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Introduction to Competition Between Continuous Cultures of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta*

O. S. Okay,[1] M. Gibson,[2] A. Gaines,[2] and A. M. Davie[3]

[1]Istanbul Technical University, Faculty of Naval Architecture and Ocean Engineering, Istanbul, Turkey
[2]Strathclyde University, Department of Civil Engineering, Environmental Health Laboratory, Glasgow, Scotland
[3]Edinburgh University, Department of Mathematics and Statistics, Edinburgh, Scotland

When the diatom, *Phaeodactylum tricornutum*, and the microalga, *Dunaliella tertiolecta*, are cultured together in a chemostat at dilution factors of $\sim$0.5 day^{-1}, the diatom develops the higher population density. At dilution factors above 1.2 day^{-1} the inability of the diatom to assimilate nutrient as fast as it flows into the chemostat results in the microalga generating the larger population. This change in population densities is accompanied by an increase in the chlorophyll content of the diatom and a decrease in the chlorophyll content of the microalga. Two species of phytoplankton can coexist when they compete for nutrient in a chemostat providing they do not otherwise interact. When the species do interact coexistence in a stable steady state is possible providing intraspecies interactions exceed the interactions between the species. Both species adjust their consumption to minimise the concentration of nutrient in the chemostat and their growth is modified to match the dilution factor of the flow.

Key Words: Continuous culture; Chemostat; Phytoplankton; Competition; Adaptation.

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Address correspondence to Strathclyde University, Department of Civil Engineering, Environmental Health Laboratory, 107, Rottenrow, Glasgow G4 0NG, Scotland; E-mail: a.f.gaines@strath.ac.uk

INTRODUCTION

Previous studies have emphasised the importance of adaptation in understanding the growth and cultivation of *Phaeodactylum tricornutum*.[1–3] Thus Figure 1, which shows the effect of the selective herbicide 2,4-dichlorophenoxy acetic acid, 2,4-D, on chemostat populations of *Phaeodactylum tricornutum* illustrates the development of resistance to the herbicide.[2] Specifically, Figure 1 shows how five to eight days after the introduction of the phytoplankton into the chemostat the culture settled down to a steady state. The cells had adapted. 100 mg/L of 2,4-D were then introduced into the nutrient flow. The population decreased and rose again to its steady-state value. On day 16, the concentration of herbicide in the inflow was increased to 300 mg/L. The cell population again decreased and then recovered. On day 24, the herbicide concentration was increased to 500 mg/L and once more the cell population declined and rose again. Not surprisingly phosphate (nutrient) concentrations rose and fell as the population densities decreased and increased. These results have been described in detail elsewhere.[2] The logarithmic plot employed in Figure 1 shows the decline in phytoplankton population on the addition of 2,4-D to have been exponential and the rate of decline to have been close to the dilution factor (0.715 per day in these experiments). Evidently the introduction of 2,4-D diminished the rate of

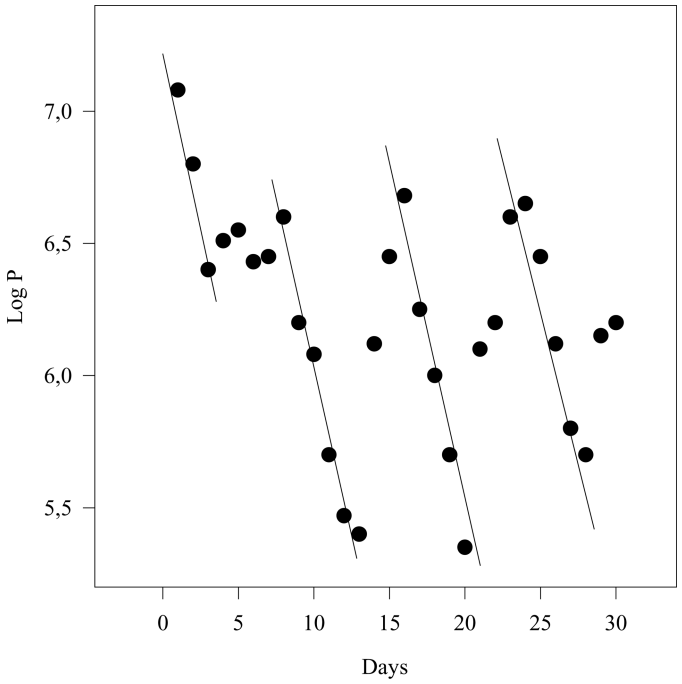

Figure 1: The effect of 2,4 dichlorophenoxy acetic acid on chemostat populations of *Phaeodactylum tricornutum* (P).

cell division, b^*, to virtually zero and the culture was washed out of the chemostat (cf Eq. (3c)). It remains necessary to find an explanation as to how it was that the culture grew again; that is, adapted to its environment by developing resistance to the herbicide.

