THE CONCEPT OF MICROBIAL AFFINITY FOR LIMITING NUTRIENTS IN STEADY STATE AND RHYTHMIC SYSTEMS

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Lewis A. Molot, B.Sc., M.Sc.

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THESIS ABSTRACT

THE CONCEPT OF MICROBIAL AFFINITY FOR LIMITING NUTRIENTS IN STEADY STATE AND RHYTHMIC SYSTEMS

Lewis A. Molot, Ph.D.
University of Alaska, Fairbanks, 1981
Supervisor: Edward J. Brown

To evaluate the role of biological rhythms in competition for survival, rhythms in cell division and limiting nutrient transport ability induced by light/dark (LD) cycles were investigated for three species of pelagic, freshwater algae growing in phosphoruslimited continuous and serially diluted batch (SDB) cultures.

Nutrient transport ability of nutrient-starved microbial populations was measured as the initial slope (affinity) of a plot of limiting nutrient transport rate (V) versus extracellular limiting nutrient concentration (S). A method was devised for the determination of the affinity in continuous culture (a_T) by monitoring S with time.

Cell division was asynchronous for the green alga, *Selenastrum capricornutum*, grown in LD continuous cultures and a rhythm in a_T for soluble reactive phosphate (Pi) was greatly affected by choice of biomass parameter. Division was strongly phased in LD SDB culture and weakly phased in continuous light (LL) SDB culture, indicating that nutrient perturbations have a greater effect on phasing of division than LD cycles for *S. capricornutum*. A rhythm in Pi transport rate in LD SDB culture was similar to the rhythm in continuous culture a_T when expressed per cell volume or cell dry weight but not when expressed per cell number.

Cell division was phased for the green alga, *Scenedesmus quadricauda*, grown in LD continuous culture. A rhythm in a_T for Pi was not greatly affected by choice of biomass parameter.

Cell division was also rhythmic in LD for the blue-green alga, *Synechococcus* Nageli.

Synechococcus was an extremely efficient Pi transporter at low Pi concentrations in LD continuous culture, indicating that it should be widespread in oligotrophic systems and has probably been overlooked in past floristic studies.

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LIST OF SYMBOLS

Symbol	Meaning
a	Affinity defined by equation (4)($l \cdot biomass^{-1} \cdot time^{-1}$).
a_S	Steady state affinity defined by equation (5).
a_T	Affinity defined by equation (7).
a_T^{MAX}	Maximum a_T.
b_i	Net rate constant for availablity of transport sites ($time^{-1}$).
C_t	Cell concentration at time t ($cells \cdot l^{-1}$).
D	Dilution rate ($time^{-1}$).
K	First order rate constant for nutrient transport ($time^{-1}$).
K_μ	Half saturation constant for growth (Monod model)(μM).
K_v	Half saturation constant for limiting nutrient transport (Michaelis-Menten model)(μM).
LD	Light/dark cycle.
LL	Continuous light.
N	Biomass concentration ($biomass \cdot l^{-1}$).
P	Phosphorus.
PAAP	Provisional algal assay procedure.
Pi	Soluble reactive phosphorus.
Q	Intracellular limiting nutrient concentration (ng-at P$\cdot biomass^{-1}$).
Q_o	Minimum Q.
r	Division rate ($time^{-1}$).
S	Extracellular limiting nutrient concentration.
S*	Radiolabelled extracellular limiting nutrient concentration ($cpm \cdot l^{-1}$).

Symbol	Meaning
S_o	Reservoir concentration of limiting nutrient.
S_T	Threshold concentration of limiting nutrient below which V = 0.
SDB	Serially diluted batch culture.
t	Time.
T_i	Concentration of available transport sites of system i.
V	Initial transport rate of limiting nutrient per unit biomass.
V_{MAX}	Asymptotic value for maximum V (Michaelis-Menten model).
$\bar{W}$	Mean cell size ($\mu m^3 \cdot cell^{-1}$).
μ	Growth rate ($time^{-1}$).
μ_{MAX}	Asymptotic value for maximum μ (Monod and Droop models).

