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A simple model of the growth of phytoplankton populations in light/dark cycles

Daniel Vaulot[1] and Sallie W.Chisholm[2]

Ralph M.Parsons Laboratory 48-425, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[1]*Present address: Station Biologique Roscoff, CNRS, Roscoff 29211, France*
[2]*To whom requests for reprints should be sent*

Abstract. Phytoplankton populations have been shown to be entrained by alternating periods of light and darkness in natural waters as well as in laboratory cultures. A simple model for the growth of such populations, as reflected by cell division, is presented here. The model takes as its structural unit the single cell, using Spudich and Sager's transition point hypothesis for the coupling between received light and cell cycle progression. A stochastic component is also included to account for cell-to-cell variability. The model predicts that the characteristics of cell division patterns in populations entrained by photocycles depends mainly on the position of the transition point within the cell cycle, rather than on the characteristics of the photocyclic regime. The model simulates successfully the major features of observed division patterns of several phytoplankton species. In addition, the model can be used to predict division patterns in high frequency photocycles and during transients induced by shifts in light regime. Under these conditions, the simulated patterns are also consistent with the hypothesis of a circadian clock controlled cell cycle, except in the case of free running transients.

Introduction

Light/dark cycles are known to synchronize or phase cell division in phytoplankton populations in cultures and *in situ*. When average population growth rates are less than or equal to one doubling per day, most species (with the exception of diatoms) undergo mitosis and cytokinesis during a defined interval relative to the light/dark cycle. This interval typically corresponds with the dark period. When populations are growing faster than one doubling per day, two division bursts are usually seen during each 24 h period (Chisholm, 1981; Chisholm *et al.*, 1984). In diatoms grown on typical 24 h photocycles, cell division is not restricted to a certain time interval regardless of the average population growth rate. Dividing cells are observed throughout the light/dark cycle, although entrained populations do display 24 h periodicities in the specific rate at which division occurs (Chisholm and Costello, 1980).

One hypothesis invoked to interpret the periodic responses of cell populations to periodic forcing variables makes reference to an endogenous oscillator (the circadian clock), which synchronizes cell division in the population by restricting mitosis to a specific phase of the entraining photocycle. The existence of such an oscillator is well established in several genera such as *Euglena, Gonyaulax, Chlamydomonas* and *Pyrocystis*. Its control is not limited to cell division, but extends to other physiological functions, such as bioluminescence, photosynthetic capacity and motility (Edmunds, 1966; Sweeney and Hastings, 1958; Bruce, 1972; Sweeney, 1981; Lonergan, 1984). Although the 'clock coupling' hypothesis can explain intuitively the division patterns in populations doubling less than or exactly once per day, it cannot be invoked easily for faster

growing populations. Moreover, it cannot interpret the diatom division patterns under any conditions, since this group of organisms does not display a restricted division gate (Chisholm *et al.*, 1980).

Spudich and Sager (1980) presented experimental evidence for an alternative mechanism of photocycle entrainment in *Chlamydomonas* which does not invoke coupling to an endogenous clock (see also Bernstein, 1964; Heath and Spencer, 1985; Donnan *et al.*, 1985; McAteer *et al.*, 1985). They hypothesized that only a limited segment of the cell cycle, bounded by two points A and T (for arrest and transition respectively), is light dependent (Figure 1).

In the present paper Spudich and Sager's hypothesis is incorporated into a model of population growth designed to predict population behavior under different photocyclic regimens, and its validity is tested against experimental data. The approach used is an extension of a preliminary work by Slocum (1980) (also discussed in Chisholm *et al.*, 1980).

Model description and formalism

Cell cycle model

In the classical image of the cell cycle (Figure 1), the phases S and M correspond to well defined events (DNA replication and mitosis respectively), whereas G_1 and G_2 are not associated wtih any precise physiological or biochemical processes (Prescott, 1976). A primary concern for the model formulation is to be able to define the position of a cell in this cycle. This would be relatively simple under constant environmental conditions, since all cells would experience exactly the same environment and it would then be possible to establish a one-to-one relationship between the age of a cell and its position in the cycle. In spatially and temporally varying environments, however, cells of the same age may be located at widely different positions in their cycles because they have different past histories. This type of heterogeneity among cells of the same age is called external variability, because it is a direct consequence of external (environmental) conditions.

In order to describe the state of a cell, we define a maturity vector **M** having the elements $m_1,\ldots,m_p$, which multidimensionalizes the concept introduced by Rubinow (1968). The element m_i quantifies any cellular chracteristic that monitors or regulates

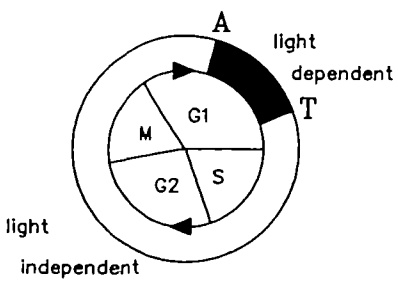


Fig. 1. The light dependent cell cycle according to Spudich and Sager (1980). Point A marks the beginning of the light dependent segment of the cell cycle. Cells between A and T are arrested in the dark, but those which have passed the transition point T can proceed through their cycle independent of light (see text).

the progression of a cell through its cycle such as age, size (Lord and Wheals, 1981), protein (Puiseux-Dao, 1981), mitogen (Cooper, 1982), etc. The specification of **M** indicates the absolute position of the cell in its cycle, and the maturation velocity vector **U**, with elements $u_1,\ldots,u_p$, is equal to the rate of increase of the maturity vector experienced by a cell (i.e. $d\mathbf{M}/dt$). **U** may be dependent on both the maturity vector **M** and the external conditions experienced by the cell.

All parts of the cell cycle are not equally affected by external conditions. In most eukaryotic cells progression through G_1 is dependent on external factors, whereas progression through S + G_2 + M is not (Sisken and Kinosita, 1961; Prescott, 1976; Johnston *et al.*, 1980). This observation has led to hypotheses suggesting the existence of one or more transition points (also called restriction points) in the cell cycle (Prescott, 1976; Pardee *et al.*, 1978); cells deprived of a growth factor while in the part of the cycle located prior to this point are arrested, while the other cells successfully complete mitosis. This basic concept has been used to construct various cell cycle models such as those of Smith and Martin (1973), Cooper (1979) and Valleron *et al.* (1981).

In our present model we assume the existence of a single transition point (T) (Figure 1), which can only be traversed if the cell has fulfilled a certain number of biochemical prerequisites (in our case coupled to a defined amount of light exposure). The actual length of the first part of the cell cycle (a_1) before T is dependent, therefore, on exposure to light. In contrast, the time necessary to complete the second part of the cycle (a_2) is independent of external conditions. This type of transition point usually occurs in late G_1 in mammalian cells (Pardee *et al.*, 1978) and yeast cells (Johnston *et al.*, 1980), but evidence for transition points in G_2 is beginning to appear (Nurse *et al.*, 1983; Puiseux-Dao, 1981). Measurements of cell cycle phase expansions in phytoplankton in response to light limitation (Olson *et al.*, 1986) suggest that light controlled transition points might be located in G_1 in some species (e.g. the coccolithophorid *Hymenomonas carterae*) or in G_2 in others (e.g. the diatom *Thalassiosira weissflogii*); thus we have not imposed restrictions on the location of T within the cell cycle in our model.