2,4-D is a modification of a naturally occurring plant hormone which consequently inhibits the growth of vegetation, though low concentrations of the water-soluble amine salt may enhance growth since the assimilation of amine can replace a deficiency of nitrogen.[2] Plant cells attempt to detoxify 2,4-D by hydroxylation of the aromatic ring, demethylation and excretion of the metabolites after their conjugation with glutathione.[4] Each of these biochemical reactions is enzyme controlled and the induction of this enzyme activity can lead to the development of resistance to the herbicide. The development of such resistance by higher plants has been observed to take years but, as Figure 1 suggests, appears to take a much shorter time to develop in phytoplankton cells. Resistance to herbicides being the development of enzyme activity, we surmise that we are discussing the expression of genes and postulate that resistance develops as a consequence of the formation of alleles by mutation (cf. [10], for example). Suppose the rate of mutation is of the order of 10^{-6} per gene per generation, similar to the rate in other, better studied, organisms. Then, if the phytoplankton density is of the order of 10^6 per ml (Fig. 1), it becomes probable that at least one cell present in the population will possess any given specified allele. Since a healthy phytoplankton cell may divide two or three times a day, a single phytoplankton cell possessing an allele favoured by the environment may generate a population of from 1,000 to 60,000 in 10 days. We suggest this to be the basis for the rapid evolution of phytoplankton cells resistant to 2,4-D. A few cells, initially present in the chemostat culture possessing alleles commanding sufficient enzyme activity to metabolise the herbicide, subsequently divide so as to resist washout and dominate the population. This arithmetic is consistent both with previous considerations of the genetic variation within phytoplankton species[5,6] and with recent measurements of the actual genetic diversity.[7,8] Both field[4,9–15] and chemostat[3] observations indicate the development of resistance by phytoplankton to herbicides to be fairly general and not restricted to 2,4-D. It is reasonable to suppose that phytoplankton share the rapid development of resistance to xenobiotics through gene expression shown by bacteria and insects, all being organisms with the potential to possess high population densities together with high rates of growth (In addition, of course, bacteria possess specific modes of developing resistance). The extent to which gene expression is similarly involved in the adaptation of phytoplankton to changes in the concentrations of nutrients in their environment is of considerable significance; it may well be that intraspecies variation in rates of consumption and growth is a consequence of differences in alleles.[16–19]

We now extend our understanding of the growth of *Phaeodactylum tricornutum* by studying how its growth is modified by competition with another

phytoplankton species. This is a significant theoretical problem. Contrary to classical ecological theory,[20–21] many different phytoplankton species live together in the field[22,23] and several species may coexist together in the same chemostat.[24,25] Practically, too, the problem is of importance in understanding how to control populations of phytoplankton weeds or of invasive species. It has been seen that phytoplankton species, because they may develop resistance rapidly, are difficult to control by herbicides. It therefore becomes important to understand the factors that regulate competition between phytoplankton "weeds" and phytoplankton "crops" and that underlie biotic resistance to invasion.[26] Here we report attempts to obtain simultaneous, stable, continuous, axenic cultures of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* in the same chemostat. Whereas *Phaeodactylum tricornutum* is a star-shaped diatom, possessing a significant siliceous cell wall and a small organic centre, *Dunaliella tertiolecta* is a larger, spherical microalga without a cell wall, though, of course, possessing a cell membrane. Cells of the two species are readily distinguished and counted under the microscope. The discussion of the results considers the importance of the adaptation of the cells to and by their environment and also the criteria that have to be satisfied to ensure the simultaneous stability of both cultures.

MATERIALS AND METHODS

Axenic samples of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* were taken from large batch cultures maintained in the laboratories of the Marmara Research Center (Turkey). Continuous cultures of phytoplankton were generated in two 550 ml glass chemostats mounted in a small temperature controlled room ($25 \pm 2^{\circ}$C) supplied by stabilised electricity. These experiments were performed exactly as described previously by Okay et al.[1] save that both species of phytoplankton were injected simultaneously into the modified f/2 culture medium[3,27] in the chemostats. The nutrient flowing into the chemostat always contained constant concentrations of nitrate and phosphate of 1300 and 850 $\mu g\,l^{-1}$, respectively. A constant illuminance of light was maintained at the chemostat day and night.

The continuous cultures were monitored by daily determinations of (i) nitrate and phosphate concentrations in the outflow using a Technicon Autoanalyser II,[28] (ii) the Chlorophyll-a fluorescence intensity (Ex = 430 nm, Em = 663 nm; Shimadzu Model RF 540 fluorescence spectrophotometer) of the outflow and the population densities of both species in the outflow as counted through a microscope, (iii) the total phytoplankton population density as measured by a Coulter Counter (Model Beckman Z2), and (iv) the rate of liquid flow using a measuring cylinder and a stop clock. Table 1 recapitulates the parameters that may be determined from the measurements.

Table 1: Parameters observable by measurements of the stationary states of continuous cultures of phytoplankton.

Parameter	Explanation
b_S^* (=D)	Average rate of cell division per cell whether alive or dead; rate of growth per living cell, g_S
1/D	Residence time of phytoplankton
P_S, Q_S	Phytoplankton population density; flux density of phytoplankton issuing from the chemostat (number per unit volume)
DP_S, DQ_S	Rate of increase of phytoplankton cells (number per unit volume per unit time)
N_S	Concentration of limiting nutrient; flux density of nutrient issuing from the chemostat (moles per unit volume)
$D(N_O-N_S)$	Rate of consumption of nutrient (moles per unit volume per unit time)
(N_O-N_S)	Flux density of consumption (moles per unit volume)
$D(N_O-N_S)/P_S$	Rate of consumption per cell (moles per cell per unit time); C_S^* (average rate of consumption per cell whether living or dead)

RESULTS

General Description

The experiments, summarised in Table 2, compared the behaviour of mixtures of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* at dilution factors, D, of around 0.5 and 1.9 per day. Table 2 and Figure 2 show that at values of D of around 0.5 per day, whereas the diatom persisted in the chemostat, albeit at a lower population density than when it was cultured as a pure species,[1] the population density of the microalga declined dramatically though, after a few days the population density stabilised at a comparatively low value. Previously, it was observed that pure cultures of the diatom were washed out of the chemostat when D was 2.25 per day[1] and Table 2 and Figure 2 illustrate accordingly how, when D was 1.9 per day, it was the microalga which persisted in the chemostat and the diatom whose population declined to a low level. A further experiment established that the microalga was the dominant

Table 2: The ratios of the population densities of *Phaeodactylum tricornutum* to *Dunaniella tertiolecta* at different dilution rates.