LIST OF EQUATIONS

1. $$V = \frac{V_{MAX}\ (S-S_T)}{K_V + (S-S_T)}$$

2. $$\mu = \frac{\mu_{MAX}\ (S-S_T)}{K\mu + (S-S_T)}$$

3. $$\mu = \frac{\mu_{MAX}(Q-Q_o)}{Q}$$

4. $$\alpha = \lim_{S \to S_T} \left(\frac{\partial V}{\partial S}\right) Q = Q_o$$

5. $$\alpha_S = \frac{DQ}{S-S_T}$$

6. $$\frac{dS}{dt} = D(S_o-S) - VN$$

7. $$\alpha_T = \frac{D(S_o-S)-\frac{dS}{dt}}{N(S-S_T)}$$

8. $$\frac{dQ}{dt} = V - DQ$$

9. $$\frac{dT_i}{dt} = b_i T_i - D\ T_i$$

10. $$T_{i,t} = T_{i,o} \exp\left[\int b_i dt - Dt\right]$$

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INTRODUCTION

One of the major goals of investigators studying the population dynamics of pelagic microorganisms (microplankton) is the prediction of species succession and/or coexistence and hence survival competition in waters whose productivity is limited by low nutrient inputs. Successful prediction of the population dynamics of the primary trophic level would facilitate prediction and manipulation of the population dynamics of all trophic levels and thus has great importance for commercial fisheries, aquaculture and pollution abatement.

Nutrient transport across a cell membrane is of primary importance in nutrient limited systems since microorganisms must acquire a growth-limiting resource before growth can occur. There have been many investigations of the transport and growth kinetics of numerous algal species. The majority of these investigations, however, were not concerned with the effects of light/dark cycles on the kinetics. Recently, there have been several reports on the influence of light/dark cycles on soluble reactive phosphorus (Pi) transport (Chisholm and Stross 1976a, 1976b, Chisholm et al. 1975, Gotham 1977, Sundberg and Nilshammar-Holmvall 1975).

This study examines the Pi transport ability and the cell division rates of three pelagic species of freshwater algae grown in phosphorus (P) limited systems exposed to both continuous light (LL) and light/dark cycles (LD). This study also examines the effects of repeated nutrient perturbations in both LL and LD on cell division rates and transport ability. A kinetic parameter other than the commonly used Michaelis-

Menten half saturation constant is employed in this study because the parameter is useful for investigating biological regulation of transport ability in continuous culture systems. Phosphorus was chosen as the growth-limiting nutrient because the productivity of many aquatic systems is limited by the supply of P and because sensitive radiolabelling techniques have been developed for the detection of low amounts of P in solution (Brown and Button 1979).

LITERATURE REVIEW

INTRODUCTION

The growth response of microorganisms in nutrient-limited, aquatic systems is mediated primarily by the nutrient in shortest supply, but a host of other factors such as light quality, duration and intensity, temperature and chemical composition of the environment can also influence growth (Goldman 1979, Goldman and Mann 1980, Hobson 1974, Senft 1978, Tilman 1977, Williams 1971, Zevenboom 1980). Satisfactory resolution of how these interacting components affect growth has yet to be achieved, even with laboratory models. Predation also influences microplankton growth in natural systems.

Rhythms in metabolic processes of microorganisms may also affect population dynamics of aquatic ecosystems. For example, exposure of eucaryotic organisms to 24 hour LD cycles results in 24 hour (circadian) rhythms in many physiological processes (Bunning 1973). The purposes of many of these rhythms are not entirely understood. However, it seems logical to investigate circadian rhythms in laboratory systems when one considers that aquatic systems at all latitudes are exposed to natural LD cycles.

