

The Growth of Continuous Cultures of the Phytoplankton *Phaeodactylum Tricornutum*

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Abstract

Continuous cultures of *Phaeodactylum tricornutum* (Bohlin) have been grown in a basic chemostat, that is, in a well-stirred, continuous flow, biochemical reactor. The effects of changes in dilution rate, nutrient concentration and illumination on the growth of the cultures have been studied by monitoring cell populations, chlorophyll fluorescence and nitrate-limited nutrient concentrations in the reactor. Three types of stationary state have been observed corresponding to light limitation and to two regimes of nutrient limitation analogous to bottom-up and top-down control in field experiments. In the first nutrient-limited regime, the phytoplankton share and consume all the available nutrient, whereas in the second regime the phytoplankton are removed from the euphotic zone before consumption is complete.

Phytoplankton adapt to their environment, a process which takes a few hours when cell division is light limited but a few days when cell division is nutrient limited, partly by the modification of gene expression and partly through the preferential washout of dead cells from the chemostat.

Key words: Phytoplankton growth, continuous culture, *Phaeodactylum tricornutum*.

Introduction

Laboratory study of continuous cultures provides such a convenient method of studying phytoplankton growth and adaptation under rigorously controlled conditions that it is chastening to realise that, despite 30 years of investigation (Droop, 1968, 1974; Capeyron and Meyer, 1972; Jones *et al.*, 1978; Droop *et al.*, 1982; Grover, 1991; Geider *et al.*, 1996), less is known about the growth of phytoplankton in a chemostat than about the growth of bacteria (Bailey and Ollis, 1986; Grigorova and Norris, 1990; Blanch and Clark, 1996). In this investigation of phyto-

plankton growth, undertaken as part of a study of ecotoxicology in coastal waters (Okay *et al.*, 1996), a diatom, *Phaeodactylum tricornutum*, has been cultured in a chemostat at a constant temperature. The flow rate, U (or the corresponding dilution factor, D), the concentration, N_O , of the nutrient in the inflow and the intensity of illumination have been varied systematically. The observed steady states have been characterised by measurements of the concentration of nutrient, N_S , in the outflow and of the population densities of the cells, P_S , and of their chlorophyll fluorescence.

Theoretical background

Suppose the continuous cultures of phytoplankton to be homogeneous (well-stirred), free of bacteria, contained at a constant temperature and a constant illumination in a basic chemostat of volume V and fed nutrient with a volume flow of U , nutrient and phytoplankton being simultaneously removed at an identical volume flow; then the equations of conservation of phytoplankton population density, P (number of cells per unit volume) expressed in terms of the rate of change of P with respect to time, t , are

$$VdP\Phi/dt = -UP\Phi + bP\Phi V - mP\Phi V \quad (1a)$$

for live phytoplankton,

$$= -UP\Phi + gP\Phi V \quad (1b)$$

and

$$VdP(1 - \Phi)/dt = -UP(1 - \Phi) + mP\Phi V \quad (1c)$$

for dead phytoplankton,

addition giving,

$$VdP/dt = -UP + bVP\Phi \quad (1d)$$

$$= -UP + b^*VP. \quad (1e)$$

Φ is that fraction of P which is alive and 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. The first term on the right hand side of Equations (1) is the rate at which phytoplankton is removed from the chemostat. These equations make no assumptions about the behaviour of either b , m or g but they neglect 'remineralisation', the conversion of dead phytoplankton cells into minerals, a process which, in the absence of bacteria, may be considered slow.

It is customary and convenient to divide Equations (1) by V whence,

$$dP\Phi/dt = -DP\Phi + gP\Phi \quad (2a)$$

$$dP(1 - \Phi)/dt = -DP(1 - \Phi) + mP\Phi \quad (2b)$$

$$dP/dt = -DP + b^*P \quad (2c)$$

where $D = U/V$ is often termed the 'dilution factor'.

If b^* is a constant, integration of (2c) gives

$$\ln P = (b^* - D)t + \text{constant}, \quad (3)$$

the constant of integration being the value of $\ln P$ when t is zero. P increases or decreases exponentially with time and there is no interesting stationary state. Experiments reveal a stationary state, characterised by stable, constant values of both P and of the concentration of nutrient in the chemostat, N , to exist for each value of D up to an upper limit D_W , termed 'washout', when the high rate of flow leaves no phytoplankton in the chemostat. Hence b^* cannot be constant but must vary with time – the phytoplankton adapt or become adapted – until $b^* = D$ when a stationary state occurs.

When the cultures are in a stationary state neither P nor Φ varies with time and Equations (2) yield

$$b_S^* = g_S = D, \quad (4a)$$

(the suffix, S , indicating a stationary state value)

$$\text{and } \Phi_S = b_S^*/b_S = g_S^*/b_S^* = g_S/b_S \quad (4b)$$

Furthermore, in this stationary state $1/D$ is the residence time of the phytoplankton cells in the chemostat.

