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Estimate of phytoplankton division rates by the mitotic index method: The f_{max} approach revisited

Abstract—The mitotic index method is re-examined by solving an idealized case analytically. A lower bound for the daily division rate of a phased cell population can be computed as $\ln[(1 + f_{max})/(1 + f_{min})]$ where f_{max} and f_{min} are the maximal and minimal fractions of cells in a terminal phase of the cell cycle (e.g. mitosis) over a light: dark cycle. This new formula extends the previous analysis of McDuff and Chisholm to the case of slow-growing cells that spend more than 1 d in the terminal phase. It should be useful in the case of phytoplankton populations growing in oligotrophic waters. Further, the error between this lower bound and the actual value of the division rate is expressed as a function of the durations of the terminal phase and of the division burst.

Phytoplankton growth rate is a key parameter necessary to gain a detailed understanding of aquatic food webs. Although biochemical rate measurements are useful to determine its magnitude at the community level, estimates at the population level are essential to assess growth variability among taxa and size classes (Furnas 1990). One appealing approach at the population level consists in deriving the value of the division rate from time series of the fraction of cells in a terminal phase of the cell cycle, usually mitosis (Gough 1905; Swift and Durbin 1972). Since clarification of the theory underlying this technique by McDuff and Chisholm (1982), it has been increasingly applied in the field (e.g. Braunwarth and Sommer 1985). More recently, the possibility of measuring per-cell DNA distributions by epifluorescence microscopy or flow cytometry (Carpenter and Chang 1988; Boucher et al. 1991) to obtain precise determinations of the fraction of cells in the different cell cycle phases has increased the applicability of the method. In the present note, an idealized case is solved analytically, allowing one to extend the analysis of McDuff and Chisholm to the case of slow-growing populations, a case relevant to oligotrophic environments, and to investigate the precision of the estimated division rate.

Consider a population for which the division rate, $\mu(t)$ (*see list of notation*), is entrained to a periodic stimulus (period t_p), which is often light ($t_p = 24$ h), but can be nutrient supply (Olson and Chisholm 1983). Assume there is a terminal phase of the cell cycle with a *fixed* length t_d, corresponding for example to the duration of mitosis. The fraction of cells in this phase, $f(t)$, is also periodic and such that (McDuff and Chisholm 1982):

$$\ln[1 + f(t)] = \int_t^{t+t_d} \mu(\tau)\, d\tau, \qquad (1)$$

which leads to the approximate equation:

$$\mu_p \approx \frac{1}{nt_d} \sum_{i=1}^{n} \ln(1 + f_i) \qquad (2)$$

where n is the number of samples collected at fixed intervals during a given entraining period t_p.

This equation is only valid when all cells within the population have the same t_d. For example, it is not applicable when the length of the terminal event is affected by darkness, as in *Synechococcus,* for which some cells are arrested in G_2 and in the paired cell stage during the dark period (Armbrust et al. 1989): cells that do arrest will have a longer t_d than cells that do not. If t_d is invariant over the population, Eq. 2 is always valid but requires knowing t_d. When the species of interest can be cultivated, the value of t_d can be measured in the laboratory and, if it is independent of environmental conditions, it can be introduced into Eq. 2 (Chang and Carpenter 1985; Campbell and Car-

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penter 1986). In the few cases where t_d has been determined, however, it does not seem to be independent of growth conditions. In *Synechococcus,* the duration of the paired cell stage increases significantly at slow growth rates (Campbell and Carpenter 1986). In the dinoflagellate *Gymnodinium* cf. *nagasakiense,* the duration of mitosis is proportional to the generation time (Videau and Partensky 1990). In this latter case, Eq. 2, although valid, cannot be used to compute division rates (*see* Videau and Partensky 1990).

McDuff and Chisholm (1982) remarked that, if there is a time window during which all cells that are going to divide during the current period can be found in the terminal phase, i.e. when the terminal phase (t_d) is long compared to the division burst (t_c), then

$$\mu_p = \frac{1}{t_p}\ln(1 + f_{\max}) = \mu_{f\max}. \qquad (3)$$

This formula, or some of its earlier variations (McDuff and Chisholm 1982), has been used extensively in the past because of its convenience (e.g. Gough 1905; Swift and Durbin 1972; Weiler and Chisholm 1976). It does not require knowledge of t_d, a major source of uncertainty in Eq. 2 and an unknown in the case of species that cannot be grown in the laboratory. Moreover, as pointed out by Antia et al. (1990), $\mu_{f\max}$ is a lower bound for the actual division rate because $f_{\max}$ decreases when the terminal phase becomes shorter than the time window during which cells divide (*see* figure 1, McDuff and Chisholm 1982). They failed to recognize, however, that Eq. 3 is only valid when there is at most a single cell cohort in the terminal phase, i.e. when the terminal phase is shorter than the entraining period ($t_d < t_p$). Moreover they did not analyze in detail the error associated with this estimate when phasing becomes less tight.