CIRCADIAN RHYTHMS

Biological rhythms with periods of less than one day to more than one year are well documented (Bünning 1973) although the underlying

molecular mechanism(s) is not well understood (Hastings and Schweiger 1976). When eucaryotic microbial populations are exposed to regularly pulsed environmental variables such as light, temperature or nutrient inputs, certain metabolic processes may become entrained with the same rhythm (Hansche 1964, Herrmann and Schmidt 1965, Rooney et al. 1977). For example, cell division may be restricted to a certain part of the day (synchronized division) when populations are exposed to LD cycles (Cook and James 1960, Ehret et al. 1973, Grant et al. 1978, Marano et al. 1978). Well synchronized populations can be used for age-dependent biochemical studies since relatively large numbers of cells with narrow age distributions can be obtained. For synchronously growing populations of *Euglena gracilis* in enrichment medium exposed to a cycle of 14 hours of light and 10 hours of darkness (LD 14:10), dry weight, RNA, protein, chlorophyll a and cartenoids per cell increased linearly during the light period, while total P per cell increased exponentially. DNA per cell remained constant during the first 8 hours of light and then increased linearly. All measured components decreased during the dark period (Edmunds 1965). Similar findings have been made for other organisms (e.g. Hitchcock 1980, Takabayashi et al. 1976).

A biological clock or pacemaker has been inferred from analyses of the behavior of biological rhythms to be the underlying controlling mechanism. As yet, the mechanism(s) is not well understood but the presence of a clock is assumed when a rhythm meets the following operationally defined criteria (Sweeney 1976a):

1. After a rhythm is established with pulsating environmental conditions (entrained), the rhythm persists under non-pulsed

environmental conditions with an approximately 24 hour rhythm (free-running conditions).

2. The period of the rhythm is not influenced by temperature within the physiological range of the organism (temperature compensation).

3. When a rhythm free-running in darkness (for example) is perturbed by a brief light pulse (15 minutes), its phase is gradually shifted to a new 'local' time after several cycles. A phase response curve can be generated by plotting the phase advance or delay against the time the pulse was administered.

A rhythm meeting the above criteria is called a circadian rhythm. Circadian rhythms are unique to eucaryotic cells and have yet to be found in procaryotic organisms (Brinkmann 1973). Some procaryotes have been synchronized (Goodwin 1969, Lindsey et al. 1971), but synchronization is considered to be a direct response to the transition or on/off step pulsing of an environmental variable rather than a result of entrainment of a circadian clock (Sweeney 1976a). The absence of circadian rhythms in procaryotes may yield clues about the nature of the clock because of fundamental differences in the biological organization of procaryotes and eucaryotes.

PHYSIOLOGICAL STUDIES OF THE BIOLOGICAL CLOCK

Information regarding the nature of the biological clock has been obtained by treating entrained organisms with substances known to affect membranes or with other various inhibitors and by work with

mutants. Treatment of entrained cells with substances that affect membranes has been reviewed by Sweeney (1976b). Some substances, such as heavy water (D_2O) and Li^+, lengthened the cycling period in the dinoflagellate, *Gonyaulax*, and in the plant, *Kalanchoe*, respectively, presumably because of diffusion rate differences compared to H_2O and K^+. Methanol and ethanol lengthened the cycling period of bean leaf movement rhythm whereas digitonin shortened the period. Ethanol caused phase changes in bean leaf movement and *Gonyaulax* bioluminescence rhythms; methanol caused phase changes in bean leaf movement rhythm and compound action potential frequency rhythm in the eye of the gastropod *Aplysia;* valinomycin, an ionophore which promotes the movement of K^+ across membrane barriers, caused a phase change in bean leaf movement and *Gonyaulax* rhythms. Gramicidin, which alters membrane permeability to Na^+ and K^+, and A23187, an ionophore for Ca^{+2} and Mg^{+2}, were ineffective in shifting the phase of bioluminescence in *Gonyaulax*. CCmP (carbonyl cyanide m-chlorophenyl hydrazone), an uncoupler of photo- and oxidative-phosphorylations, also caused a phase shift in *Gonyaulax* bioluminescence. A membrane model of a circadian clock has been proposed (Njus et al. 1974, Njus 1976) in which a photosensitive ion gate allows a particular ion to move across cellular membranes towards equilibrium. Membrane transport of the ion and biochemical coupling of various rhythms to the ion gradient rhythm is also postulated.