The equation of conservation of a single limiting nutrient is

$$VdN/dt = U(N_O - N) - CP\Phi V \quad (5a)$$

$$\text{or } dN/dt = D(N_O - N) - C^*P \quad (5b)$$

where N and N_O are the concentrations of limiting nutrient in the vessel and in the inflow, respectively, and C is the rate of consumption of N per single living cell and C^* is the observed rate of consumption per cell whether living or dead. Equations (5) continue to neglect 'remineralisation' of dead phytoplankton.

In a stationary state not only will P and Φ be constants, so also will N

$$\text{and } C_S^* = D(N_O - N_S)/P_S \quad (6)$$

The parameters that may be observed in studies of continuous cultures of phytoplankton in stationary states are summarised in Table 1.

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

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	=	phytoplankton population density
	=	flux density of phytoplankton issuing from the chemostat (number per unit volume)
DP_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)
$(N_O - N_S)/P_S$	=	flux of consumption per cell (moles per cell)
	=	C_S^*/b_S^* (ratio of the rates of cellular consumption to division)

Materials and Methods

All cultures were monitored in a room possessing stabilised electricity and a constant temperature, 20 ± 1 °C. The room was small, with matt, white walls and illuminated by white, fluorescent strip-lighting from the ceiling; in addition four such lamps were mounted horizontally 1 m above the reactor. Illuminance was measured by a hand-held digital lightmeter DLM2. Illumination was continuous throughout the day and night.

Samples of healthy, stable cultures of *Phaeodactylum tricornerutum* were injected into sterile, transparent, roughly spherical, glass reaction vessels having a vertical inlet tube entering from the top and an outlet tube emerging horizontally. All the glassware, pumping tubes etc. were autoclaved to prevent bacterial growth. Continuous stirring by Teflon-coated magnets, in addition to a vigorous daily manual stir, ensured cultures remained homogeneous, that adsorption on the reactor walls was minimal and all phytoplankton cells enjoyed the same intensity of illumination. Preliminary experiments showed small changes in the rate of stirring to have no effect on the behaviour of the cultures.

Sterile, $f/2$ culture medium (Guillard and Rytter, 1962) was fed continuously into the reactor inlet at a rate carefully controlled by a peristaltic pump. The concentrations of the constituents were modified (Okay *et al.*, 1994) so that the medium was 'nitrate limiting'. Unconsumed culture medium, together with phytoplankton, flowed continuously from the outlet from where it was sampled once or twice a day. Two reactors having volumes of 560 ml and 3 l could be operated independently. Nutrient concen-

trations in the outflow were determined by a Technicon Autoanalyser II according to standard methods modified for continuous sea water analysis (Technicon Industrial Methods, 1977 a and b). Phytoplankton cells were counted through a microscope. The chlorophyll fluorescence (FI) (Ex = 430 nm; Em = 663 nm) of the phytoplankton suspended in the outflow was monitored by a Shimadzu Model RF 540 fluorescence spectrophotometer. Rates of liquid flow were determined daily with a measuring cylinder and stop-clock.

Experiments consisted of continuously monitoring the phytoplankton cultures in the reactor, usually over a period of a month, and observing the changes which accompanied the deliberate variation of the environmental parameters. In the first standard experiments a viable population of a batch culture of *Phaeodactylum tricornerutum* was introduced into the stirred reaction vessel and fed nitrate-limited nutrients at a constant rate, at a constant temperature, 20 ± 1 °C, and under constant illuminance, 4000 lux. Some days after the culture had started growing, a steady state was attained. Thereafter the rate of flow of nutrients, and thus the dilution rate, was systematically increased – or decreased – although the concentration of nutrients in the inflow, N_O , was maintained constant. Each time the dilution rate was changed the phytoplankton culture adjusted its consumption and growth and a new stationary state developed. A stationary state has been defined experimentally by the observation of a constant nutrient concentration and phytoplankton population in the reactor for a duration of at least 2 days. Preliminary experiments confirmed the results to be independent of the volume of the reactor (when this

was varied by a factor of five). This was in conformity with Equations (1) and (2) and it confirmed that processes at the surface of the chemostat had no significant effects.

Results and Discussion

The effect of dilution rate on phytoplankton population density

Figures 1-5 illustrate the effects of stepwise change in the dilution rate on the stationary state functions listed in Table 1. The same graphs were obtained, within experimental error, whether the dilution rate experienced stepwise increase or stepwise decrease. One could proceed reversibly from one stationary state to another. Any stationary state plotted in P_S, N_S space is accessible from any other and this constrains simulations of phytoplankton growth to models which give a community matrix having real negative roots.

Figure 1 shows the steady, essentially linear, decrease in the stationary phytoplankton population that was observed as the dilution rate was increased. The Figure displays the results of dilution rate experiments under the same standard conditions but with two different concentrations, N_O , of nitrate limited nutrient in the inflow differing by a factor of three.