Parameters	$D = 0.445\ d^{-1}$		$D = 0.524\ d^{-1}$	$D = 0.545\ d^{-1}$		$D = 1.19\ d^{-1}$	$D = 1.87\ d^{-1}$	
R Initial	1.02	1.63	0.02	0.69	1.86	1.8	0.49	1.55
Final*	12.2	20.7	1.8	49	55	0.02	0.006	0.60
N	35	30	7	12	10	12	26	4

R = Ratio of the population densities of *Phaeodactylum tricornutum* to *Dunaliella tertiolecta*
* = Experiments lasted from 7 to 16 days until it was clear which species was dominant, though a steady state was not necessarily attained.
N = Final concentration of nitrate in the outflow from the chemostat ($\mu g\, l^{-1}$); the concentration of nitrate in the inflow was always 1300 $\mu g\, l^{-1}$.

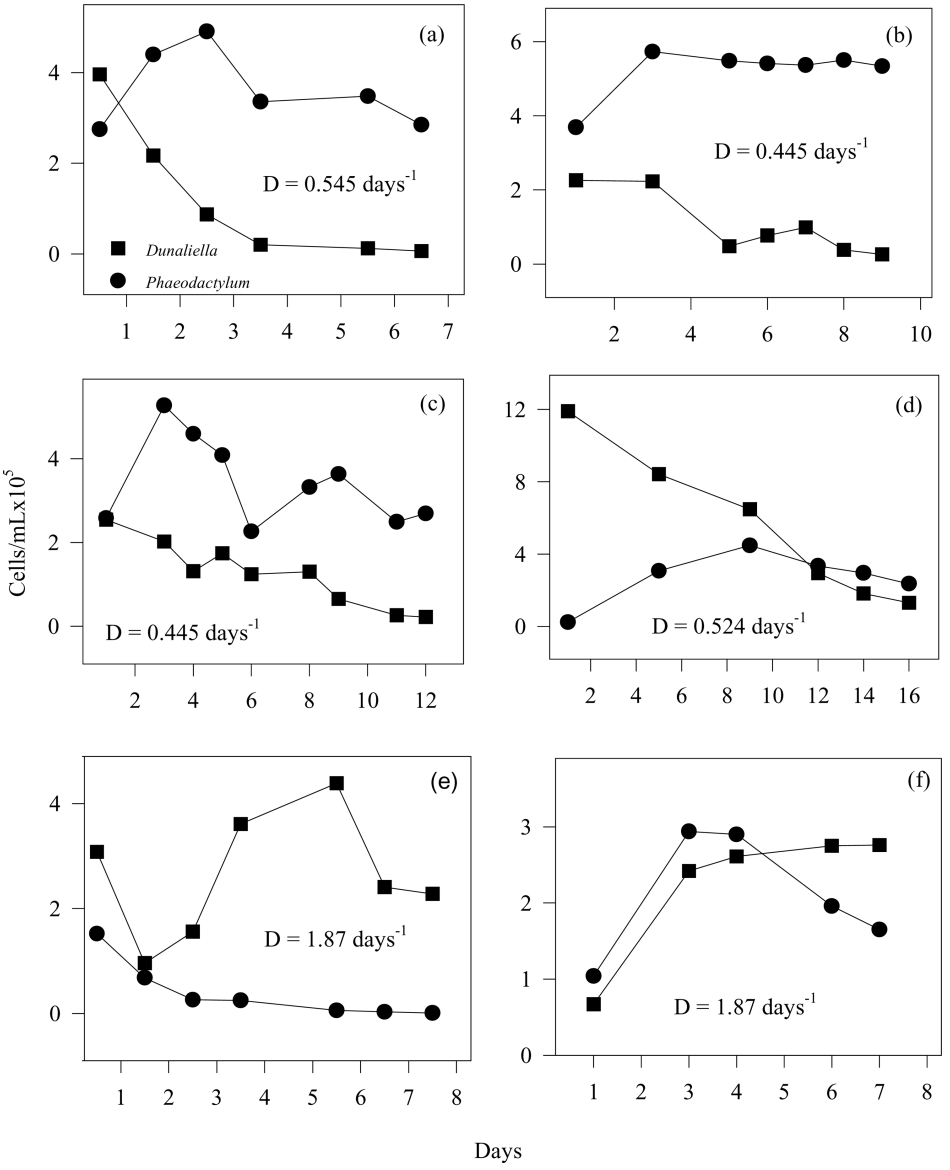

Figure 2: Observed population densities of *Phaeodactylum tricornutum*, ●, and *Dunaliella tertiolecta*, ■ with different initial cell populations. (a) Dilution factor 0.545 days^{-1}, (b) Dilution factor 0.445 days^{-1}, (c) Dilution factor 0.445 days^{-1}, (d) Dilution factor 0.524 days^{-1}, (e) Dilution factor 1.87 days^{-1}, (f) Dilution factor 1.87 days^{-1}.