Fatty acid composition of the media also influences circadian rhythms, further implicating membrane involvement because of the high fatty acid content of membranes. The period of the free-running spore-forming or conidiation rhythm in a fatty acid-requiring mutant of the mold *Neurospora*

crassa (cel^-) increased with addition of unsaturated fatty acids to the medium (Brody and Martins 1979). Addition of saturated fatty acids had no effect. A rhythm in the ratio of unsaturated to saturated fatty acids of the total lipids in the growing frontal region of a *Neurospora* mycelial mat has also been found (Brody and Martins 1976).

Other studies have shown that oligomycin-resistant mutants of *Neurospora* (oli^r) have shortened period lenths in the free-running conidiation rhythm (Dieckmann and Brody 1979). This is of interest because oligomycin resistance appears to be due to changes in the primary structure of a mitochondrial ATP synthetase protein. Further, this mutant maps very close to another mutant (frq) with an altered conidiation periodicity (Feldman et al. 1979). Growth inhibition by oligomycin of oligomycin-sensitive *Neurospora* can be reversed with the addition of unsaturated fatty acids to the medium (Brody and Forman 1980). The effect of unsaturated fatty acids on the cel^- mutant discussed above is lost when the cel^- and oli^r mutants are crossed. This suggests that unsaturated fatty acids affect the mitochondrial ATP synthetase complex.

Vanden Driessche et al. (1970) found that rifampicin, an inhibitor of mitochondrial and chloroplastic RNA polymerase, did not prevent circadian rhythmicity of photosynthesis in nucleate and anucleate *Acetabularia* (a Charophyte). Actinomycin D, an inhibitor of nuclear DNA transcription, did affect the rhythm in nucleate cells only when treatment with actinomycin D exceeded one week (Vanden Driessche 1966). Therefore the operation of the clock is not dependent on short-lived mRNA. However, short-term pulsing with various protein synthesis inhibitors affected rhythms (Karakashian and Schweiger 1976a, 1976b,

Sullivan 1977, Jacklett 1980), thus supporting the hypothesis that daily protein systhesis is required for expression of circadian rhythms.

CIRCADIAN RHYTHMS AND THE 'PARADOX OF THE PLANKTON'

The 'Paradox of the Plankton', as first stated by Hutchinson (1961), states that coexistence of very large numbers of algal species, apparently occupying the same ecological niche, is a contradiction of the competitive exclusion principle. Laboratory work as well as theoretical models have shown that species cannot coexist under steady state conditions unless the number of growth-limiting substrates is equal to the number of species (Hansen and Hubbell 1980, Kilham and Tilman 1979, Taylor and Williams 1975, Tilman 1977, Titman 1976, Van Gemerden 1974). Therefore, aquatic systems are limited by several factors and/or the systems are never in equilibrium. Williams (1971) postulated that circadian rhythms in nutrient transport rates could account for long-term coexistence of algal species if the times of maximum nutrient transport differed among the species, thereby reducing competition. It is possible, therefore, that natural LD cycles permit coexistence by continually perturbing otherwise steady state aquatic systems.

There are several reports in the literature of rhythms in nutrient transport by eucaryotic algae. *Navicula pelliculosa* exhibited a rhythm in $S_i(OH)_4$ transport rate under a 12 hour LD cycle (Sullivan 1977). The minimum half-saturation constant for transport (K_v) occurred 3 hours and the largest apparent maximum transport rate (V_{MAX}) occurred 5 hours after the beginning of the cell cycle (see page 14, equation[1]). The maximum

ratio, V_{MAX}/K_v, occurred at 4 hours. Chisholm and Stross (1976a, 1976b) and Gotham (1977) found periodicities in K_V and V_{MAX} for Pi transport in *Euglena gracilis* grown under LD 14:10 at 26°C and 24°C respectively. Sundberg and Nilshammar-Holmvall (1975) found a rhythm in the transport rate of Pi at 150 µM KH_2PO_4 for *Scenedesmus obtusiusculus* grown in LD 15:9 at 30°C. A rhythm in the incorporation of radiolabelled phenylalanine into the protein fraction of *Euglena* has been reported for LD 12:12 at 25°C (Feldman 1968). This rhythmicity could represent changes in the rate of synthesis of proteins incorporating phenylalanine, phenylalanine transport rate or the size of the intracellular phenylalanine pool.