Unsurprisingly, the population densities were higher at the higher value of N_O – increasing the food supply increases growth. Within experimental error, complete washout of the phytoplankton population occurred at the same rate of dilution, $D \sim 2.25$, at both values of N_O and the equation

$$\begin{aligned} P_S/P_{SO} &= 1 - D/D_W; \\ D/D_W &= 1 - P_S/P_{SO} \end{aligned} \quad (7)$$

describes both graphs, P_{SO} , the value of P_S when $D = 0$, being the maximum population density which the nutrient concentration, N_O , can support. If one supposes $P_{SO} = 0$ when $N_O \sim 0$, then P_{SO} was approximately proportional to N_O over the range we have examined. Pragmatically D_W gives the highest rate of division of which the cells are capable and this is apparently independent of N_O over the three-fold range we have considered. These relationships should be explored further. Dilution factors causing ‘washout’ being the maximum rates of cell division, it would be interesting to compare these with the rates of growth of batch cultures of phytoplankton in the log phase.

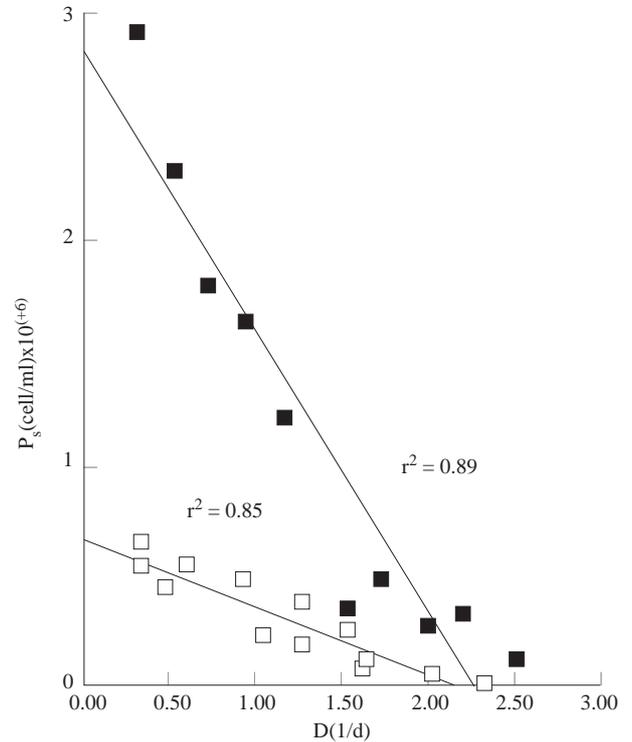


Figure 1. Change in stationary state population densities of phytoplankton with dilution factor.

Y-axis: P_S , (cells $\times 10^6$ /ml); X-axis: D , (per day).

Upper graph, $N_O = 1500 \mu\text{g/l}$; lower graph, $N_O = 500 \mu\text{g/l}$.

Standard deviation of an individual result: Upper graph, 2.75×10^5 cell/ml; lower graph, 7.5×10^4 cell/ml.

Figure 2 shows the total rate of cell division of the culture per unit volume, DP_S , as a function of D . P_S decreasing linearly with increase in D (Equation (7)), DP_S is a quadratic function of D , in fact it is a parabola having its maximum value when D is essentially $0.5 D_W$, the value predicted using Equation (7).

How phytoplankton cell division is adapted by and adapts to the chemostat

Phytoplankton cells growing in a stationary state are cells which have adapted – or have been adapted – to their environment. Adaptation from one stationary state characterised by a dilution factor and an input concentration to another always took from 1 to 5 days, while it took longer for the diatom *Phaeodactylum tricornutum* than for the green alga *Dunaliella*

tertiolecta (Butcher) and appeared sensitive to the presence of pollutants (Okay and Gaines, 1996).

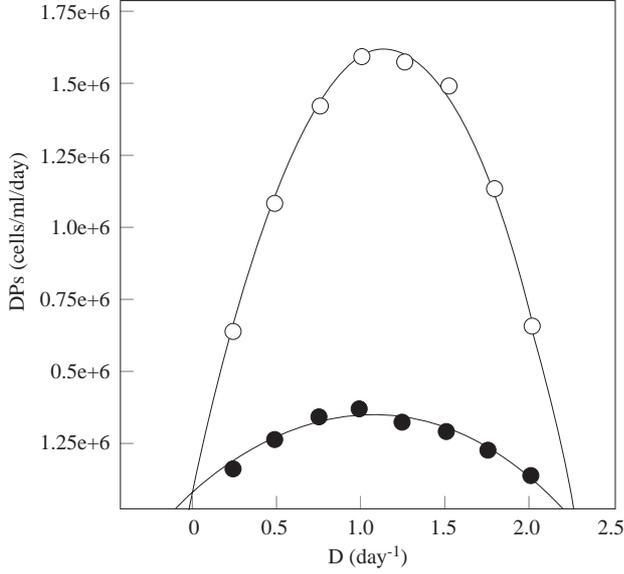


Figure 2. Change in total rate of cell division in the stationary state with dilution factor.

Y-axis: DP_S , (cells $\gg 10^6$ /ml/day); X-axis: D , (per day).