culture when D was 1.2 per day. Table 2 indicates the dominance of the diatom and the microalga at D values of 0.5 and 1.9, respectively, to be independent of the proportions of the cultures initially injected into the chemostat. Virtually all the nitrate flowing into the chemostat was consumed in each experiment (Table 2). We were indeed observing classical competition for a limiting

nutrient between two different species of phytoplankton—which might be considered as a crop and a weed. The results were similar to those observed in competition between cultures of the bacteria, *Bacillus subtilis* and the yeast *T. utilis*.[29] Our description of our results, however, is rather different to the description of interspecies competition within a chemostat given by previous authors.

Chlorophyll Fluorescence

Figures 3 show the variation in the intensity of chlorophyll fluorescence, F, throughout the experiments as a function of the ratio of the population

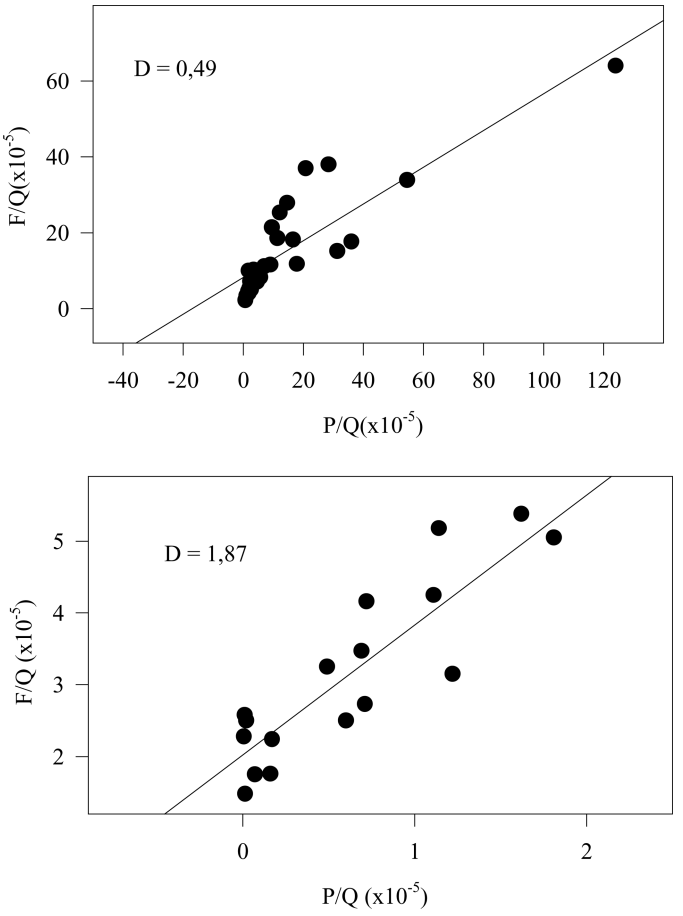

Figure 3: Intensities of chlorophyll fluorescence by mixed cultures of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta*, where F = Fluorescence intensity (arbitrary units), Q = population density of D*unaliella tertiolecta* ($\times 10^5$ cells per ml) and P = population density of *Phaeodactylum tricornutum*. ($\times 10^5$ cells per ml) (a) Dilution factor 0.49 $\pm$ 0.03 days^{-1} and (b) Dilution factor 1.87 days $^{-1}$.

densities of *Phaeodactylum tricornutum*, P, to *Dunaliella tertiolecta*, Q. Suppose the observed fluorescence intensities, F, to be the sum of the intensities of the individual cells. That is

$$F = k_P P + k_Q Q \tag{1}$$

Then,

$$F/Q = k_P P/Q + k_Q \tag{2}$$

Figure 3 shows graphs of F/Q against P/Q to be reasonable straight lines, thereby justifying the assumptions inherent in equation (1). Accordingly, k_P and k_Q, the respective chlorophyll intensities of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* cells are given by the slopes and intercepts deduced from Figures 3 and displayed in Table 3. When D was about 0.5 per day, k_Q, the chlorophyll fluorescence of *Dunaliella tertiolecta* cells was larger than k_P, the chlorophyll fluorescence of *Phaeodactylum tricornutum* cells. At dilution factors near 1.9, however, the reverse was true. The increase of k_P, the chlorophyll fluorescence of cells of *Phaeodactylum tricornutum*, with increase in the dilution factor was previously observed when pure continuous cultures were studied.[1]

The initial value of the fluorescence of the individual cells recorded in Figure 3 was observed within 24 h of the injection of the cultures into the chemostat. The change in the values when the dilution factor was changed was due to the adaptation of the cells to the changed conditions and not to a change in the parent batch cultures. This was shown by the results of running a sequence of chemostat experiments with dilution factors of around 0.5, 1.9, and around 0.5 starting with injections from the same parent batch cultures. The cells gave the same chlorophyll fluorescence intensity when the dilution factor was around 0.5 both before and after the experiments with a dilution factor of 1.9. The changes in the intensity of chlorophyll fluorescence were caused presumably by changes in the concentrations of chlorophyll molecules per cell. The rapidity of the adaptation of the chlorophyll concentrations to the magnitude of the dilution factor recalls the rapidity of adaptation to changes in illuminance observed previously and discussed in terms of the expression of the Olive gene.[1]

Table 3: Intensities of chlorophyll fluorescence per cell (arbitrary units).