To investigate the general case valid for any length of the terminal phase, consider an idealized situation (Fig. 1A) with the following assumptions. First, t_d is fixed for all cells. If t_d is longer than the photoperiod t_p, t_d' is defined as t_d modulo t_p, such that $t_d = mt_p + t_d'$ where m is a positive integer. Second, all cells divide in phase at the beginning of the entraining period, i.e.

Notation

t_c	Duration of division burst, h
t_d	Duration of terminal phase, h
t_p	Duration of entraining period (24 h usually), h
t_d'	t_d mod t_p (e.g. if $t_d = 30$, $t_p = 24$, then $t_d' = 6$), h
m	t_d div t_p (integer, e.g. if $t_d = 30$, $t_p = 24$, then $m = 1$)
$\mu(t)$	Instantaneous division rate, h^{-1}
μ_p	Avg division rate over the photoperiod t_p, h^{-1}
$\mu_{\max}$	Maximum of $\mu(t)$ over t_p, h^{-1}
$\mu_{f\max}$	Estimate of division rate (Eq. 3), h^{-1}
$\mu_{f\min,f\max}$	Estimate of division rate (Eq. 7), h^{-1}
$f(t)$	Fraction of cells in terminal phase
$f_{\min}$	Minimum of $f(t)$ over t_p
$f_{\max}$	Maximum of $f(t)$ over t_p
S	DNA synthesis phase
G_2	Gap at the end of the cell cycle before mitosis
M	Mitosis

$$\mu(t) = \mu_p \sum_{i=-\infty}^{+\infty} \delta\left(\frac{t - it_p}{t_p}\right) \qquad (4)$$

where $\delta(t)$ is the Dirac function.

If we restrict our analysis to a single entraining period and apply Eq. 1, we find that

$$\begin{aligned} \ln[1 + f(t)] &= m\mu_p t_p \\ &\qquad 0 < t < t_p - t_d' \qquad (5a) \\ &= (m + 1)\mu_p t_p \\ &\qquad t_p - t_d' < t < t_p. \qquad (5b) \end{aligned}$$

These equations indicate that m cohorts are present in the terminal phase between times 0 and $t_p - t_d'$ and $(m + 1)$ cohorts during the rest of the entraining period. Therefore $f(t)$ oscillates between two values $f_{\min}$ and $f_{\max}$ (Fig. 1A) given by

$$\begin{aligned} f_{\min} &= \exp[m\mu_p t_p] - 1 \\ &\qquad 0 < t < t_p - t_d' \qquad (6a) \\ f_{\max} &= \exp[(m + 1)\mu_p t_p] - 1 \\ &\qquad t_p - t_d' < t < t_p. \qquad (6b) \end{aligned}$$

Combining Eq. 6a and b yields

$$\mu_p = \frac{1}{t_p}\ln\left(\frac{1 + f_{\max}}{1 + f_{\min}}\right) = \mu_{f\min,f\max}. \qquad (7)$$

Equation 7 is more general than Eq. 3.