Here we consider a spatially homogeneous environment with light as the only limiting factor for growth. It is 'supplied' according to a prescribed photocycle and the light intensity is maintained constant during the light period. Let k_1 be the minimum time a cell has to be exposed to light (of fixed intensity) in order to cross T. Besides fulfilling its energy requirement (and the concomitant biosynthesis of reduced carbon via the Calvin cycle), the cell must complete a number of other biochemical processes before being able to cross T. Let n_1 be the minimum time devoted to these required processes and let n_2 be the minimum time elapsed between transition (T) and division (Figure 2a). In terms of the maturity formalism described above, this translates into a maturity vector $\mathbf{M} = (k,n)$, where k corresponds to the light requirement and n to the non-light dependent requirements. It obeys the differential system:

$$d\mathbf{M}/dt = \mathbf{U}(k,n,t) = (u_k,u_n) \quad (1a)$$

with:

$$u_k = 1 \text{ when light on, } k < k_1 \quad (1b)$$

$$u_k = 0 \text{ when light on, } k = k_1 \quad (1c)$$

or light off

$$u_n = 1 \text{ when } n < n_1 \text{ or } n > n_1 \text{ or } n = n_1,\ k = k_1 \quad (1d)$$

$$u_n = 0 \text{ when } n = n_1,\ k < k_1 \quad (1e)$$

The cell passes through T when $k = k_1$ and $n = n_1$, and undergoes mitosis when $n = n_1 + n_2$ (Figure 2a). Since n is not regulated by changing external conditions in the case considered here, there is an unequivocal relationship between n and cell age

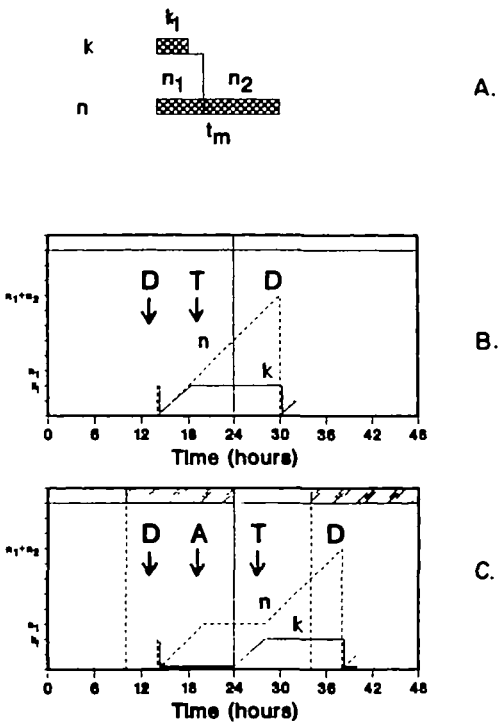


Fig. 2. (A) Representation of the cell cycle using the maturity variables k and n, which define the position of a cell in its cycle in terms of specific cellular characteristics. k is a light dependent maturity variable, and k_1 is the minimum duration of light exposure required to progress into the second half of the cell cycle. n is light independent and n_1 is the minimum time devoted to non-light requiring processes before the transition point T can be crossed, which can occur only when both $k = k_1$ and $n = n_1$. Division takes place n_2 units of time later. The minimum generation time (t_m) is equal to $\max(k_1,n_1) + n_2$. In this example k_1 = 4 h, n_1 = 6 h and n_2 = 10 h such that t_m = 16 h (see b and c below). (B) Typical evolution of the two maturity variables k (solid line) and n (dashed line) for a cell in continuous light. The cell is born at t = 14 (D) and both maturity variables are reset to zero. k and n increase concomitantly until $k = k_1$. When $n = n_1$ (and $k = k_1$) transition (T) occurs and is followed by division (D). This cell is never arrested and the actual generation time is equal to the minimum generation time (t_m, equation 3). (C) Typical evolution of the two maturity variables of a cell in a forcing photocycle (L:D 10:14). The cell is born in the dark (D). Since n refers to light independent processes it begins to increase immediately, while k does not. At time t = 20, n reaches its threshold value n_1. It cannot proceed any further since $k < k_1$, and the cell is arrested (A). When light is provided (t = 24), k begins to increase. At time t = 28, k reaches its threshold value k_1 and transition occurs (T). Division takes place n_2 units of time later (D). Since this cell has been arrested in the dark, the actual generation time is longer than the minimum generation time (t_m) and equal to the period of the forcing photocycle (24 h).

(a) and thus the same hypothesis could have been translated in terms of a maturity vector (k,a).

Let a_m be the actual cell cycle length (i.e. cell age at division), such that:

$$a_m = a_1 + a_2 \tag{2a}$$

Obviously then (Figure 2a):

$$a_1 \geq k_1 \tag{2b}$$

$$a_1 \geq n_1 \tag{2c}$$

$$a_2 = n_2 \tag{2d}$$

With these hypotheses, the actual cell cycle length is the shortest when the cell is placed in continuous light, since under these conditions it is never arrested (Figure 2b). This minimum length (t_m) is obtained using equations (2a–d):

$$t_m = \min(a_m) = \max(k_1,n_1) + n_2 \tag{3}$$

Under a photocyclic regime, the actual cell cycle length will be in general larger than t_m since cells may be arrested in the dark (Figure 2c).

One subtle but critical difference between our assumptions and that of Spudich and Sager (1980), also incorporated in the model of Heath and Spencer (1985), is that here cells can start to fulfill their light requirement (k_1) right at the beginning of the cell cycle (Figure 2) and not only in the segment AT, as Spudich and Sager (1980) hypothesized. If $n_1 > k_1$ and therefore if A is not located at the beginning of the cell cycle (Figure 2), this implies that cells have a certain flexibility in the timing of the fulfillment of their light requirement. Some cells might fulfill it at the beginning of their cycle, as always occurs in continuous light (Figure 2b), while others might fulfill it just before transition, as may occur in photocyclic conditions (Figure 2c).

For the sake of simplicity, cells in our model are totally arrested in their cycle when they are between A and T in the dark, and we assume that processes involving light are independent (in their timing) of other cellular processes. This hypothesis may seem unrealistic, but we note that Spudich and Sager (1980) have observed that in *Chlamydomonas* the amount of time a cell spends in the arrested state does not influence the timing of cell cycle events after the removal of the block. Moreover, although dark-arrested cells were observed to shrink in size in their experiments, they had very low rates of endogeneous respiration and starch degradation relative to cells which had passed the transition point. More recent observations in *Chlamydomonas* (McAteer *et al.*, 1985) and other species (Vaulot *et al.*, 1986) have shown, however, that cells in the dark can modify slightly their cell cycle characteristics.

As mentioned earlier, exposure of a cell population to variable external conditions will create 'external' variability in cell generation times. In addition to this type of variability, there exists inherent variability in the generation times of cells which is independent of external conditions. This 'internal' variability can be taken into account mathematically, by considering the parameters of the cell cycle (k_1,n_1,n_2) as being stochastic variables. The distribution of these parameters is not easily determined experimentally, but that of t_m, the total length of the cycle, is readily measured. Its distribution under constant environmental conditions is usually bell-shaped and sometimes positively skewed (e.g. Powell, 1956; Cook and Cook, 1962), and several

functional forms have been proposed to fit the observed data, including: Gaussian (Hersh and Kitos, 1980), decreasing exponential (Smith and Martin, 1973), gamma (Prescott, 1959), and quantized (Klevecz, 1976). The actual form of the distribution has little influence on the qualitative features of the results presented in this paper, thus we use a Gaussian distribution in most examples.