Species	Fluorescence intensity at $D = 0.49 \pm 0.03\ d^{-1}$	Fluorescence intensity at $D = 1.87\ d^{-1}$
Phaeodactylum tricornutum	1.5	3.7
Dunaliella tertiolecta	2.8	0.15–1.5

Results with Dilution Rate, D, of ~0.5 Per Day

When D was about 0.5 per day studies of pure species indicate nutrient to be consumed as fast as it impinged on the cell surface. Cells of *Phaeodactylum tricornutum* had the larger surface area (and the smaller volume of organic material) and their rate of consumption was therefore greater than the rate of consumption by cells of *Dunaliella tertiolecta*. Since the cells of *Dunaliella tertiolecta* had the larger mass of organic material and thus the larger chlorophyll fluorescence per cell they needed more nutrient per cell in order to reproduce. For this reason, although in the steady state its rate of division per cell, b^*, would be the same as that of *Phaeodactylum tricornutum* and equal to the rate of dilution, D, (Eq. (3c) applied to each species), the population density of *Dunaliella tertiolecta* was the smaller (Fig. 2). Consistent with this description, in the stable chemostat system of phytoplankton species studied by Hegarty and Villareal,[25] the lowest population densities corresponded to the species having the largest cells.

This section has set out the essential explanation of the distribution of the stable populations of two phytoplankton species—a crop and a weed—under circumstances when both species can consume nutrient as fast as it impinges on the cells.

Results with Dilution Rate, D, of Between 1.2–1.9 Per Day

When the dilution factor was 1.2 or more, pure cultures of *Phaeodactylum tricornutum* were incapable of assimilating nutrient as fast as the nitrate impinged on the cells[1] even though the cells adapted by increasing their content of chlorophyll.[30,31] Accordingly the rate of consumption was enzyme controlled. When *Dunaliella tertiolecta* was also present the nitrate concentration in the chemostat remained trivial (Table 2) and the population density of the microalga now exceeded that of the diatom (Fig. 2). Given that in the steady state the rates of division per cell, b^*, of both the microalga and the diatom were the same (=D), it is clear that the microalga was not merely mopping up the nitrate the diatom was unable to consume, it was consuming much more nitrate than the diatom. One supposes that the microalga was still able to consume nitrate as fast as it impinged on the cells even at these comparatively high dilution factors. Apparently, the absence of effective competition from the diatom permitted the *Dunaliella tertiolecta* cells to diminish their chlorophyll content.

The failure of *Phaeodactylum tricornutum* at the higher dilution factors to consume nitrate as fast as it impinged on the cells could have been be due either to the impediment of its siliceous cell walls to the flow of nitrate into the cells or to a relative failure of the assimilation biochemistry of the diatom compared with the microalga. Further studies should distinguish these possibilities.

This section has described how when one species of phytoplankton is able to consume nutrient as fast as it impinges on the cells and the other species

cannot, it is the stable population of the former species that is naturally the larger.

DISCUSSION

Adaptation

The low nitrate concentrations observed in all the experiments recorded in Table 2 and Figure 2 testify to the adaptation of the phytoplankton cells so as to minimise the concentration of nutrient.[1] Simultaneously with the adaptation of consumption by the cells, their growth became adapted by the chemostat environment until the phytoplankton cultures persisted in a stable state with a rate of cell division per cell (whether living or dead), b^*, equal to D. The number of cells of each species was conserved (Eq. (3c)). One sees also that adaptation involved changes in the chlorophyll content of the cells. We suggest the adaptation of phytoplankton cells by and to their environment to be important to the persistence and diversity of phytoplankton species in the field.[32,33,5–8,19]

Conditions for a Stable Steady State

So far, the behaviour of the culture in the chemostat has been described as if one may accept that the two competing species of phytoplankton ultimately coexisted in a steady state. Since the work of Volterra it is often averred that when two species of phytoplankton compete for a single nutrient one of the species must die out.[29] The experiments we report did not extend beyond two weeks and, had the experiments been continued indefinitely, it might be supposed that the species having the lower population density would ultimately have been washed out of the chemostat. Apparently paradoxically, species of phytoplankton coexist naturally in the field,[22] though low population densities can often be wiped out by a random fluctuation in the environment. Stable assemblages of phytoplankton species have been cultured in chemostats at a single, low dilution factor.[24,25] Consider, therefore, the circumstances necessary to ensure the existence of a steady state in the controlled environment of a chemostat.