Upper graph, $N_O = 1500 \mu\text{g/l}$; lower graph, $N_O = 500 \mu\text{g/l}$.

From Equations (4) one obtains $\Phi_S = D/b_S$. Suppose b_S to be constant. When D is small most of the phytoplankton cells in the stationary state are dead. As D increases, dead cells must be removed preferentially from the chemostat and in this way both the fraction of cells which are live and b_S^* increase; the phytoplankton culture is adapted by the chemostat. Equation (7) yields

$$P_S = P_{SO}(1 - b_S\Phi_S/D_W) \quad (8)$$

(if $b_S = D_w$, the maximum value of b_S^* , then $P_S = P_{SO}(1 - \Phi_S)$ and $\Phi_S = 1$ when $D = D_W$)

and one sees that changing D changes the population density of the phytoplankton cells in the stationary state because of the alteration in Φ . However, b_S may well vary with D .

Thus,

- increasing D decreases the residence time and therefore the maximum age of the phytoplankton cells in the stationary state. Young and old cells are not expected to divide at the same rate.

- changes in D may change the amount of nutrient assimilated by the phytoplankton and consequently alter the rate of cell division.

Rates of consumption

Figure 3 shows the variation in the concentrations of the limiting nutrient, nitrate, in the chemostat as the dilution factor, D , was changed systematically. These results are remarkable. Providing D was less than about 1 there was very little nitrate to be observed in the chemostat. The phytoplankton cells were consuming the nitrate as fast as it entered the chemostat. We have maintained stable, healthy phytoplankton cultures for as long as a month in the apparent absence of significant nitrate concentration in the chemostat. Presumably the way the phytoplankton cells were behaving in the chemostat corresponds to the behaviour of well-illuminated phytoplankton in the open sea supplied comparatively slowly with nutrient (Chisholm, 1992; Reynolds, 1994). To take examples well known to us, this appears to be the behaviour of phytoplankton in the surface waters inside the Rim Current of the Black Sea and at the Rhodes Gyre in the eastern Mediterranean, observed nutrient concentrations being low at both locations. One should distinguish between the rate at which food is supplied (by rivers, from the atmosphere or by upwelling in field experiments and defined precisely by UN_O or DN_O in chemostat experiments) and the concentration of nutrient in the surrounding medium. Increasing the food supply, N_O , naturally increases the phytoplankton population density, P (Figure 1), but a change in N may not imply a change in either N_O or P .

When the dilution factor, D , exceeded about 1.5, the steady state values, N_S , of the nutrient concentration in the chemostat increased abruptly, reaching the value of N_O at washout. In this second, nutrient-limited regime, although the cells were dividing rapidly ($b_S^* = D$), there were insufficient live cells to consume all the available nutrient in the relatively short residence times available. Equation (6) enables this to be expressed quantitatively. The concentration of nutrient consumed in the stationary state, $(N_O - N_S)$, is equal to $C_S^*P_S/D$ ($1/D$ being the residence time for consumption). All the phytoplankton cells share all the available nutrient and, throughout the first regime, as P_S diminishes C_S^* increases steadily to make this possible. As the increase in D shifts the stationary states to the second

regime, C_S^* increases still further (Figure 5) but, despite this adaptation, the living cells are no longer capable of consuming all nutrient as fast as it arrives at the cell's surface; the product $C_S^*P_S/D$ becomes increasingly insufficient to consume all the available nutrient.

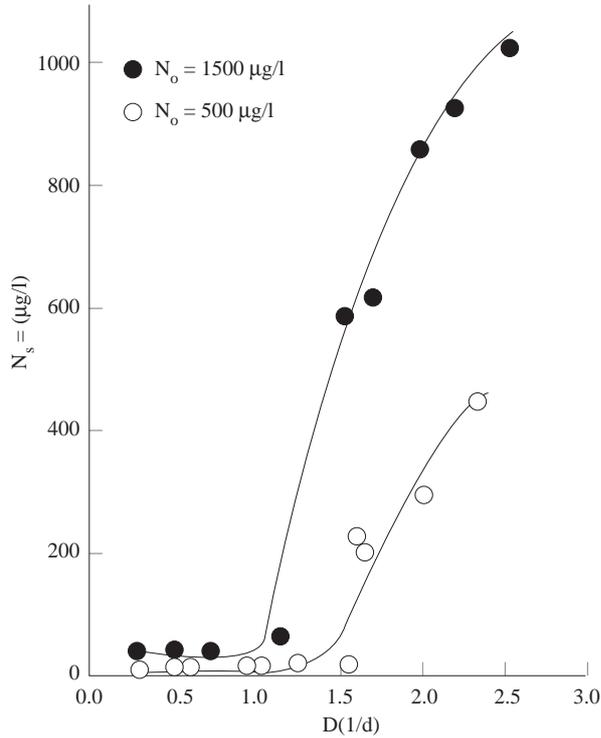


Figure 3. Change in stationary state nitrate concentration in the outflow with dilution factor. Y-axis: N_s , ($\mu\text{g/l}$); X-axis: D , (per day). Upper graph, $N_o = 1500 \mu\text{g/l}$; lower graph, $N_o = 500 \mu\text{g/l}$. Standard deviation of an individual result: Upper graph, $D < 1$ $13 \mu\text{g/l}$, $D > 1$ $60 \mu\text{g/l}$; Lower graph $D < 1.5$ $5 \mu\text{g/l}$, $D > 1.5$ $77 \mu\text{g/l}$.