Once the distribution of t_m (under constant conditions) has been prescribed, the proportions of this variability attached to the first and the second part of the cell cycle have to be defined. It is customarily assumed that the internal variability lies mostly in the G_1 part of the cycle (Smith and Martin, 1973), as is often the case for the external variability described above. However, a recent review of the existing data for mammalian cells (Guiguet *et al.*, 1984) reveals that in reality all cell cycle phases are equally variable. In our case, in order to keep the model formulation as simple as possible, we will locate all of the internal variability in the second part of the cycle. Thus, in most cases we have:

$$k_1 = N(K_1,0) \tag{4a}$$

$$n_1 = N(N_1,0) \tag{4b}$$

$$n_2 = N(N_2,\sigma_2) \tag{4c}$$

$$t_m = N(T_m,\sigma_2) \text{ with } T_m = \max(K_1,N_1) + N_2 \tag{4d}$$

where upper case symbols indicate mean values of lower case stochastic variables and where $N(K,\sigma)$ indicates the normal distribution with mean, K, and standard deviation, σ. Finally, we note that the stochastic cell cycle parameters will always be assumed to be uncorrelated and independent of the total duration of the cell cycle.

Population evolution

In order to describe the time evolution of a cell population, a generalization of the continuous age-structure formalism (Nisbet and Gurney, 1982) has been chosen from several possible choices, including discrete matrix formalism (Leslie, 1945) and renewal formalism (Bronk *et al.*, 1968). The population is described by its maturity structure as follows. If $f(k,n,t)$ is the density of cells of maturity (k,n) at time t, the driving equation is a continuity equation:

$$\frac{\partial f}{\partial t} + \frac{\partial (u_k f)}{\partial k} + \frac{\partial (u_n f)}{\partial n} + hf = b \tag{5}$$

where $h(k,n)$ is the division rate of cells of maturity (k,n) and $b(k,n,t)$ the incoming flux of newborn cells. Equation (5) is valid in the domain $k > 0$, $n > 0$, $t > 0$ and the initial condition is:

$$f(k,n,0) = f_0(k,n) \qquad t = 0 \tag{6}$$

$N(t)$, the total number of cells per unit volume at time t, is given by:

$$N(t) = \iint_0^{+\infty} f(k,n,t).\mathrm{d}k.\mathrm{d}n \tag{7a}$$

and $\mu(t)$, the division rate of the population, as:

$$\mu(t) = 1/N(t).\mathrm{d}N/\mathrm{d}t \tag{7b}$$

The driving terms of the population dynamics are the cell division rate $h(k,n)$, the incoming flux of newborn cells $b(k,n,t)$ and the maturation velocity $\mathbf{U}$ (u_k,u_n) which couples the population to the time variability of the environment.

For our model of the cell cycle, $\mathbf{U}$ is given by equation (1) and b is equal to:

$$b(k,n,t) = 2\delta(k).\delta(n).\iint_0^{+\infty} h.f.\mathrm{d}k'.\mathrm{d}n' \tag{8}$$

(where $\delta(x)$ is the Dirac function) meaning that each cell divides into two newborn cells with maturity vector (k,n) reset to (0,0) and that no cell death occurs. Finally $h(k,n)$ is equal to:

$$h(k,n) = \delta(k-K_1).g(n)/\iint_n^{+\infty} g(n').\mathrm{d}n' \tag{9}$$

since there is no internal variability attached to the light-dependent part of the cycle (equation 4a). $g(n)\mathrm{d}n$ is the proportion of cells dividing between maturity n and $n + \mathrm{d}n$, given for the case of the Gaussian internal variability by:

$$g(n) = 0 \qquad n < N_1 \tag{10a}$$

$$g(n) = \frac{\exp(-(n - N_1 - N_2)^2/2.\sigma_2^2)}{\sqrt{(2\pi).\sigma_2}} \qquad n \geq N_1 \tag{10b}$$

At first glance, equation (5) looks like a simple wave equation; however the use of equation (8) transforms equation (5) in an integro-differential equation in $f(k,n,t)$, preventing an easy search for analytical solutions. Thus equation (5) has been solved numerically along its characteristics with a time step ranging from 0.25 to 1 h for an average cell cycle length of 1 day.

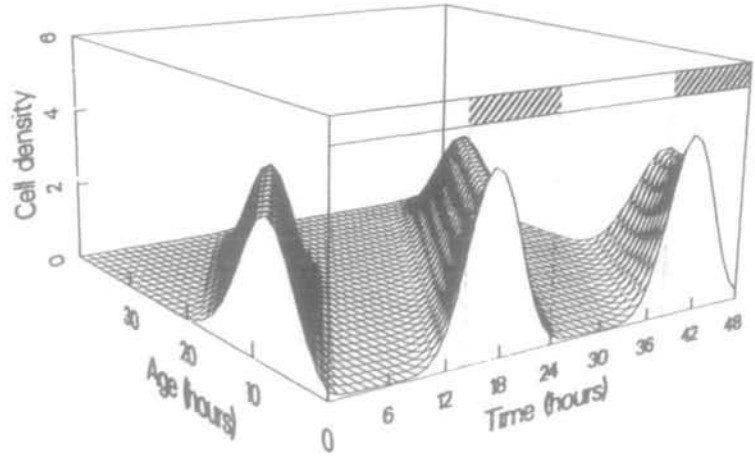


Fig. 3. Evolution of the maturity (measured here by age) distribution as a function of time for a population of species A entrained to 14:10 L:D photocycle. The instantaneous population division rate $[\mu(t)]$ is given by the curve in the forefront plane.

In summary, the model inputs are the cell cycle characteristics, which in this case are uniquely determined by four parameters: K_1, N_1, N_2 and σ_2; the model output is the maturity structure $f(k,n,t)$ at each time step, from which the cell number, $N(t)$, and population division rate, $\mu(t)$, can be obtained easily through equation (7).

Photoperiodic forcing conditions: the persistent behavior

When cultured under light/dark photocycles, phytoplankton populations exhibit periodic division rates which persist indefinitely in the presence of the entraining photocycle (Chisholm, 1981; Edmunds and Adams, 1981). As an initial test of our model we have applied it to photocyclic regimes investigating only the long-term persistent dynamics of the population. In the following simulations, a population with given cell cycle parameters (K_1, N_1, N_2, σ_2), and with an arbitrary initial maturity structure, is subjected to periodic light/dark conditions. (For simplicity the photocycle is always 24 h long, but all time variables can be non-dimensionalized by normalizing to the length of any forcing period.) After a certain number of periods, the normalized cell density $[F(k,n,t) = f(k,n,t)/N(t)]$ becomes entrained to the forcing period in a pattern which is independent of the initial conditions. As shown in Figure 3, the cell density motion is wave-like. Each crest corresponds to a distinct cohort of cells. If age is taken as the maturity variable, the relationship between the maturity structure and the population division rate is given by:

$$\mu(t) = 1/2\ F(0,t) \tag{11}$$

and thus μ is obtained by taking half the projection of the normalized density on the forefront plane $a = 0$ (Figure 3).