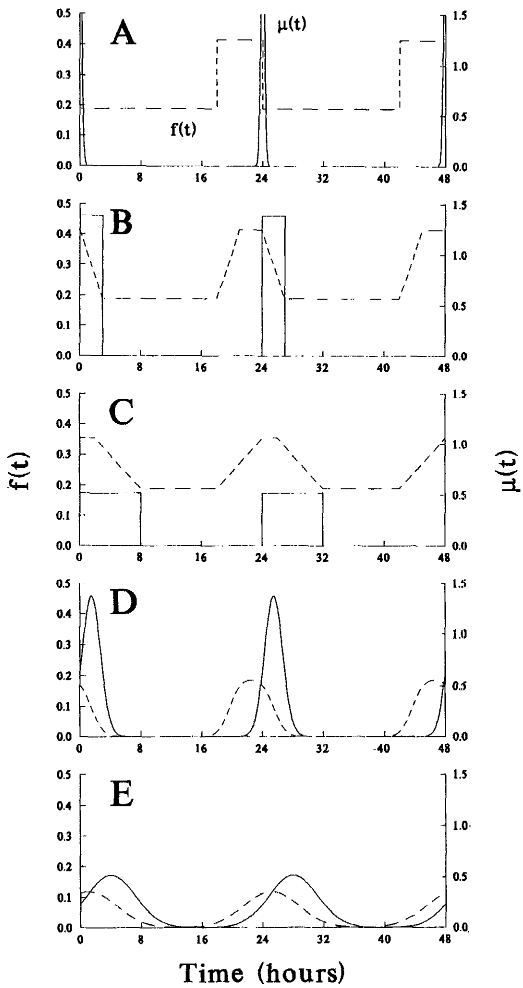


Fig. 1. Instantaneous division rate, $\mu(t)$ (d^{-1}), and fraction of cells in terminal phase, $f(t)$, for phased populations with generation time of 96 h ($\mu_p = 0.173\ d^{-1}$) and periodicity of 24 h. A. Perfectly phased population ($t_c = 0$). $\mu(t)$ is described by a sum of Dirac functions (Eq. 4). The length of the terminal phase, t_d, is 30 h ($m = 1$, $t_d' = 6$ h). B. Unperfectly phased population ($t_c = 3$ h). Other parameters as in panel A. C. Unperfectly phased population ($t_c = 8$ h). Other parameters as in panel A. D. Unperfectly phased population ($t_c = 3$ h). The division rate is no longer constant over the division burst, but shows Gaussian variation. Other parameters as in panel A except for $t_d = 6$ h ($m = 0$). E. Unperfectly phased population ($t_c = 8$ h). Other parameters as in panel D.

When $t_d < t_p$, then $m = 0, f_{\min} = 0$, and Eq. 7 gives Eq. 3.

What happens when the second assumption is not met, i.e. when division is not perfectly phased and extends over a finite interval during the day? Take a schematic case, where $\mu(t)$ is described by a step function:

$$\mu(t) = \mu_p t_p / t_c \qquad 0 < t < t_c \tag{8a}$$
$$= 0 \qquad t_c < t < t_p \tag{8b}$$

where t_c is the time window during which cells divide (Fig. 1B). In what follows, t_d' is assumed smaller than $(t_p - t_d')$, i.e. $t_d' < t_p/2$; the case $t_d' > t_p/2$ is symmetrical. If the division burst is short, i.e. if $t_c < t_d'$, Eq. 1 yields

$$\ln[1 + f(t)] = m\mu_p t_p + \mu_p t_p (t_c - t)/t_c \qquad 0 \le t < t_c \tag{9a}$$
$$= m\mu_p t_p \qquad t_c \le t < t_p - t_d' \tag{9b}$$
$$= m\mu_p t_p + \mu_p t_p (t - t_p + t_d')/t_c \qquad t_p - t_d' \le t < t_c + t_p - t_d' \tag{9c}$$
$$= (m + 1)\mu_p t_p \qquad t_c + t_p - t_d' \le t < t_p. \tag{9d}$$

As long as $t_c < t_d'$, Eq. 7 can still be used to compute μ_p because there are two time windows during which either m (Eq. 9b) or $m + 1$ (Eq. 9d) cohorts are present in toto in the terminal phase (Fig. 1B). As t_c increases (i.e. as phasing becomes less precise), the equations lose their validity. When t_c becomes larger than t_d' but is still lower than $t_p - t_d'$, then the system of equations becomes (Fig. 1C):

$$\ln[1 + f(t)] = m\mu_p t_p + \mu_p t_p t_d'/t_c \qquad 0 \le t < t_c - t_d' \tag{10a}$$
$$= m\mu_p t_p + \mu_p t_p (t_c - t)/t_c \qquad t_c - t_d' \le t < t_c \tag{10b}$$
$$= m\mu_p t_p \qquad t_c \le t < t_p - t_d' \tag{10c}$$
$$= m\mu_p t_p + \mu_p t_p (t - t_p + t_d')/t_c \qquad t_p - t_d' \le t < t_c + t_p - t_d'. \tag{10d}$$

This result implies that Eq. 6a is still valid (*see Eq. 10c*), but Eq. 6b is replaced (*see Eq. 10a*) by

$$f_{\max} < \exp[(m + 1)\mu_p t_p] - 1. \quad (11a)$$

When finally t_c becomes larger than $t_p - t_d'$, then the equations are modified again and as a result Eq. 6a loses its validity:

$$f_{\min} > \exp[m\mu_p t_p] - 1. \quad (11b)$$

These two inequalities imply that the following relation always holds:

$$\mu_p \geq \frac{1}{t_p}\ln\left(\frac{1 + f_{\max}}{1 + f_{\min}}\right) = \mu_{f\min,f\max}. \quad (12)$$

Therefore $\mu_{f\min,f\max}$ can be considered as a lower bound on the specific division rate.