Figure 3 shows a threefold change in the concentration of nutrient in the inflow, N_o , did little to change the distinction between the two regimes of phytoplankton growth. For example, when a concentration of $1500 \mu\text{g/l}$ of nitrate at $D = 1.8$ was diminished to a concentration of $500 \mu\text{g/l}$ of nitrate (also at $D = 1.8$) the rate of consumption remained in the second regime even though the reduced flux was equivalent to $1500 \mu\text{g/l}$ of nitrate at $D = 0.6$, which gave a steady state in the first regime. Similarly, increase of a flux comprised of $500 \mu\text{g/l}$ of nitrate at $D = 0.6$ to a flux of $1500 \mu\text{g/l}$ also at $D = 0.6$ (equivalent to a flux of $500 \mu\text{g/l}$ of nitrate at $D =$

1.8) did not produce a change to the second regime. Changes in N_o did not produce the same effects as changing D .

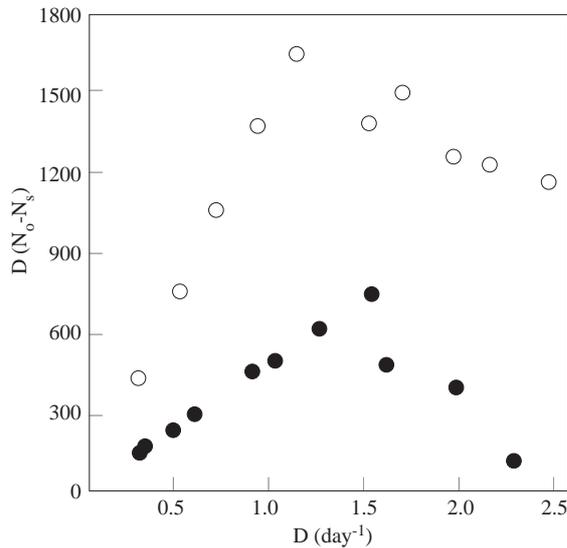
In the second regime, the ratio of nitrogen to phosphorus consumed was significantly higher ($p < 0.05$) than in the first regime (Table 2). Since in the first regime all the nitrate was consumed as fast as it entered the reactor, this indicates that at the higher dilution factors assimilation of phosphorus was slower than assimilation of nitrate. (Since the nutrient was nitrate limited, no nitrate: phosphate ratios approached the Redfield value.)

The correspondence between the second regime observed in the chemostat, ($D > 1.5$, $0 < N < N_o$), when residence times were too small for the relatively sparse populations of phytoplankton to consume all the nutrient available in the chemostat, and phytoplankton behaviour in the field is subtle and important. In a calm ocean the phytoplankton are removed from the euphotic zone not by being washed out as in the chemostat but either by sedimentation or by being consumed by predators. Settling velocities of phytoplankton in the ocean vary from 1 to 200 m per day (Jannasch *et al.*, 1996) and, if the behaviour of *Phaeodactylum tricoratum* in the chemostat is representative of that of other species of phytoplankton, then, when the ratio of the settling velocity of the phytoplankton to the thickness of the euphotic zone exceeds about 1.5, phytoplankton may well have insufficient time to consume all the available nutrient. Again, whenever predation is so strong that the residence time of the phytoplankton in the euphotic zone is less than a day, the cells may be unable to consume all the available nutrient. An obvious example of this effect of predation occurs at the end of a phytoplankton bloom. Should future work show most phytoplankton species to behave similarly to *Phaeodactylum tricoratum*, then the presence of significant concentrations of unconsumed nutrient in a well illuminated euphotic zone will be a significant indicator of strong predation. The behaviour of phytoplankton in the chemostat is the behaviour of phytoplankton experiencing a constant rate of predation. In the terminology of those who model populations in the oceans, the first regime in the chemostat when all the nutrient is consumed as fast as it reaches the phytoplankton surface corresponds to 'bottom-up' control, whereas the second regime with limited residence times corresponds to 'top-down' control.

Table 2. Average N/P ratios of consumed nutrient.

N/P ratio supplied* (N_O/P_O , $\mu\text{g/l}/\mu\text{g/l}$)	$D < 1$ per day	$D > 1$ per day
500/1000	1.18 ± 0.26	2.97 ± 0.02
1500/1000	1.82 ± 0.22	3.14 ± 1.10

*Nitrate limiting nutrient was supplied in all the experiments; phosphate was always present.


Figure 4. Change in rate of consumption of nitrate in the stationary state with dilution factor.