Field evidence for periodicity in Pi transport by aquatic microorganisms was obtained by Stross (1980). He found that the time delay between enrichment of P-limited phytoplankton samples with 0.1 µM NaH_2PO_4 and stimulated $^{14}CO_2$ fixation was dependent upon the time of day at which the phosphate was administered.

Even if Williams' (1971) hypothesis is verified in the laboratory, field verification will be extremely difficult, particularly for P-limited aquatic systems, because of difficulties in assigning specific Pi transport rates in multispecies assemblages and in measuring low concentrations of Pi commonly found in P-limited systems. Qualitative information about nutrient competition may be obtained, however, if nutrient transport has a definable phase relationship with some easily measured cellular process such as cell division (Weiler and Chisholm 1976). For example, if the difference between the time of maximum cell division and maximum nutrient transport rate (phase relationship) is species specific and independent of the physiological state of the

organism as well as environmental factors such as temperature and day-length, then a rhythm in cell division can be used as a 'marker' for a transport rhythm in a natural system. Phase relationships have been found to be constant and independent of growth rate in four species of algae (Gotham and Rhee 1980). A constant phase relationship is consistent with the hypothesis that unicellular rhythms are all outputs of a single 'master' clock (McMurry and Hastings 1972). Recently, however, Edmunds and Adams (1981) postulated that rhythms in cell division are not strictly circadian but may, instead, be due to a coupling of a cell division cycle clock and a circadian clock. This would account for synchronously dividing populations in which division occurs daily during a regular time interval but not every cell divides during each interval (phased division).

There are problems with the idea of using cell division rhythms as markers for transport rhythms. Circadian rhythms are known to persist in non-dividing (stationary phase) cells (Chisholm and Stross 1976b, Sulzman and Edmunds 1972). It is not known whether asynchronously dividing populations of eucaryotes have circadian rhythms. Perry (1976) found that division in the diatom, *Thalassiosira pseudonana*, was asynchronous in continuous culture with LD 15:9 and Marra (1980) reported that a diatom, *Lauderia borealis*, maintained in continuous culture with LD 11:13 did not synchronize so it is possible that synchronization may not be universal in natural systems. Table 1 lists some reports of naturally occurring rhythms in aquatic systems, including cell division.

It is thus apparent that circadian rhythms are widespread among eucaryotic microorganisms and that circadian rhythms in the cellular transport of a limiting nutrient may play a role in the species composi-

TABLE 1. Diel rhythms of phytoplankton in natural systems.

Rhythm	Location	Reference
Photosynthesis	North Sea	Dohler and Rosslenbroich (1979)
	East Pacific	Malone (]97])
	Eastern North Pacific	Prezelin and Ley (1980)
	Baltic Sea	Gargas et al. (1979)
	North Atlantic	MacCaul and Platt (1977)
	Lake Taupo, N.Z.	Paerl and MacKenzie (1977)
	Lake Ontario	Harris (1973)
	Lake George, N.Y.	Stross et al. (1973)
		Stross and Pemrick (1974)
		Stross (1980)
	Arctic Ponds, Alaska	Stross (1975)
N_2 Fixation	Western North Pacific	Saino and Hattori (1978)
Nitrate Reductase Activity	Peru Current	Eppley et al. (1970)
Dinoflagellate Vertical Migration	North Atlantic	Staker and Bruno (1980)

TABLE 1 (Continued). Diel rhythms of phytoplankton in natural systems.

Rhythm	Location	Reference
Cell Division	Michigan	Staley (1971)
	North Atlantic	Doyle and Poore (1974)
	Puget Sound	Williamson (1980)
	Eastern North Pacific	Weiler and Chisholm (1976)
		Lewin and Rao (1975)
	North Atlantic	Smayda (1975)

tion of the phytoplankton community. The next section of this thesis discusses the kinetic theory of transport since kinetic theory is the basis for interpretation of transport phenomena.