Two hypothetical species, A and B, are considered first. They have the same light requirement ($K_1 = 4$ h) and total cycle length ($T_m = 18$ h). Species A exhibits an early transition point T and species B has a late transition point. As pointed out earlier, this results in a greater flexibility for species B to fulfill its light requirement. The internal variability, represented by a Gaussian distribution in n_2 ($\sigma_2 = 3$ h), is the same for the two species. The results of the simulations (Figure 4) show that the two species have distinctly different division patterns resulting solely from the positioning of the transition point. Their responses to increasing photoperiods are also different. Species A has a single division peak in the dark, which is barely affected by an increase in the duration of the photoperiod. Species B exhibits a division peak in the light for short photoperiods and as the photoperiod is extended, the amplitude of this peak declines and a second (smaller) peak appears during the dark period. Division phasing is weaker for species B than species A, and the daily averaged division rate of species B is much more sensitive to the length of the photoperiod than that of species A (Figure 4).

If $K_1 + N_2$ and K_1 are small enough, and the light period long enough, the major peak occurs during the day and some cells may see enough light to be able to cross the transition T before the dark period. In this case a second peak of division occurs in the dark. A longer light period increases the number of cells crossing T before the dark and in some cases, the secondary peak can be moved to the next light period, deceptively preceding the main division burst.

The previous examples demonstrate how the location of the transition point, T, within the cell cycle determines the final division pattern and the average population division

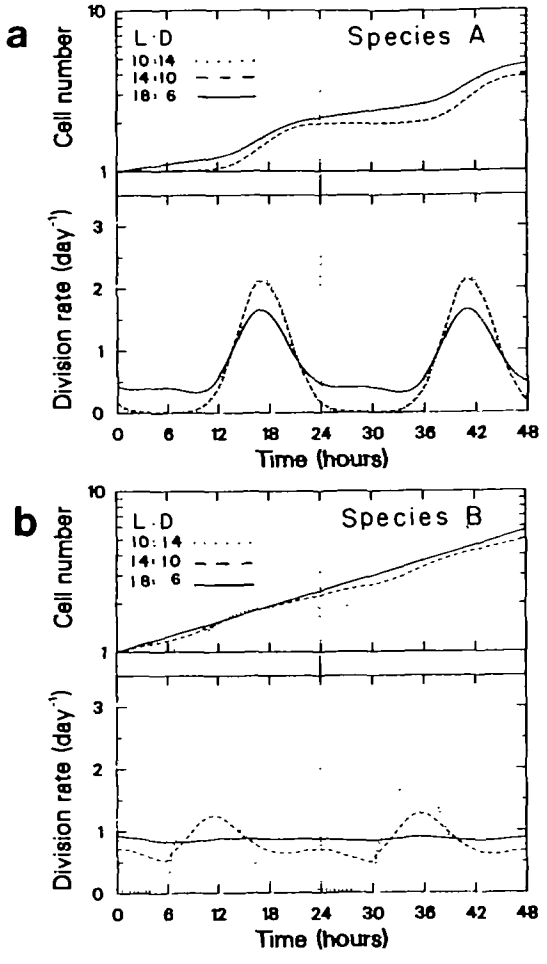


Fig. 4. Model simulations of the division pattern of a hypothetical species A grown on three different photocycles: L:D 10:14 (dotted curves), 14:10 (dashed curves) and 18:6 (solid curves). Dawn always occurs at 0 and 24 h. An arbitrary initial population was entrained for at least five photocycles and allowed to reach an asymptotic state independent of the initial conditions. Relative changes in cell number and in the instantaneous population division rate are shown for two consecutive photocycles. μ_{24} is the average population division rate (day^{-1}) over the 24 h photocycle (note that $\mu_{24} = 0.69\ day^{-1}$ for populations doubling once per day). (a) Species A has an early transition point and its cell cycle parameters are equal to: $K_1 = 4$ h, $N_1 = 4$ h, $N_2 = 14$ h and $\sigma_2 = 3$ h, such that $T_m = 18$ h. Note that the dotted and dashed lines overlap. (b) Species B has a late transition point but its light requirement and total cycle length are the same as those of species A. $K_1 = 4$ h, $N_1 = 10$ h, $N_2 = 8$ h and $\sigma_2 = 3$ h, such that $T_m = 18$ h.

rate. Conversely, the model provides a framework for the interpretation of division patterns observed experimentally, facilitating the selection of parameter values for the simulation. A given division pattern can be described by a set of a few variables (Figure 5). P_1, the position of the mean peak relative to the onset of the light, defines the phase angle of the pattern. Its significance arises when other environmental factors, such as nutrient supply, are periodic as well. These factors may have variable effects on population growth depending upon their phase relationships with the division pattern (Olson

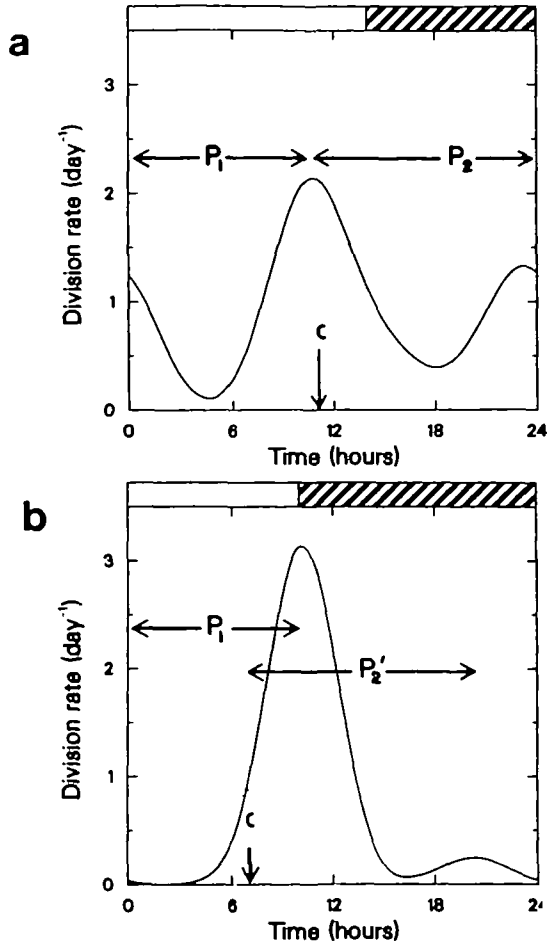


Fig. 5. Analysis of characteristics of division patterns with respect to the cell cycle parameters used: K_1 = 3 h, N_1 = 6 h, N_2 = 8 h, σ_2 = 2 h (T_m = 14 h). (a) 14:10 L:D photocycle. The main peak takes place P_1 hours (= 10.5 h) after the onset of light which is roughly equal to $K_1 + N_2$ (= 11 h). Cells born at the critical point C (which occurs just after the main division peak, K_1 hours before the dark period) are the mother cells for the second division burst. The distance between the two peaks (P_2 = 13 h) is close to the minimum cell cycle length ($N_1 + N_2$ = 14 h). (b) 10:14 L:D photocycle. The critical point C occurs before the main peak. Here, the distance between C and the secondary peak (P_2') is equal to $N_1 + N_2$.

and Chisholm, 1983; Wheeler *et al.*, 1983). P_1 is roughly equal to $K_1 + N_2$ (Figure 5). If a secondary division peak is present, it results from the division of cells born K_1 hours before the dark period which we define as the critical point (C) of the photocycle (Figure 5). These cells are not blocked and thus divide roughly T_m hours after their birth. If the mean peak of division occurs before the critical point, C (Figure 5a), the distance between the two peaks (P_2) is equal to T_m. If not, the distance between C and the secondary peak (P'_2) is equal to T_m (Figure 5b). Finally the degree of synchrony of the culture is given by the breadth of the peaks and is trivially proportional to the internal variability in cell cycle length (σ_2) (Figure 6).