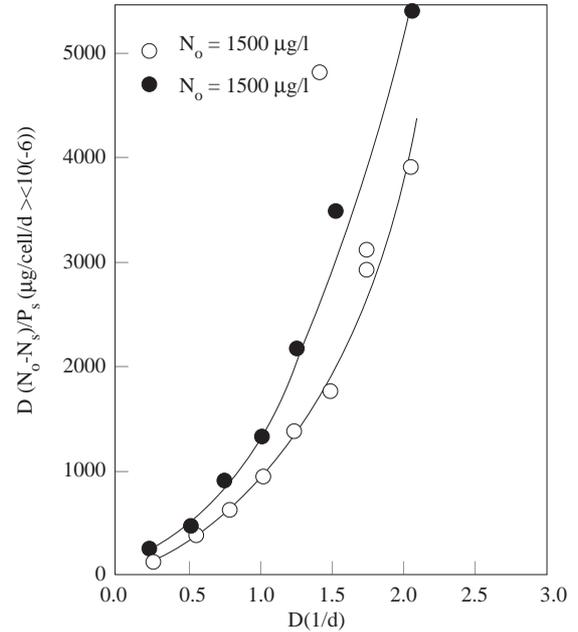
Y-axis: $D(N_O - N_S)$, ($\mu\text{g/l/d}$); X-axis: D , (per day).

Upper graph, $N_O = 1500 \mu\text{g/l}$; lower graph, $N_O = 500 \mu\text{g/l}$.

Standard deviation of an individual result: Upper graph $D < 1$ $25 \mu\text{g/l/d}$, $D > 1$ $60 \mu\text{g/l/d}$; lower graph $D < 1.5$ $8 \mu\text{g/l/d}$, $D > 1.5$ $100 \mu\text{g/l/d}$.

Figures 4 and 5 show the rate of consumption in the stationary state, $D(N_O - N_S)$, and the rate of consumption per cell, $D(N_O - N_S)/P_S (=C_S^*)$, as functions of the dilution factor, D . As we have already discussed, the rate of consumption per cell, C_S^* , shown in Figure 5 increases markedly with D . The rate was generally larger at the lower value of N_O when there were fewer phytoplankton cells to consume the available nutrient (Figure 5). The increase in C_S^* with D in both regimes suggests a definition of adaptation; cells adapt to or are adapted by changes in environmental parameters so as to minimise the concentration of nutrient in the surrounding medium. The minimisation of the concentration

of nutrient in the medium is presumably a consequence of the minimisation of the free energy of the chemostat system. The cells in the second regime have average residence times of less than a day in the chemostat and are thus younger than the cells in the first regime. One observes the chlorophyll-a fluorescence per cell to have been larger, $12 \gg 10^{-6}$, in the second, and smaller, $4 \gg 10^{-6}$, in the first regime. Thus the younger cells of *Phaeodactylum tri-cornutum* were better adapted to the second regime by their increased ability to photosynthesise and thereby consume more nutrient per cell.


Figure 5. Change in rate of consumption per cell in the stationary state with dilution factor.

Y-axis: $D(N_O - N_S)/P_S$ ($\mu\text{g/cell/d} \gg 10^6/\text{d}$); X-axis: D (per day).

Upper graph $N_O = 1500 \mu\text{g/l}$; Lower graph $N_O = 500 \mu\text{g/l}$.

Standard deviation of an individual result: Both graphs $145 \gg 10^6 \mu\text{g/cell/d}$.

The effect of light intensity

Figures 6a and 6b show how a sequence of stationary states was generated when the intensity of light in the chemostat was diminished but the dilution factor and the inflows of nitrate and phosphate were maintained constant at $1500 \mu\text{g/l}$ and $1000 \mu\text{g/l}$ per day respectively. The dilution factor was chosen so that under full illumination the cells were just able to consume all the available nutrient. When the illuminance was first diminished the population den-

sity, P_S , of the continuous culture fell abruptly to around 10^6 per ml but subsequently declined slowly to about half this value at 610 lux (Figure 6a). The changes in nutrient concentration in the chemostat were more dramatic. Starting from an illuminance of 3285 lux, a sequence of stationary states was generated in which all the nutrient was consumed as fast

as it entered the reactor. It was not until the illuminance was reduced to 2855 lux that the cells were no longer able to assimilate all the phosphate (Table 3). Complete assimilation of nitrate ceased when the illuminance was further reduced to 2160 lux (Figure 6a) and thereafter rates of consumption were light

Table 3. The effect of light intensity on consumption.

Illuminance	A	B	C	D	E	F
Nitrate consumption:						
$(N_O - N_S)$	1500	1500	1500	950	850	600
$(N_O - N_S)/P_S \gg 10^6 (=C^*/b^*)$	430	1500	1500	1188	1133	860
Phosphate consumption						
$(P_O - P_S)$	985	985	850	525	550	425
$(P_S - P_S)/P_S \gg 10^6 (=C^*/b^*)$	280	985	985	656	735	610

P = Phosphate in nutrient ($\mu\text{g l}^{-1}$)

A = 3285 lux; B = 2855 lux; C = 2160 lux; D = 1518 lux;

E = 610 lux; F = 425 lux.