Under optimal culture conditions or when a short terminal event is chosen (e.g. mitosis), t_d is usually shorter than the entraining period (Chang and Carpenter 1985; Campbell and Carpenter 1986) and $m = 0$. The above analysis is still perfectly valid: for a given t_d, as t_c is made to increase (i.e. as phasing becomes less tight), first $f_{\max}$ decreases (Eq. 11a), and then $f_{\min}$, which was initially equal to zero, increases (Eq. 11b). In this case since $m = 0$, Eq. 11a can be used alone and reduces to the classical relation

$$\mu_p \geq \frac{1}{t_p}\ln(1 + f_{\max}) = \mu_{f\max}. \quad (13)$$

In the ocean where cells might have generation times much longer than 1 d (Furnas 1990) as a result of either nutrient limitation in the upper euphotic zone, light limitation near the bottom of the euphotic zone, or temperature limitation in winter, t_d might be longer than the photocycle length t_p and Eq. 12 should be used instead of Eq. 13 to obtain reasonable estimates of minimum cell division rates. If $f_{\min}$ is different from 0, as observed for example for the fraction of *Synechococcus* dividing cells in coastal waters in winter when temperature is probably limiting (Carpenter and Campbell 1988), it is likely that t_d will be longer than the entraining period.

The case of a long terminal event also has some relevance in the context of the recent improvement of the mitotic index method devised by Carpenter and Chang (1988). Their strategy is to use a terminal phase encompassing two cell-cycle phases (e.g. S and G_2 + M, or mitotic and paired cells); the duration of the prolonged terminal phase is determined as twice the time lag between the maxima of the two cell-cycle fractions (Carpenter and Chang 1988)—and not as 1 times this lag as stated incorrectly, for example, by Braunwarth and Sommer (1985). When the sum of the two cell-cycle phases is larger than the two entraining periods, however, this time lag is longer than the entraining period. The correct lag is therefore obtained as the difference between the two maxima plus an integer number of entraining periods. Failing to correct for this effect would cause a dramatic underestimate of t_d and therefore a dramatic overestimate of μ_p. This situation is likely to occur when applying Carpenter and Chang's (1988) method in the field. For example, in the diatom *Thalassiosira weissflogii,* S + G_2 + M lasts 5.9 h under optimal conditions, but can extend to >35 h under temperature-limited conditions (Olson et al. 1986). In a recently isolated strain of prochlorophyte—a dominant photosynthetic procaryote in oligotrophic oceanic waters (Chisholm et al. 1988)—G_2 is always >24 h even under optimal growth conditions (unpubl. observations).

It is possible to go one step further and assess how good the estimates $\mu_{f\max}$ and $\mu_{f\min,f\max}$ are. First, let us consider the case $t_d > t_p$ ($m > 0$), for which $\mu_{f\min,f\max}$ (Eq. 12) is the most appropriate estimate of μ_p (Fig. 2A). When $t_c < t_d'$,

$$\frac{\mu_{f\min,f\max}}{\mu_p} = 1. \quad (14a)$$

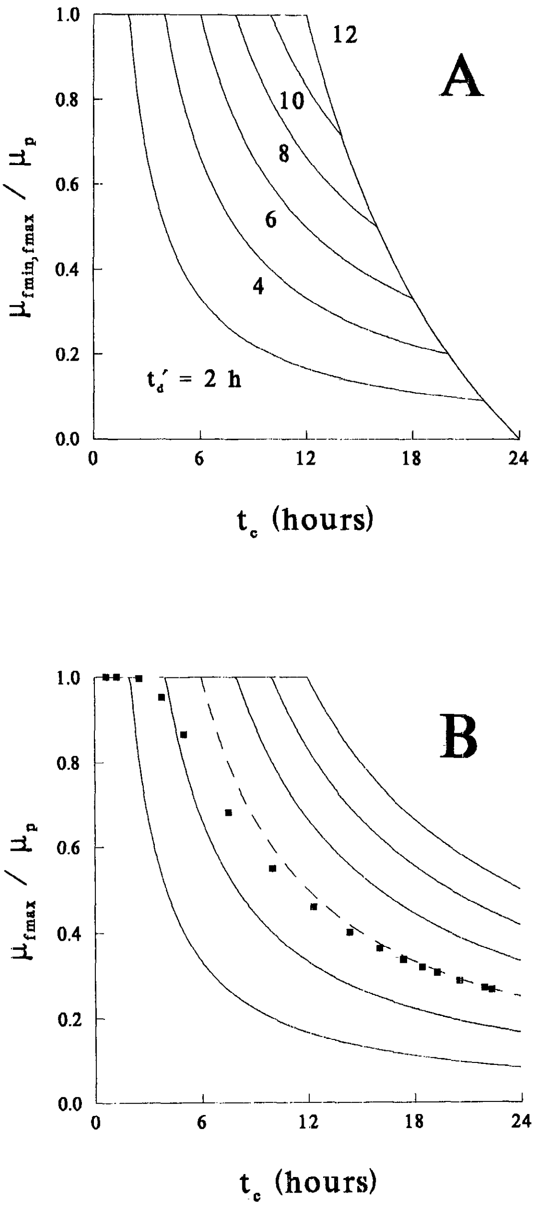
When $t_d' < t_c < t_p - t_d'$, $f_{\max}$ is given by Eq. 10a and