In addition to predicting the timing of division in a light/dark phased culture, the

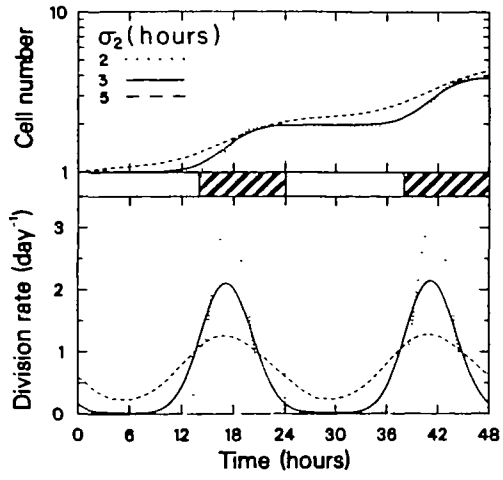


Fig. 6. Effect of changing the internal variability on the division pattern of a population grown on a 14:10 L:D photocycle. Model parameters are: K_1 = 4 h, N_1 = 4 h, N_2 = 14 h (T_m = 18 h); σ_2 = 2 h (dotted line), σ_2 = 3 h (solid line) and σ_2 = 5 h (dashed line). Daily averaged division rates (μ_{24}) are respectively 0.66, 0.67 and 0.73 day^{-1}.

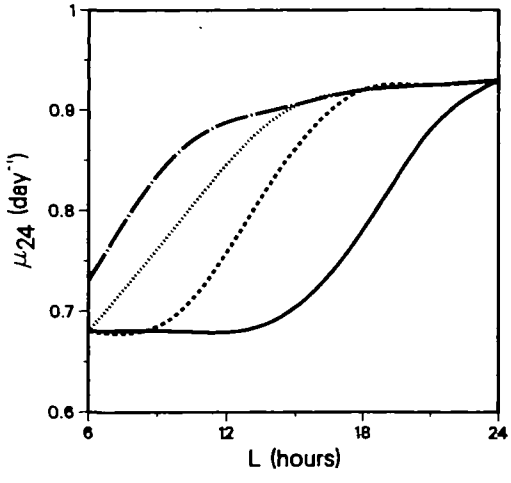
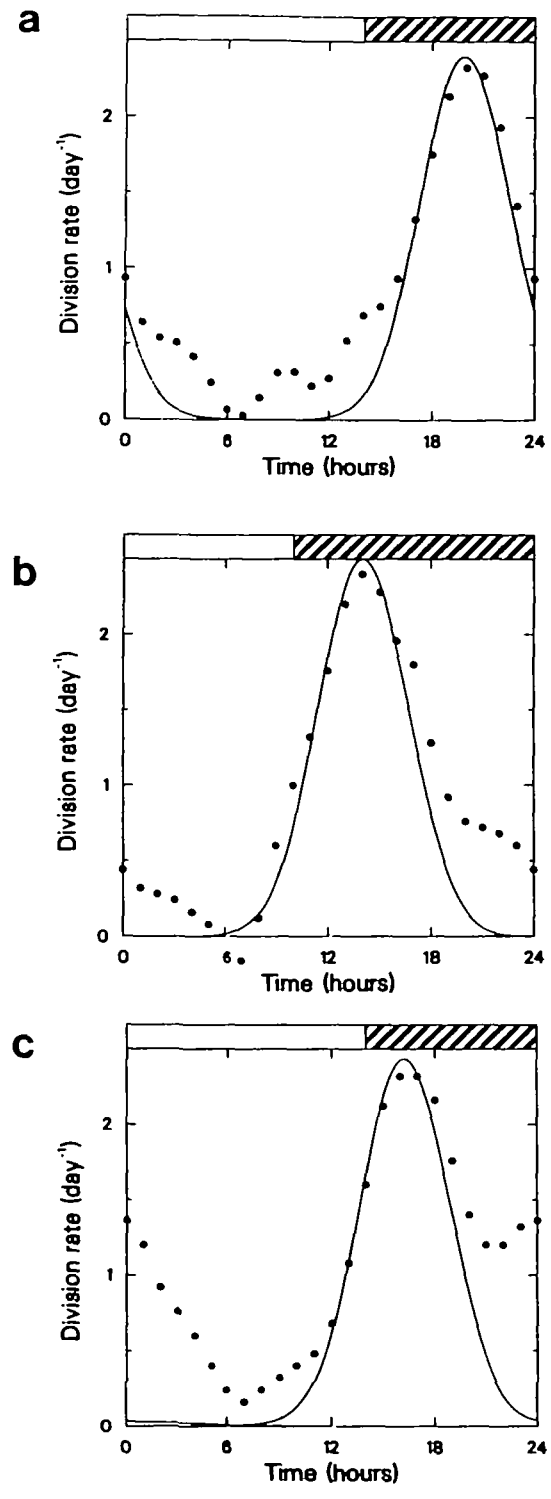


Fig. 7. Daily averaged population division rate (μ_{24}) as a function of the length of the light L period for four different cell populations with the same total cell cycle length (T_m = 18 h) and the same internal variability (σ_2 = 3 h). Solid curve: K_1 = 4 h, N_1 = 4 h, N_2 = 14 h; dashed curve: K_1 = 4 h, N_1 = 9 h, N_2 = 9 h; dotted curve: K_1 = 4 h, N_1 = 12 h, N_2 = 6 h; dash-dotted curve: K_1 = 1 h, N_1 = 9 h, N_2 = 9 h.

model also allows us to examine the dependence of the daily averaged population division rate (μ_{24}) on the length of the photoperiod and on the cell cycle parameters. This dependence is the net result of the interaction between each cell in the population and the conditions it experiences during a photocycle. All factors which favor an increase in the proportion of cells born before the critical point C (Figure 5), will increase μ_{24} even though T_m is fixed. As a consequence, there are several ways to increase μ_{24} by adjusting cell cycle parameters. One is to spread out the distribution of cell generation times (σ_2), and thus reduce the degree of phasing in the population (Figure 6). Another