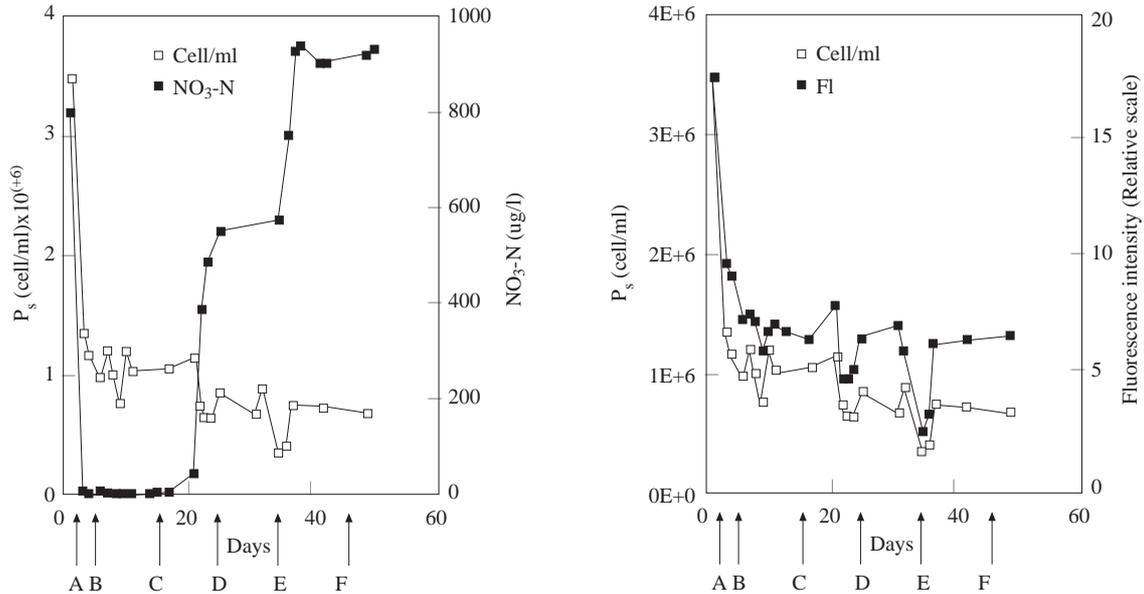


Figure 6. The effect of light intensity.

a. Variation in stationary state phytoplankton population densities and nitrate concentrations in the outflow as a function of illuminance.

Y-axes: (Left) P_S , (cell $\gg 10^6$ /ml), (Right) $\text{NO}_3\text{-N}$ ($\mu\text{g/l}$)

X-axis: days.

Standard deviations of an individual result: $2.5 \gg 10^5$ cell per ml and (for significant nitrate concentrations) $60 \mu\text{g/l}$.

b. Variation in stationary state phytoplankton population densities, P , and chlorophyll fluorescence per cell as a function of illuminance.

Y-axes: (Left) P_S , (Right) Fluorescence intensity (Relative scale)

A: 3285 lux, B: 2855 lux, C: 2160, D: 1518 lux, E: 610 lux, F: 425 lux.

$N_O = 1500 \mu\text{g/l}$.

controlled. Figures 6 and Table 3 show that the rates of consumption per cell, C_S^* ($= D(N_O - N_S)/P_S$), now decreased sharply.

The change from nutrient to light limitation was accompanied by a steady increase in the chlorophyll fluorescence per cell (Figure 6b) similar to the increase in chlorophyll content observed in phytoplankton as one proceeds from the top to the bottom of the euphotic zone in the open ocean. Clearly the cells adapted to minimise the reduction in nutrient consumption evoked by the diminution in illuminance. There was little change in the ratio of nitrate to phosphate consumed, the ratio being 1.5 to 1.7 throughout the experiment.

It has already been noted in the Rates of consumption section that the chlorophyll fluorescence per cell increased in the second regime when the dilution factor was increased. The chemostat observations that the chlorophyll fluorescence per cell changes both with changes in light intensity and with changes in dilution factor, the other parameters being held constant in each situation, are significant since comparable field observations can be complicated by changes at the top and bottom of an euphotic zone not only in illuminance but also in nutrient supply and in temperature.

Unlike the adaptation to changes in the dilution factor, which occupied several days, adaptation to each change in light intensity took only a few hours. The rapid adaptation of the phytoplankton cells to changes in light intensity recalls the rapid adaptation of the Olive gene observed in *Antirrhinum majus*, which shows extensive homology to the bchH gene in purple photosynthetic bacteria (Hudson *et al.*, 1993). These genes control the insertion of magnesium atoms into chlorophyll molecules during their biosynthesis and gene expression is inhibited by light. Homologous genes have been identified in both photosynthetic bacteria and in wild plants and so one would expect future work to discern homologous genes in phytoplankton; such observations would clearly provide a mechanism for the adaptation of phytoplankton cells to changes in illuminance.