Kinetic Theory of Nutrient Transport

Previous investigators of nutrient transport have, in general, interpreted observations in terms of Michaelis-Menten enzyme theory. This section outlines some of this theory, discusses its problems, proposes an alternative kinetic approach and then applies the alternative approach to measurements of transport rates.

Any model of nutrient-limited microbial growth applicable to natural systems must incorporate a transport function as a first step in simulating population dynamics and must not be restricted to steady-state transport. The most widely used transport function is the Michaelis-Menten equation from simple enzyme kinetics, as modified by Caperon and Meyer (1972),

$$V = \frac{V_{MAX}(S-S_T)}{K_V + (S-S_T)} \quad , \qquad (1)$$

where V is the transport rate of a nutrient per unit biomass, V_{MAX} is the maximum transport rate, S is the extracellular limiting nutrient concentration, S_T is the intercept on the abscissa ($S_T = S$ when $V = 0$), and K_V is the half-saturation constant equal to $S-S_T$ when $V = 1/2\ V_{MAX}$. Inhibition effects of intracellular fractions may be included in equation (1) (Engel 1977, Rhee 1980) in which case V_{MAX} and K_V are statistically determined when inhibition is absent (Rhee 1974).

Two widely used equations for the prediction of growth rate are the Monod equation, which relates growth rate (μ) to S. The first (modified from Monod 1942) is,

$$\mu = \frac{\mu_{MAX}\ (S-S_T)}{(S-S_T) + K_\mu}\ , \qquad (2)$$

where μ_{MAX} is the maximum growth rate and K_μ is the half-saturation constant for growth ($K_\mu = S-S_T$ when $\mu = \mu_{MAX}/2$). The second is the cell quota equation, which relates μ to the intracellular limiting nutrient concentration (Q),

$$\mu = \frac{\mu_{MAX}(Q-Q_o)}{Q} \qquad (3)$$

where Q_o is the minimum Q (Droop 1968, 1973, 1974, 1975). Equations (2) and (3) are steady state formulations.

The applicability of models derived from steady state equations to describe natural systems is questionable although they are sometimes used (e.g. Titman 1976, Tilman 1977) since it is implied that organisms can adapt instantaneously to a perturbation and that circadian rhythms are insignificant. The enrichment experiments of Stross (1980), however, showed that time delays between phosphate enrichment and stimulated $^{14}CO_2$ fixation in a P-limited lake occur and the magnitude of the delay depends upon the time of day the fertilzer is applied. Therefore a more accurate predictor of growth rate would be time-dependent (Chisholm and Nobbs 1976).

Kinetic models are potentially useful for predicting the outcome of competition between species for a limiting nutrient (Taylor and Williams 1975, Tilman 1977, Titman 1976). Some investigators have compared species on the basis of their values for K_V (Hecky and Kilham 1974, Sharp et al. 1979). Thus a species with a large K_V is assumed to be less efficient

transporting a limiting nutrient at low concentrations than a species with a smaller K_V. However, such comparisions are valid only when the values of V_{MAX} are identical for all species compared (Tilman 1977, Titman 1976).

An alternative kinetic parameter is the initial slope of a V versus S plot. The discussion below provides a general theoretical framework for the interpretation of initial slopes and describes the utility of this parameter for continuous culture studies.

The initial slope of a V versus S plot is,

$$\alpha = \lim_{S \to S_T} \left(\frac{\partial V}{\partial S}\right)_{Q=Q_o} \quad , \tag{4}$$

with α being the microorganism's affinity for a limiting nutrient (initial slope). Affinity, then, is the maximum slope of a plot of transport rate versus limiting nutrient concentration and is measured in a non-rhythmic, nutrient-starved population to avoid inhibition of transport by intracellular fractions (Button 1978, Law and Button 1977) and to avoid circadian rhythms.