Previously the growth of an axenic culture of *Phaeodactylum tricornutum* in a chemostat has been shown to satisfy the equations:

$$\mathrm{dP}\phi/\mathrm{dt} = -\mathrm{DP}\phi + \mathrm{gP}\phi, \tag{3a}$$

$$\mathrm{dP}(1-\phi)/\mathrm{dt} = -\mathrm{DP}(1-\phi) + \mathrm{mP}\phi, \tag{3b}$$

$$\mathrm{dP}/\mathrm{dt} = -\mathrm{DP} + \mathrm{b}^*\mathrm{P}, \tag{3c}$$

$$\mathrm{dN}/\mathrm{dt} = \mathrm{D}(\mathrm{N_O} - \mathrm{N}) - \mathrm{C}^*\mathrm{P}, \tag{4}$$

where ϕ is that fraction of P which is alive, b, m and g (=b − m) are the rates of cell division, death and growth per living phytoplankton cell and b^* is the rate of cell division per cell whether living or dead. D is the dilution factor, essentially the rate at which cells are removed from the chemostat, this removal being equivalent to the cells suffering steady predation. N and N_O are the concentrations of limiting nutrient in the vessel and in the inflow, respectively, and C^* is the observed rate of consumption of N per cell whether living or dead. Equations (3a–3c) are the equations of conservation of phytoplankton cells and equation 4 is the equation of conservation of a single limiting nutrient (1). Equation (3c) shows that in a steady state the rate of growth per cell, b^*, is equal to the dilution rate at which the cells are removed from the chemostat.

Suppose Eqs. (3) and (4) to be extended to both species in the present experiments. Thus,

$$dP/dt = -DP + b_P{}^*P = f1, \tag{3d}$$

$$dQ/dt = -DQ + b_Q{}^*Q = f2, \tag{3e}$$

and

$$dN/dt = D(N_O - N) - C_P{}^*P - C_Q{}^*Q = f3. \tag{4a}$$

We now derive the community matrix. First, since the two species are supposed independent,

$$\delta f1/\delta Q = \delta f2/\delta P = 0$$

Second, in the study of pure cultures of *Phaeodactylum tricornutum* the rates of growth were determined by the flux of nutrient entering the chemostat and hence by D and N_O and not by the concentration of nutrient within the chemostat.[1] We therefore postulate

$$\delta f1/\delta N = \delta f2/\delta N = 0$$

Consequently, the community matrix gives

$$[P_S(\delta b_P^*/\delta P)_S - \lambda][Q_S(\delta b_Q^*/\delta Q)_S - \lambda][D + X + \lambda] = 0, \tag{5a}$$

where

$$X = P_S(\delta C_P^*/\delta N)_S + Q_S(\delta C_Q^*/\delta N)_S \tag{5b}$$

The suffix, S, indicates evaluation at the steady state. Thus, λ has negative values and consequently the steady state is stable if—and only if—$(\delta b_P^*/\delta P)_S$ and $(\delta b_Q^*/\delta Q)_S$ are both negative and (D + X) is positive. For all phytoplankton species under most conditions db^*/dP is observed to be negative and all chemostat studies that we are aware of find dC^*/dN to be positive for pure

cultures—and thus X will be positive. It would appear that the steady state is stable.

One notes that the special case when the growth and consumption of both species is described by

$$b^* = Dw(1 - P/P_O) \quad \text{and} \quad C^* = ANDw(1 - P/P_O), \tag{6}$$

relationships that describe the growth and consumption of pure cultures of *Phaeodactylum tricornutum* in the chemostat,[1] Dw being the value of D that washed all the cells from the chemostat, P_O being the value of P_S when D was zero and A being a constant parameter regulating consumption, indeed gives negative roots for the community matrix, consistent with the preceding analysis.

It is trivial to extend this analysis to a system of n independent species of phytoplankton competing for a single limiting nutrient in a chemostat; the conclusions are little changed. We surmise, therefore, that when two (or more) phytoplankton species whose cells may have different sizes and different surface to volume ratios, compete for nitrate in a chemostat then, providing the two species grow independently, a steady state can develop. Competition for nutrient does not cause one of the species to be washed out of the chemostat. When washout occurs it must be because one of the species interacts with the other's growth. Clearly interaction will always be less pronounced at low population densities; that is, when the nutrient influx is low and when the dilution factor is relatively high.

This suggests that when the diversity of phytoplankton species in natural bodies of water is threatened by the interactions between species, then the diversity may become stabilised by such environmental changes as an increase in predation or a reduction in the influx of nutrients. These are changes that diminish the interactions and increase the independence of the species by decreasing population densities.[34–36]

Although the mathematical analysis shows independent phytoplankton species to be able to coexist in a chemostat, it has to be emphasised that in the steady state the population density of each species is precisely that which would have occurred had each species been cultured separately. Although this is possible at relatively high dilution factors when cells are unable to assimilate nutrient as fast as it impinges on their walls, it is not possible at low dilution factors when each species is able to consume nutrient as fast as it arrives in the chemostat. Clearly, the behaviour of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* in the chemostat was not that of independent species.

When interaction between species is discussed in terms of the community matrix, one uses equations (3d), (3e), and (4a) with $\delta f1/\delta N = \delta f2/\delta N = 0$ but recognises that, due to the interaction between species, $\delta b_P{}^*/\delta Q$ and $\delta b_Q{}^*/\delta P$ evaluated for the steady state are no longer zero. Under these circumstances

the community matrix has negative roots provided $(D + X)$ is positive, as before, and

$$(\delta b_P^*/\delta P)_S(\delta b_Q^*/\delta Q)_S > (\delta b_P^*/\delta Q)_S(\delta b_Q^*/\delta P)_S > 0. \tag{7}$$

Many more studies are needed to determine whether these inequalities are actually satisfied by living cultures of mixed phytoplankton species.