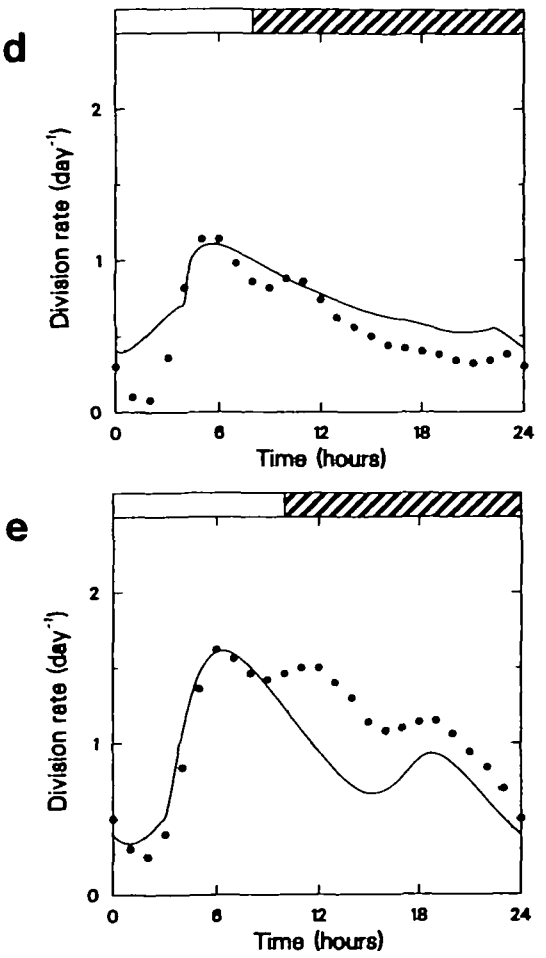


Fig. 8. Comparison between experimental data (dots) and model results (curves). (a) *A. carteri* on 14:10 L:D (data from Olson and Chisholm, 1983); model parameters are K_1 = 4 h, N_1 = 4 h, N_2 = 16.5 h, σ_2 = 2.65 h (T_m = 20.5 h). (b) *H. carterae* on 10:14 L:D (data from Chisholm and Costello, 1980); model parameters are K_1 = 4 h, N_1 = 4 h, N_2 = 10.75 h, σ_2 = 2.65 h (T_m = 14.75 h). (c) *H. carterae* on 14:10 L:D (data from Chisholm and Costello, 1980); model parameters are K_1 = 4 h, N_1 = 4 h, N_2 = 13 h, σ_2 = 2.6 h (T_m = 17 h). (d) *T. weissflogii* on 8:16 L:D (data from Chisholm and Costello, 1980). n_2 is gamma distributed; model parameters are K_1 = 4 h, N_1 = 18 h, N_2 = 5 h, n_2 mode = 1.5 h, σ_2 = 4.2 h (T_m = 23 h). (e) *T. weissflogii* on 10:14 L:D (data from Chisholm and Costello, 1980). n_2 is gamma distributed, model parameters are K_1 = 3 h, N_1 = 10.5 h, N_2 = 6 h, n_2 mode = 3.5 h, σ_2 = 3.9 h (T_m = 16.5 h).

is to move the main division peak before the critical point C by reducing $K_1 + N_2$ while still keeping T_m fixed (Figure 7, dashed and dotted curves). This, in effect, moves the transition point to a later position in the cell cycle. Conversely, one can also increase μ_{24} by moving the critical point C to a later position in the photocycle; this can be achieved by decreasing the light requirement K_1, with the position of the main peak (given by $K_1 + N_2$) remaining fixed (Figure 7, dash-dotted curve). If the light requirement K_1 is larger than the photoperiod L, a cell has to go through two cycles in order to fulfill its light requirement and μ_{24} is then less than 0.69 day^{-1}. The value $L = K_1$

acts like a threshold inducing a rapid change in μ_{24}. For a given L, μ_{24} might be more or less sensitive to the model parameters depending on the value of L considered (compare $L = 12$ and $L = 18$ in Figure 7).

To test the model against experimental data, three species are considered, a dinoflagellate (*Amphidinium carteri*), a coccolithophorid (*Hymenomonas carterae*) and a diatom (*Thalassiosira weissflogii*), for which division patterns have been studied extensively in cyclostat cultures (Chisholm and Costello, 1980; Olson and Chisholm, 1983). Qualitatively, *A. carteri* and *H. carterae* behave like our hypothetical species A with a major division burst in the dark, whereas *T. weissflogii* more closely resembles species B, with a major division burst in the light (Figure 8). It is possible to extract estimates of $K_1 + N_2$, T_m [$= \max(K_1,N_1) + N_2$] and σ_2 from these division patterns. For example in Figure 8a, the occurrence of the main division peak at 19 h indicates that $K_1 + N_2$ is roughly equal to this value. The width of the division peak suggests a value of 2.5 h for σ_2. This set of parameters can then be adjusted by trial and error in order to obtain the best fit to the experimental data. Since we have three constraints and four parameters, several sets of parameters are able to give equally good fits. K_1, for example, can be varied within a limited range (at least for the photocyclic regimens considered) without modifying the output, as long as N_1 and T_m remain constant. Independent experiments, such as direct measurement of the minimum light exposure for division (Heath and Spencer, 1985), have to be performed in order to determine the complete parameter set.

The model reproduces well the first peak of division in all three species, but has more difficulty accounting for the secondary peaks (e.g. *H. carterae* L:D 14:10, Figure 8c; *T. weissflogii* L:D 10:14, Figure 8e). These secondary peaks are most likely due to the division of cells born during the light period and traversing through the cell cycle more rapidly than the cells born during the dark period. An alternate interpretation makes use of Klevecz's (1976) quantized model of the cell cycle, in which cells have to go through a 'sub-loop' of the cell cycle an integer number of times before commiting to division. This has been modelled previously by Slocum (1980) and Chisholm *et al.* (1980) and the results fit some of the experimental data quite well. Klevecz's hypothesis, however, awaits confirmation for phytoplankton populations by direct recording of the cell generation time distributions (Chisholm *et al.*, 1984).

We conclude from the simulations thus far that the proposed model for the coupling between light and cell cycle is able to reproduce the qualitative characteristics of division patterns of diverse phytoplankton taxa (for a review see Chisholm, 1981). These patterns can also be explained in some cases by cell cycle coupling to a circadian clock (Edmunds and Adams, 1981). Our model, however, eliminates the need for the distinction between ultradian ($\mu_{24} > 0.69$ and infradian ($\mu_{24} < 0.69$ day^{-1}) growth modes (Ehret and Wille, 1970) often required to explain changes in population division patterns when the average growth rate exceeds one doubling per day (Ehret and Dobra, 1977). As the length of the light period increases in our model, a population progresses from the infradian or phased mode ($L < K_1$) to the synchronized ($L = K_1$) and then to the ultradian ($L > K_1$) mode with continuity (Figure 7). This is in accord with our experimental observations for several species (Chisholm and Costello, 1980) and for *Euglena* grown at relatively high light intensities (Edmunds and Funch, 1969).

Another general feature predicted by the model is a fixed phase relationship between

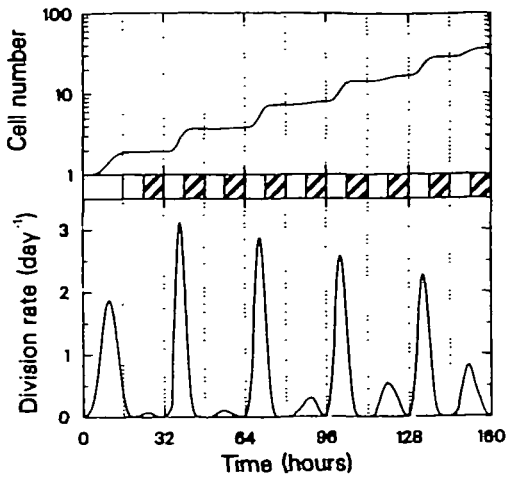


Fig. 9. Transient population behavior when the length of the light period (L) is shorter than the light requirement (K_1). Population parameters are K_1 = 12 h, N_1 = 12 h, N_2 = 4 h, σ_2 = 2 h (T_m = 16 h). A synchronous population was introduced into a 8:8 L:D photocycle at time t = 16 h. An intrinsic transient with a period of 32 h (two photocycles) is observed while the long term behavior converges to a period of 16 h (one photocycle).

the main division burst and dawn, regardless of photoperiod length, because blocked cells are released at dawn. This is in accordance with the results obtained by Edmunds (1965) and Edmunds and Funch (1969) for *Euglena*, by Paasche (1967) for *Emiliana huxleyi* and by Heath and Spencer for *Thalassiosira pseudonana*. However, some species (e.g. *Ceratium furca*, Weiler and Eppley, 1979) exhibit the opposite behavior (fixed phase with respect to dusk, or division right at dusk for short dark periods), which could be due to a different blocking mechanism (Adams *et al.*, 1984).