Equation (4) can be applied to any V = f(S). The Michaelis-Menten equation [equation (1)] is only one example of a function that can be fit to V versus S data. Other mathematical formulations are possible such as those listed by Jassby and Platt (1976). They fit eight different expressions to photosynthetic rate versus light intensity data. The relationship between photosynthetic rate and light intensity is a saturation curve similar to a V versus S curve, excluding photoinhibition. Only two parameters are needed in these equations - the initial

slope and the maximum photosynthetic rate. All of the equations are reduced to a linear form in the vicinity of the x-intercept.

There are numerous reports of short-term, perturbation studies of batch and steady state populations in which the nutrient transport rate increases as the concentration of intracellular nutrient decreases (i.e., as the period of nutrient-starvation increases) for a given extracellular nutrient concentration (Brown and Harris 1978, Chen 1974, Fuhs et al. 1972, Rhee 1973, Rosenberg et al. 1969, 1977, Vaccaro and Jannasch 1966, Zevenboom and Mur 1979a). However, steady state net transport rates (μQ) of a limiting nutrient are positively correlated with Q in several algal species (Brown and Button 1979, Droop 1973, 1974, Lang 1980, Zevenboom and Mur 1979b). There are several hypotheses that can account for this apparent discrepancy. One hypothesis is that nutrient limitation produced in nutrient-limited continuous culture is severe enough to reduce the efficiency of transport systems and that feedback inhibition does not occur until an optimal intracellular nutrient level is reached. An alternative hypothesis is that, for microorganisms with more than one transport system for a given substrate, the 'high affinity' system(s), which operates more efficiently at low S, is not affected by Q while the 'low affinity' system(s) operating at higher S is affected by Q. However, the data of Chen (1974) for the bacterium, *Corynebacterium bovis*, and Zevenboom and Mur (1979b) for the blue-green alga, *Oscillatoria agardhii*, are not consistent with the first hypothesis because they observed a decrease in V at high S with increasing dilution rate or Q. Their data are consistent with the second hypothesis. The data of Rosenberg et al. (1977)

for the bacterium, *Escherichia coli*, are not consistent with the second hypothesis because they observed intracellular nutrient inhibition of the 'high affinity' system, although the concentrations used were large.

In addition to the conflicting data which makes interpretation of transport inhibition difficult, there have also been many reports of nutrient transport that do not adhere to simple Michaelis-Menten kinetics. Deviations from simple Michaelis-Menten kinetics have been observed for Pi transport by *Neurospora crassa* (Beever and Burns 1977, Burns and Beever 1977), the green algae *Selenastrum capricornutum* rown et al. 1978) and *Chlorella pyrenoidosa* (Jeanjean 1973), nitrate transport by *Oscillatoria agardhii* (Zevenboom and Mur 1979a), histidine transport by the yeast, *Saccharomyces cerevisiae*, (Crabeel and Grenson 1970) and arginine transport by a marine bacterium, *Vibrio* sp. (Geesey and Morita 1979). Only the work of Brown et al. (1978) with *Selenastrum*, Zevenboom and Mur (1979a) with *Oscillatoria* and Geesey and Morita (1979) with *Vibrio* used environmentally realistic concentrations. The kinetic data as well as results from studies with mutants of *E. coli* (Argast and Boos 1980, Gerdes et al. 1977, Medveczky and Rosenberg 1971, Rosenberg et al. 1977, Tomassen and Lugtenberg 1980, Willsky et al. 1973, Willsky and Malamy 1980a, 1980b) are consistent with the hypothesis that two simultaneously operating, simple, Michaelis-Menten transport mechanisms, derepressed or activated selectively (Brown et al. 1978) and most likely having different values for S_T, occur in these organisms. The 'high affinity' systems are more efficient at low S and the 'low affinity' systems are more efficient at high S. Equation (4) describes the initial slope of the 'high affinity' system in a microorganism with more than one transport mechanism.

If the microorganism demonstrates simple Michaelis-Menten kinetics for transport of a nutrient (i.e. one transport system with a single affinity) then from equations (1) and (4) $a = V_{MAX}/K_V$ for a starved population (Healey 1980). However, K_V can only be accurately calculated with knowledge of S_T, although values for K_V are generally an order of magnitude greater than S_T. Therefore, a cannot be accurately extrapolated from data obtained at high concentrations. In addition, extrapolation may cause the investigator to overlook the presence of a 'high affinity' system operating only at low concentrations (Brown et al. 1978, Geesey and Morita 1979, Zevenboom and Mur 1979b). In short, there is no substitution for measurements of transport rates at low substrate concentrations.