An interesting example of interacting species of phytoplankton occurs when growth follows the Verhuls logistic equation. As we have seen the growth of *Phaeodactylum tricornutum* in a chemostat obeys Eq. (6). It would therefore seem reasonable that when *Phaeodactylum tricornutum* interacts with a second species such as *Dunaliella tertiolecta* the equations of conservation in the chemostat would be formed from a generalised Verhuls' equation, thus,

$$dP/dt = -DP + Dwp\ P(1 - x_1P - x_2Q) \tag{3f}$$

$$dQ/dt = -DQ + Dwq\ Q(1 - y_1Q - y_2P) \tag{3g}$$

$$dN/dt = D(N_O - N) - A_1Dwp\ NP(1 - x_1P - x_2Q) - A_2Dwq\ NQ(1 - y_1Q - y_2P \tag{4b}$$

where the constants Dwp, Dwq are the washout values of D for cultures of pure P and Q, (or the maximum rates of cell division achievable per cell, Table 1) and x_1, x_2, y_1, and y_2 are parameters describing the interactions within and between the two species.

The system has a stable steady state in which both species coexist, the community matrix having three negative roots, if $(D + X)$ is positive as before and, in particular, if

$$x_1y_1 > x_2y_2. \tag{8}$$

Equation (8) is, of course, a special case of Eq. (7) showing that a stable steady state in which both species coexist is possible if the intraspecies interactions exceed the interactions between species.[37] Volterra studied symmetric interactions between the two species in which $x_1y_1 = x_2y_2$ so that the community matrix possessed a zero root inhibiting the stable coexistence of both species.

This analysis of the community matrix assumes the interaction parameters, x_1, x_2, y_1, and y_2 to be constants though they may well depend on the values of D and N_O and on the distance apart of the cells. Indeed, we have seen that the chlorophyll contents per cell changed with D and that in the second regime *Phaeodactylum tricornutum*, unlike *Dunaliella tertiolecta*, could not assimilate all the nutrient as fast as it impinged on the cells and this suggests the interaction parameters x and y may change as the cells adapt to the chemostat environment.

Simulating the Experimental Data

Figure 4 shows graphs illustrating the fitting of the *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* data to a Verhuls' logistic formalism. The figures were obtained by integrating equations (3f) and (3g) numerically.