Transient behavior

Thus far we have dealt exclusively with 24 h photocycles which have a time scale of the same order as that of the cell cycle for the species considered. A large amount of experimental data has been obtained by other investigators for photocycles with periods different from 24 h and for transient situations (as in Edmunds and Funch, 1969; for an overview see Chisholm, 1981). In the following section we have applied our model to such situations in an attempt to see if we can simulate some of the responses of the cell populations in these studies using the transition point hypothesis.

Short photocycles and phase response curves

When a cell population is transferred from one light regime to another, a certain lapse of time is necessary for the population to adapt to its new environment. Our model reproduces such transient behavior. The transient phase rarely lasts for more than one photocycle if each cell is able to satisfy its light requirement within one light period ($L > K_1$). If the cells need to be exposed to light for two or more photocycles, however, the transient phase may last for a much longer time, as is demonstrated in Figure 9. Here a synchronized population with a light requirement of 10 h is shifted to a 8:8 L:D photocycle. The period of the division rate oscillation is initially 32 h (= two

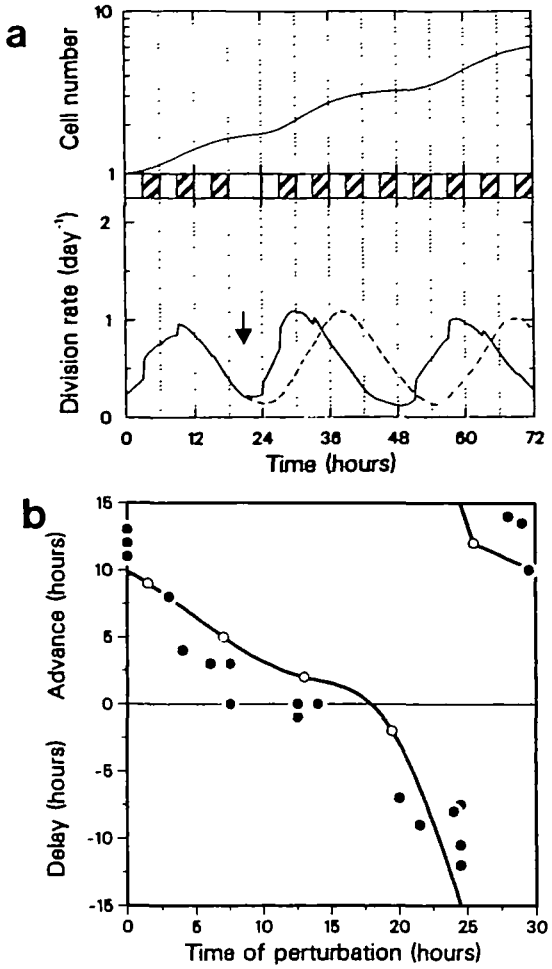


Fig. 10. Simulation of data from an experiment on *Euglena* described in Edmunds *et al.* (1982). Model parameters are K_1 = 12 h, N_1 = 12 h, N_2 = 6 h, σ_2 = 3 h (T_m = 18 h). (**a**) An initially synchronous population was introduced into a 3:3 L:D photocycle. Time 0 corresponds to the end of the eleventh 3:3 photocycle. The population exhibits a long lasting transient with a period length of 30 h (dashed curve). The omission of the dark period at t = 21 results in a phase advance for the division pattern of the population (solid curve). (**b**) Phase response curve constructed from model simulations (open circles and solid curve) compared with the experimental data of Edmunds *et al.* (1982) for *Euglena* (solid circles). Each open circle on the curve corresponds to a phase advance or delay caused by the omission of a dark interval at various phases relative to the initial division pattern. The abscissa gives the time of the perturbation relative to the time at which the inflection point in the descending part of the division curve occurs (e.g. at t = 15 in Figure 10a). Figure 10a corresponds to the second open circle from the left.

photocycles) and it evolves towards the long term value of 16 h. We note, however, that the evolving second peak in this 7 day simulation would probably go undetected in a laboratory experiment given the resolution of most methods of measuring cell concentration. The pattern could be interpreted as reflecting a steady state population with a 32 h periodicity if the experiment was not continued beyond 1 week. Experimental data for *Euglena* grown in short photocycles (Edmunds and coworkers, 1965, 1969

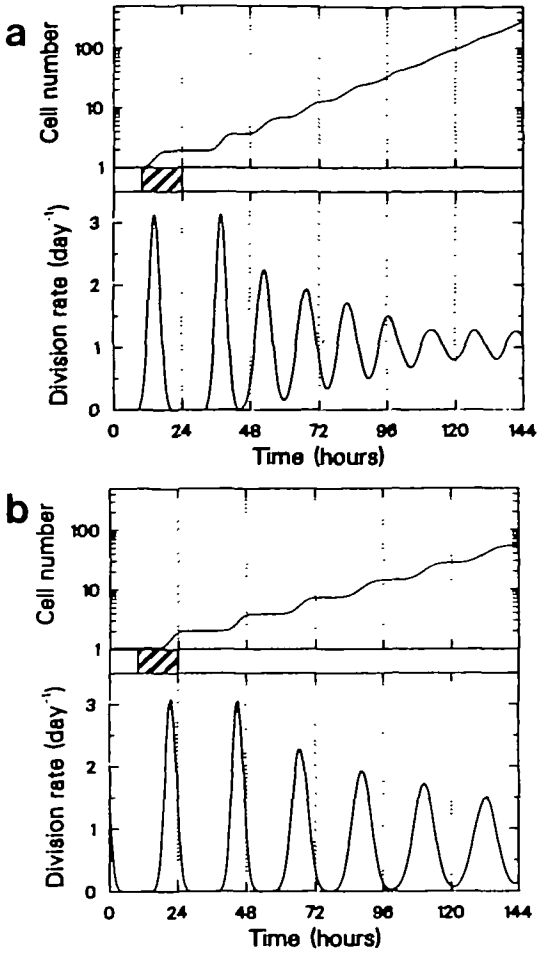


Fig. 11. Patterns of division in populations allowed to free-run in continuous light after synchronization by 10:14 L:D. The average doubling time of the population was 1 day before it was placed in continuous light. (a) Population with large internal variability. Model parameters are K_1 = 7 h, N_1 = 7 h, N_2 = 8 h, σ_2 = 2 h (T_m = 15 h) resulting in a coefficient of variation of 0.13. Note the rapidly damped oscillations of period close to T_m. (b) Population with small internal variability. Model parameters as in Figure 11a except for N_2 = 15 h (thus T_m = 22 h); the coefficient of variation is now 0.09. The oscillations are less damped but still have a period close to T_m.

and 1982) typically exhibit an entrained period which is an integer multiple of the photocycle length (Chisholm *et al.*, 1984), and could reflect such a transient. These data are therefore consistent with the transition point model, although we do not claim that they support it.