Simulation of cell behaviour

We proceed pragmatically. Experimentally, we have found that, at least for *Phaeodactylum tricornerutum*,

$$b_S^* = D = D_W(1 - P_S/P_{SO}), \quad (7)$$

$$\text{which suggests } b^* = D_W(1 - P/P_{SO}), \quad (9)$$

Whence, from Equations (2),

$$dP/dt = -DP + D_W P(1 - P/P_{SO}) \quad (10)$$

the rate of cell division being given by a logistic (Verhulst's) equation. Integration of Equation (10) yields

$$P = j/(k + Ke^{-jt}) \quad (11)$$

where $j = (D_W - D)$ and $k = D_W/P_{SO}$ and the constant, K , satisfies the value of the left-hand side of (11) when t is zero. Equation (11) shows how P changes from one steady stationary state value to another if the value of the dilution factor, D , is changed. We have monitored changes in P consistent with Equation (11) but the stepwise changes in D we employed were always small (as our Figures show) and the test of the equation was not stringent. Equations (9) and (11) show how the rate of cell division changes with time.

The Rates of consumption section has shown how in the first regime the phytoplankton cells adapted to share and consume all the available nutrient. As D was increased phytoplankton cultures in the second regime continued to adapt by increasing the consumption per cell, C^* , in a vain attempt to consume all the available nutrient. One is unable to furnish a single analytical expression for the rates of consumption in both regimes since in the first regime nutrient is consumed at the rate it arrives at the cell but in the second regime rates of consumption correspond to the slow step in the mechanism by which nutrient is assimilated. The adaptation of the cells in the second regime was a response to the nutrient concentration in the chemostat. At least to the extent that consumption provides the nutrient needed for cell division, one expects C^* to be proportional to the rate of cell division and thus Equation 5b may be written

$$dN/dt = D(N_O - N) - AD_w NP(1 - P/P_{SO}) \quad (12)$$

Equations (10) and (12) are satisfactory in that they yield a community matrix with two real, negative roots; in other words, consistent with the experiments, one can pass reversibly from one stationary value of P and N to another by changing the dilution factor, D .

While Equation (12) – and pragmatic extensions of the equation – gives a good straight line plot

(not shown) generating reasonable values for the stationary state nutrient concentrations, N_S , observed in the second regime one finds that a Monod or Michaelis-Menton formulation does not describe the consumption of nutrient by *Phaeodactylum tricornerutum*.

Conclusions

This description of the behaviour of phytoplankton cells in a well-stirred, continuous flow, biochemical reactor furnishes considerable understanding of the growth of *Phaeodactylum tricornerutum* fed nitrate-limited nutrient and suffering steady predation. Stationary states are formed for each value of the dilution factor from zero to ‘washout’.

1. When the motion of the surrounding aqueous medium is gentle – corresponding to dilution factors of less than 1 in our experiments – and given sufficient light, the phytoplankton adapt to consume essentially all the limiting nutrient as fast as it is supplied. Adaptation takes a few days and appears to take longer for diatoms than for green algae. This regime is analogous to ‘bottom-up’ control observed in the field.
2. The rate of cell division follows a logistic equation governed by the maximum population density the incoming nutrient can sustain and by the maximum rate of division that can be achieved. The latter is equal to the rate of dilution at washout.
3. When the dilution factor is increased (which is equivalent in the field to an increase in either the rate of predation or the rate of settling of phytoplankton) the regime changes abruptly and the decreased phytoplankton population has insufficient time to consume the nutrient as fast as it is supplied. This regime is characterised by relatively high concentrations of nutrient remaining in the surrounding water, by higher ratios of nitrogen to phosphorus uptake (when the nutrient is nitrate limited) and by higher chlorophyll-a fluorescence per cell; it

is analogous to top-down control observed in natural environments.

4. Light limited growth, when the phytoplankton cells have insufficient energy to consume all the available nutrient, has similar characteristics to the second, top-down regime just described but differs in that the adaptation of phytoplankton is much more rapid. These conclusions are summarised in Table 4.
5. In all regimes the *Phaeodactylum tricornerutum* cultures are adapted by the chemostat by the preferential washout of dead cells. The cultures themselves adapt to changes in their environment in the chemostat by maximising the rate of consumption of nutrient per cell. Future research needs to address the biochemical mechanisms by which this adaptation occurs as a function of gene expression.

Not only have we been guided by the considerable corpus of previous work, there are many factors which we have failed to consider, notably the consumption of ammonia rather than of nitrate, competition between phytoplankton species, the interaction of phytoplankton with bacteria and the specific changes in the protein, carbohydrate, lipid and DNA/RNA composition of the phytoplankton cells.

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Table 4. Three phytoplankton growth regimes.

Status	Nutrient	limitation	Light limitation
Control	Bottom-up	top-down	
Illuminance	strong	strong	weak
Conc. of nutr. in surrounding water	low	relatively high	relatively high
Chlorophyll fluorescence per cell	normal	high	high
Time taken to adapt to environmental change	days	days	hours

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