$$\frac{\mu_{f\min,f\max}}{\mu_p} = \frac{t_d'}{t_c}. \quad (14b)$$

Finally, when $t_p - t_d' < t_c < t_p$, the error can be computed in the same way as

$$\frac{\mu_{f\min,f\max}}{\mu_p} = \frac{t_p}{t_c} - 1. \quad (14c)$$

In the simplified case when t_d is shorter than t_p ($m = 0$), then $\mu_{f\max}$ is a better esti-



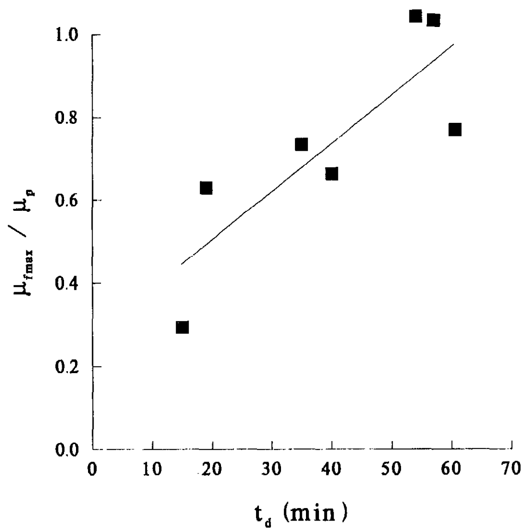


Fig. 3. Relation between $\mu_{f\max}/\mu_p$ and t_d for *Ceratium furca.* Data are extracted from Weiler and Eppley's (1979) tables 2 and 3 ($r^2 = 0.69$, $n = 7$, $P = 0.021$).

$$\frac{\mu_{f\max}}{\mu_p} = \frac{t_d}{t_c} \qquad t_d < t_c < t_p. \qquad (15b)$$

Does this error analysis apply to more realistic populations for which the division rate is a smooth curve rather than a step function? In what follows, the discussion is restricted to the case $m = 0$ ($\mu_{f\max}$); the case $m > 0$ could be treated very similarly. The growth of a population with a Gaussian division rate was simulated for varying phasing tightness (Fig. 1D and E). $f(t)$ was derived from $\mu(t)$ with Eq. 1, assuming $t_d = 6$ h ($m = 0$); $\mu_{f\max}$ was then computed in each case. In order to check the validity of Eq. 15, it is necessary to evaluate the duration of the division burst, t_c, for division rate curves that are not steplike. One approach is to take t_c as the period during which $f(t)$ is different from 0 or higher than a certain

The relation between $\mu_{f\max}/\mu_p$ and t_c established for this more realistic case fits Eq. 15 well (Fig. 2B); the only disagreement appears for t_c values close to t_d (between 4 and 8 h for $t_d = 6$ h), where $\mu_{f\max}$ underestimates μ_p more than predicted in the idealized case.

The most comprehensive experimental data set available in the literature to test these predictions is that of Weiler and Eppley (1979) for *Ceratium furca.* They provide measurements for μ_p, $\mu_{f\ \max}$, and t_d; unfortunately t_c cannot be computed from their data. Nonetheless $\mu_{f\max}/\mu_p$ appears to be linearly related to t_d (Fig. 3), as predicted by Eq. 15, assuming that t_c does not vary greatly for this dinoflagellate. The mitotic index provides a very elegant and powerful method to estimate in situ growth rates of phytoplankton, but the present analysis points out that its application must rely on a detailed understanding of both the cell cycle and the population dynamics of the investigated species.

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