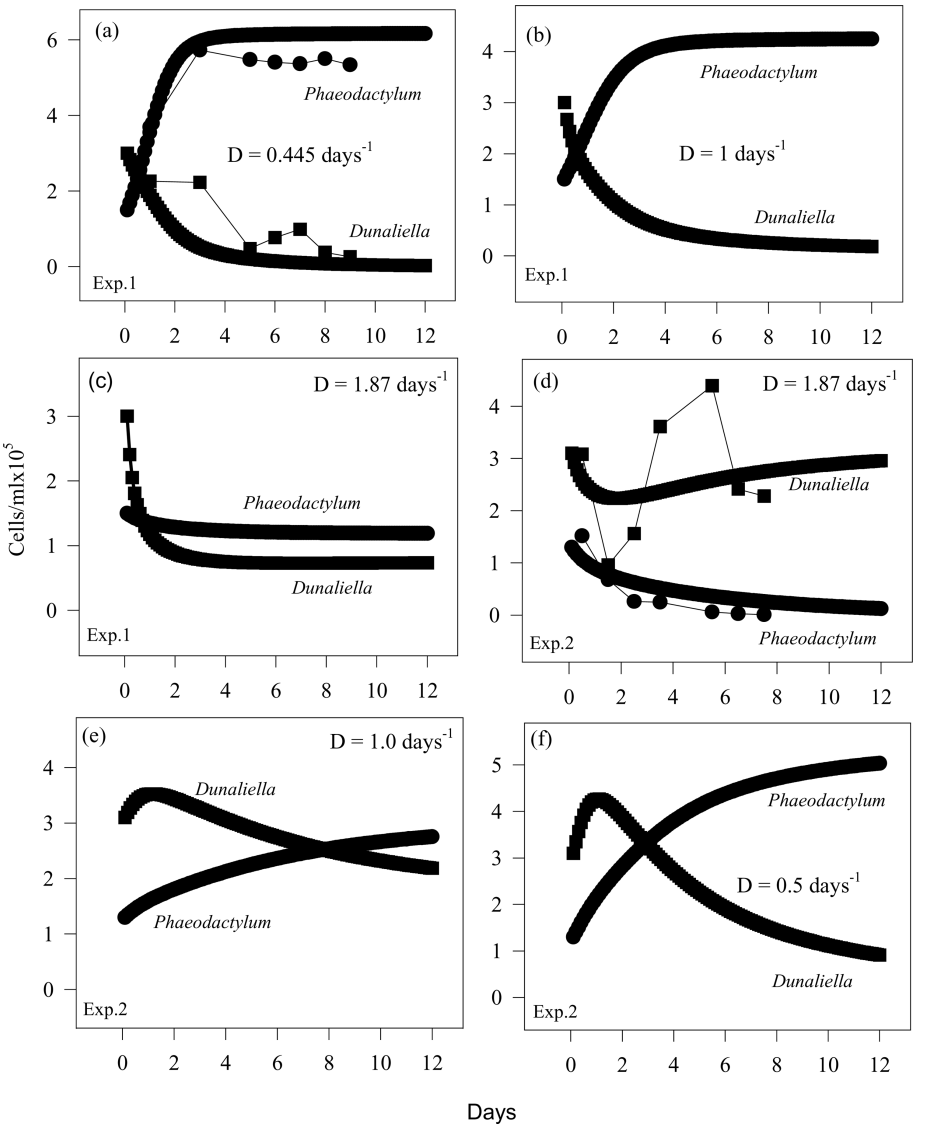

Figure 4: Simulation of the behaviour of competing populations of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* in a chemostat. Exp 1. $Po = 1.5 \times 10^5$; $Qo = 3.0 \times 10^5$; $x_1 = 1.3 \times 10^{-6}$; $y_1 = 2.7 \times 10^{-6}$; $x_2 = 0.2 \times 10^{-6}$; $y_2 = 1.5 \times 10^{-6}$; a) $D = 0.445$ days^{-1}, b) $D = 1.0$ days^{-1} and c) $D = 1.87$ days^{-1}. Exp 2. $Po = 1.3 \times 10^5$; $Qo = 3.1 \times 10^5$; $x_1 = 1.4 \times 10^{-6}$; $y_1 = 1.2 \times 10^{-6}$; $x_2 = 0.75 \times 10^{-6}$; $y_2 = 1.4 \times 10^{-6}$; d) $D = 1.87$ days^{-1}, e) $D = 1.0$ days^{-1} and f) $D = 0.5$ days^{-1}.

Write

$$P(i+1) = P(i) + dP(i)/dt\Delta t \tag{9a}$$

and

$$Q(i+1) = Q(i) + dQ(i)/dt\Delta t, \tag{9b}$$

where Δt is a small interval of time—which we have taken to be 0.1 days—and P(i) and Q(i) are the values of P and Q at time i Δt (i is an integer from 0 to about 100–120, that is iΔt becomes 10 to 12 days).

From equations (3f) and (3g)

$$P(i+1) = P(i) + DwpP(i)\ \Delta t[1 - x_1P(i) - x_2Q(i)] - DP(i)\Delta t \tag{9c}$$

and

$$Q(i+1) = Q(i) + DwqQ(i)\Delta t[1 - y_1Q(i) - y_2P(i)] - DQ(i)\Delta t \tag{9d}$$

Equations (9c) and (9d) permit simulation of the experimental observations using a spreadsheet, the parameters being chosen to give the best fit to the data. The dilution factor, D, is an experimental observation; Dwp has been taken as 2.25, the value observed in cultures of pure *Phaeodactylum tricornutum*[1]; Dwq appears to be close to 3 in all the experiments.

Appropriate values of the interaction parameters x and y and the starting values P(0) and Q(0) are given in Figure 4 as are the values of the interaction parameters which gave the best fit to the experimental results. Although the values of the interaction parameters were not the same for each experiment, x_1y_1 was always greater than x_2y_2 (Eq. (8)) so the final steady-state populations should be stable provided they were unaffected by experimental fluctuations. One sees the simulations to be reasonable though not good. They may be used to predict the effect of changes in the chemostat environment on the populations of the two species of phytoplankton. In other words the simulations facilitate prediction of the effects of weeds and of the strength of biotic resistance to invasive phytoplankton species. In fact, Figure 4 includes simulations demonstrating that when the interaction parameters x and y remain constant, the distributions of the populations of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* nevertheless become changed by alterations in the dilution factor, the population of *Dunaliella tertiolecta* becoming dominant at large dilution factors as was actually observed (Fig. 2). This confirms that it was the change in dilution factors that was primarily responsible for the change in the relative distributions of the phytoplankton species. This implies that in the field changes in the rates of predation or of sedimentation *will* change the relative populations of phytoplankton species significantly. Further studies of competing species of phytoplankton would obviously be worthwhile.

CONCLUSIONS

The diatom *Phaeodactylum tricornutum* and the microalga *Dunaliella tertiolecta* have been cultured together in a chemostat at two very different dilution factors of ~0.5 and 1.9 days^{-1}. The results are consistent with both species responding to the influx of nutrient by increasing their consumption per cell (adapting). Simultaneously, the rates of growth per cell of both species become adjusted to equal the dilution factor of the chemostat. The larger cells of *Dunaliella tertiolecta* required a larger amount of nutrient in order to divide and consequently when D-0.5 per day their population density was the lower. When D was 1.9, *Phaeodactylum tricornutum* being unable to assimilate nutrient as fast as it entered the chemostat, its population density became smaller than that of *Dunaliella tertiolecta*.

Providing two species of phytoplankton do not interact they can coexist in the chemostat in a steady state though competing for nutrient. Clearly interaction between species is minimised by reduction in population densities caused by diminution in the influx of nutrient or increase in the dilution factor (which, in the field is equivalent to an increase in the rates of sedimentation or predation). Examination of the community matrix reveals that even when the species of phytoplankton interact, both species may coexist in a stable steady state if intra species interaction exceeds the interaction between species.

Should future experiments confirm these results and show the same reasoning to be applicable to phytoplankton species in natural waters, then clearly one may explain the distribution of population densities in environmental niches. Numerical models already explain the distribution of population densities between niches in terms of rates of growth, predation, succession, disturbance (which increases extinction), and diffusion (colonisation) so as to provide the spatial distribution of niches[38] and the effects of disturbance on species diversity.[39]

The simulations of chemostat populations provided here permit prediction of the effects of phytoplankton weeds and of biotic resistance to invasive phytoplankton species.

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