also as one expected, when the washout values, D_{WX}, varied within observable limits between species, the temporal behaviour of the system of three species shows greater complexity and, especially when the flow rate through the chemostat, D, is greater than unity, species may die out before a steady state is reached. This is illustrated in Fig. 2d for $D = 0.25$, 1.0 and 1.75. Extending Eq. 10 to chemostats containing many competing species is logically straightforward and, given sufficient computer space, spread sheets could be used to provide the effects of multispecies competition in continuous flow chemostats. The effects of ranges of values—and of anomalous values—of the growth rates and interaction constants that characterise species could be studied much more rapidly than by experiment.

When describing two competing species, it was seen that Verhuls inter- and intraspecies interaction constants can be derived experimentally by studying pairs of species when D is zero. This appears impractical when more than three species—three pairs—will be competing in the continuous flow chemostat. When it is desired to study spread sheet simulations of many competing species with real rather than hypothetical values of their properties, it would seem necessary to reduce the number of species simulated to three by studying mixtures of species with experimentally determinable average values of their washout constants and interaction constants so as to avoid having more than three pairs of species to consider.

Thermodynamics of steady states

Statistical thermodynamics applied to the exponential phase of batch cultures of phytoplankton (Levich (2000), and references therein) has predicted results in at least qualitative agreement with experimental observation. Similar observations have been obtained at the surface of selected oceans (e.g. May and Leonard 1975; Ballantyne et al. 2010; Chen and Liu 2010). Whereas there is continuous flow through the chemostat, in the steady state the total mass and the mass and composition of each constituent—water, nutrient and phytoplankton—and their momentum and angular momentum remain constant (are conserved). One may, therefore, define classical thermodynamic properties for the chemostat system in the steady state and thence

suppose the distribution of phytoplankton in the steady state to be that which minimises the creation of Gibbs Free Energy—or maximises the creation of entropy (Denbigh 1951; Katchalsky and Curran 1965).

Let δG be the maximum useful chemical work that the growth (biosynthesis) of a phytoplankton cell can perform. This is normally written $\mu_i \delta n_i$, μ_i being the appropriate chemical potential. One is not considering equilibrium but an irreversible process in which free energy is being created continuously and, in the steady state, at a constant rate,

$\frac{\delta G}{\mathrm{dt}} = \sum$(maximum useful work per cell)×rate of reaction,

the rate of reaction being the rate of cell growth (or biosynthesis). Regarding the phytoplankton as a single averaged species, in the chemostat in the steady state the rate of cell growth is DP_S. One may suppose the maximum useful work per cell to be KI, where I is the light energy absorbed per cell and K is the efficiency.

Thus, $\frac{\delta G}{\mathrm{dt}} = DP_S KI$.

The steady state being stable, $\frac{\mathrm{d}}{\mathrm{d}P_S}(\delta G/\mathrm{d}t) = 0$.

In other words, in consequence of thermodynamics in the stable steady state ($DP_S KI$) is independent of P_S. As DP_S decreases, more light is utilised and as DP_S increases less light is utilised (Okay et al. 1994, 2003, 2005).

Simplifying, light is absorbed initially by one of the $\sim$400 chlorophyll molecules in one of the chloroplasts in the membrane of a phytoplankton cell. About one in eight of the absorbed photons is transferred successfully to the photosystem at the centre of the chloroplast and each such photon furnishes an electron that travels through the photosystem losing around half of its energy during the process. The electron participates in the decomposition of a water molecule at the end of the photosystem and thence forms molecules of ATP and NADPH which transport chemical energy for biosynthesis. Unsurprisingly, K, the efficiency of light utilisation is only a few percent. One expects the light energy absorbed per cell, I, to be proportional to the logarithm of the number of chlorophyll molecules per cell; the efficiency of light utilisation, K, is more difficult to define experimentally.

The distribution of different phytoplankton species in a batch culture (exponential phase) is a consequence of the nature of the environmental resources (Reynolds et al. 2001; Holtgrieve et al. 2010). Given comfortable illumination, the relative abundances of the species depend only on the ratios of the nutrients that limit growth. The relative abundance of a species takes its greatest possible value if the environmental resource ratios coincide with the ratios of the species' demands for the same resources (nutrients) (Levich (2000); Gaines et al. (2006)). We have observed that the populations of *Phaeodactylum tricornutum* and *Dunaliela tertiolecta* in the steady state increase with the concentration of nutrient in the incoming flow, N_O. The intraspecies interaction parameters (x_1 and y_1 for two species, x_m for m species) should, therefore, diminish as N_O increases. The thermodynamics of irreversible processes would suggest that when growth was nutrient limited the intraspecies interaction parameters should diminish as the demands of consumption are more nearly matched by the ratios of the limiting nutrients supplied. Interspecies interaction parameters should behave in the same way. Similarly, the 'washout' dilution rates for individual species, D_{WX}, should increase with increase in the rate of consumption and increase in the match between resource demand and supply. These predictions need testing; many batch cultures examined have been so dilute that interactions between cells could be neglected. This description of steady states in a continuous flow chemostat suggests that the thermodynamics of irreversible processes may also be applied to observations of steady states of phytoplankton in regions of the oceans where D may be due to a defined current or to a constant rate of predation, though the effect of periods of darkness (night) on the rates of cell division and death, and the extent of remineralisation by bacteria need consideration (Ruiz-Gonzalez et al. 2010).

Conclusions

Phytoplankton growth in an axenic, continuous flow chemostat evolves by the adaptation of the rates of cell division and growth to the environmental parameters of the chemostat to yield a steady state for both the population of phytoplankton and the concentration of nutrient. The adaptation inhibits the integration of the equations of conservation of phytoplankton population and nutrient concentration. The pragmatic modelling of phytoplankton growth in a chemostat using a Verhuls formulation with intra- and inter- species interaction

constants has been shown to facilitate the prediction of cell growth and steady-state populations. Phytoplankton responses to deliberate changes in such environmental parameters as the rate of flow through the chemostat can be modelled very much more quickly than by direct observation when several days are required to generate a steady state. In principle, the intra- and interspecies interaction parameters can be established by observation of phytoplankton cultures when flow through the chemostat is slow. These parameters determine whether steady states are stable. Nevertheless, it is essential to establish that the Verhuls formulation does indeed model the growth of a wide range of phytoplankton species. Experimental determination of the evolution of those fractions of phytoplankton cultures that are live and able to divide would be welcome. In a stable steady-state in a continuous flow chemostat, the population of phytoplankton, the concentration of nutrient, their momenta and their angular momenta are all conserved. It is, therefore, possible to define classical thermodynamic quantities for the stable chemostat and to suppose that the distribution of phytoplankton in the steady state is that which minimises the creation of Gibbs Free Energy by the system. In consequence, the utilisation of light energy by stable cultures of phytoplankton cells in a steady state in a continuous flow chemostat varies inversely with the rate of cell growth, DPS. Previous statistical thermodynamic studies of batch cultures of phytoplankton may be extended to embrace the steady states of cultures in a continuous flow chemostat and predict that the Verhuls interaction parameters should diminish as the demands of cell consumption become more nearly matched by the ratios of the limiting nutrients.

It would be desirable to have more information about the dependence on N of the rate of consumption A, and also about the effect of N on the growth rate of the populations, so that the rôle of the nutrient can be made more explicit in the mathematical models.

Conflict of interest The authors have no competing interests.

Author's contribution OO performed all the experimental work. DG produced all the spread sheets and Figures under the supervision of RB. AD and EO monitored and extended the mathematics. AG developed the mathematical model and put everything together.

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