Another type of transient arises when an entrained population is perturbed by a single discrete change in light regime. The population usually recovers its initial periodicity, but with a phase shift with respect to the absolute timing of the original photocycle. The mapping of this phase shift as a function of the perturbation time constitutes a phase response curve (PRC), as has been described by Edmunds *et al.* (1982) for *Euglena*

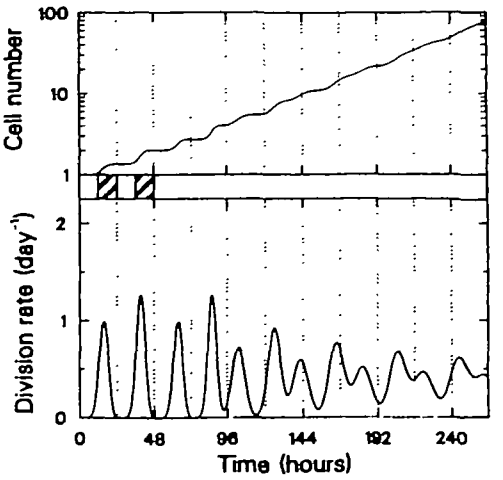


Fig. 12. Patterns of division in populations allowed to free-run in continuous light after phasing by 12:12 L:D. The average doubling time of the population was 2 days before it was placed in continuous light. Model parameters are K_1 = 10 h, N_1 = 10 h, N_2 = 30 h, σ_2 = 3 h (T_m = 40 h). Damped oscillations of period 40 h (= T_m) are observed which have two components 24 h out of phase.

populations submitted to a 3:3 L:D cycle. Under these conditions, the period length of the entrained population division pattern was 30 h.

The model has been run under the photocyclic conditions of the Edmunds *et al.* (1982) experiment using the following cycle parameters: $K_1 = N_1 = 12$ h, $N_2 = 6$ h, $\sigma_2 = 3$ h. A long oscillatory transient of period 30 h is observed (Figure 10a). This entrained transient has a very long decay time (in contrast with the free running transients discussed below) and is sustained for more than 15 oscillations. When a dark period is omitted at different times relative to the trough of the division pattern, as was done in the Edmunds *et al.* (1982) experiment, the division rate oscillation (Figure 10a) is phase shifted in advance (if the perturbation is mainly experienced by cells located before transition point T in their cycle) or in delay (if the perturbation is experienced by cells advanced beyond T). Using the results of our simulations, a phase response curve can be generated which is in good agreement with that obtained experimentally by Edmunds *et al.* (Figure 10b). This demonstrates that phase resetting and the phase response curve can also be consistent with the transition point model if long-lived transients are observed in populations entrained to short photocycles.

Constant light and free running populations

For the sake of completeness, we now consider the behavior of an entrained population after release into constant light. In this case the population division rate (μ) becomes constant such that (Painter and Marr, 1967):

$$T_m = \ln 2/\mu + \mu.\sigma_2^2/2 \quad (12)$$

This long-term steady state is not reached immediately, however, but after a transient phase, the characteristics of which are dependent on the initial photocyclic conditions and on the cell cycle parameters. First let us consider the release into continuous light of a well synchronized population (Figure 11). Its dynamics are in accordance

with those described by Bronk *et al.* (1968): the division rate exhibits decaying oscillations which have a period close to one cell cycle length (T_m). The loss of synchrony can be characterized by a damping time (T_d) given by (Bronk *et al.*, 1968):

$$T_d = \pi^2/2.(\sigma_2/T_m)^{-2}.T_m \quad (13)$$

The persistence of the transient oscillations is an inverse function of the internal variability in generation times in the population (compare Figure 11a and b). When the initial population is phased rather than synchronized, the transients may be more complicated. This situation is illustrated in Figure 12 where cells with a long generation time (T_m = 40 h) phased to a 12:12 L:D cycle are released into continuous light. Although the division pattern exhibits an overall 40 h period, it consists of two components dephased by 24 h.

Such free running oscillations are not consistent with a clock controlled cell cycle. In the case of a clock-controlled cell cycle the period length of the free running oscillations is equal to that of the circadian clock and therefore independent of the generation time, while in our model the period length is equal to the generation time of the population.

Conclusions

The model presented here was designed to explore the consequences of the transient point hypothesis (Spudich and Sager, 1980) for light entrained phytoplankton populations. This hypothesis has recently received additional experimental support in several phytoplankton species (Heath and Spencer, 1985; McAteer *et al.*, 1985; Donnan *et al.*, 1985; Vaulot *et al.*, 1986). The model involves two critical components: (i) a coupling between cell cycle and light exposure relying on the transition point hypothesis, and (ii) a provision for random variability in cell cycle length among individual cells. Despite its simplicity, the model reproduces a wide range of cell division patterns that have been observed experimentally in several phytoplankton species. These include: (i) entrainment of cell division to photoperiodic forcing, (ii) a fixed phase relationship between the timing of the division peak and beginning of the light period, (iii) entrained periodicity equal to the length of the photocycle or an integer multiple thereof, and (iv) phase shifting upon perturbation.

These characteristics are also consistent with the hypothesis of a clock entrained cell cycle (Edmunds and Laval-Martin, 1984), thus they could not be used to discriminate between the two hypotheses. Perhaps the best criterion for distinguishing between the clock controlled and transition point controlled models of the cell cycle is the free running behavior. In cells which are not clock controlled, the rhythm will not persist indefinitely, and the period length will be equal to the mean generation time of the population. The clock and transition point models are not mutually exclusive, however, and in some species they may coexist. Indeed, Adams *et al.* (1984) have proposed that cell division in *Euglena* and *Ceratium* is jointly regulated by both a clock-type mechanism ('circadachron') and a transition-point-type mechanism ('cytochron'). The former is reset by the light/dark transition, which defines the temporal division gate, and the latter prevents individual cells from dividing within the division gate until they meet a certain light requirement.

The model provides an interpretation for the difference observed between species

dividing during the dark period, such as the dinoflagellates and the coccolithophorids, and species dividing during the light period, such as the diatoms (Chisholm and Costello, 1980; Olson and Chisholm, 1983). The location of the light controlled transition point in the cell cycle dictates when a cell will divide relative to the light/dark cycle.

The model has many oversimplifications which contribute to its inability to reproduce exactly some characteristics of the experimental data, such as secondary division peaks. By hypothesis, the mean value of the cell cycle parameters (K_1,N_1,N_2) and their variability (σ_2) are fixed. These parameters are undoubtedly more 'history dependent' than we allowed for, since rates of the biochemical processes which mediate the conversion of light energy into cell cycle progression obviously are. Indeed recent experiments with *H. carterae* (Vaulot *et al.*, 1986) have revealed that prolonged darkness results in a slight increase in generation time (T_m) after release into continuous light. Cell cycle parameters may also be cell lineage dependent as evidenced by the correlations between mother and daughter cell cycle lengths observed in other types of cells (e.g. Palsson and Himmelstein, 1981). But more fundamentally, light control over phytoplankton cell cycle may be more complex than assumed here: several light dependent segments may coexist, each corresponding to a different light requiring process, as we suspect to be the case in the diatom *T. weissfloggi* (Vaulot *et al.*, 1986).

Despite these limitations, the model points out and clarifies the effects of two kinds of cell-to-cell variability on the average population behavior. The external variability among cells induced by temporal (and spatial) changes in the environmental conditions results in the segregation of an initially homogeneous population into subpopulations having different life histories (Figure 3). In addition, the internal variability, which is due to differences in the intrinsic characteristics of individual cells, results in a continuous exchange between subpopulations and thus counteracts the effect of the external variability. In the case of regular, periodic conditions discussed in this paper, the interplay between the two mechanisms leads to the establishment of stable population division patterns. These modelling concepts could be easily extended to more complicated environments with both temporal and spatial variability, which would mimic more closely natural conditions.

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