An approximation of affinity as defined in equation (4) can be obtained in nutrient-limited, steady-state, continuous culture for a given temperature and light (if applicable) regime. The steady-state affinity, a_S, has been defined by Brown and Button (1979) as,

$$a_S = \frac{DQ}{S-S_T} \quad , \tag{5}$$

where D is the dilution rate. Although a_S does not strictly adhere to the conditions that $S \rightarrow S_T$ and $Q = Q_o$, it nevertheless is a good approximation of a as long as the culture is nutrient limited. For example, the maximum Q (Q_{MAX}) for P in P-limited, steady state continuous culture of *Selenastrum* is only 6.4% of Q_{MAX} for P in nitrogen-limited batch

culture. Extracellular Pi concentrations range from 15 to only 60 nM in P-limited, steady state continuous culture (Brown and Button 1979). Steady state, Pi affinities have been obtained for several microorganisms. The values for *Selenastrum*, the yeast *Rhodotorula rubra*, the green alga *Scenedesmus quadricauda* and the blue-green alga *Synechococcus* Nageli are 2.5, 30.7, 4.7 and 68.6 $l \cdot (mg\ dry\ weight \cdot day)^{-1}$ respectively (Brown and Button unpublished data, Lang and Brown 1981, Robertson and Button 1979). In practice, S_T and a_S are statistically determined with a geometric mean regression analysis (see Ricker 1973) using data obtained from several dilution rates (Lang and Brown 1981). It may also be possible to measure S_T in a substrate-limited, batch culture in stationary phase.

Zevenboom and Mur (1979b) found that perturbations of nitrogen-limited steady state *Oscillatoria agardhii* cultures with nitrate resulted in biphasic hyperbolic V versus S plots. The initial slope of the 'high affinity' system was approximated by a_S. The initial slope of the curve fit to high values of S underestimated a_S.

One would expect Pi concentrations of P-limited, natural systems to be in the neighborhood of the threshold values of the dominant microplankton species. The Pi threshold values for *Selenastrum*, *Rhodotorula*, *Scenedesmus* and *Synechococcus* using radiolabelling, tracer techniques are 10, 3, 36, and 2 nM Pi respectively (Brown and Button 1979, Lang and Brown 1981, Robertson and Button 1979) which are below the limit of Pi detectability using the spectrophotometric method. Therefore, a system with Pi detectable by the spectrophotometric method may not be P-limited, although the spectrophotometric can overestimate Pi concentrations (Brown et al. 1978).

It is theoretically possible to calculate a for a limiting substrate in continuous culture at a given dilution rate provided the threshold is known for the organism in question. This follows from a consideration of the rate of change of substrate in the growth vessel,

$$dS/dt = D(S_o - S) - VN \qquad (6)$$

where S_o is the incoming substrate concentration, S is the substrate concentration in the growth vessel, V is the transport rate per unit biomass and N is the biomass concentration. Rearranging equation (6) and substituting into equation (4),

$$a_T = \frac{D(S_o - S) - dS/dt}{N(S - S_T)} \qquad (7)$$

Calculation of a_T from equation (7) requires a series of measurements over time. Therefore, equation (7) could prove useful for monitoring circadian rhythms in affinity. N and S are the mean values of two successive measurements and dS/dt can be approximated by $\Delta S/\Delta t$ although functions can be fit to the time series data. Frequent measurements should be made in order to minimize biomass changes between measurements. Equation (7) has not, to my knowledge, been used to calculate affinities. The time series data of Gotham (1977) for *Euglena gracilis* grown in P-limited continuous culture and LD 14:10 were used to compare V_{MAX}/K_V and a_T (Fig. 1), assuming a threshold value of 10 nM Pi. The curves are very similar.

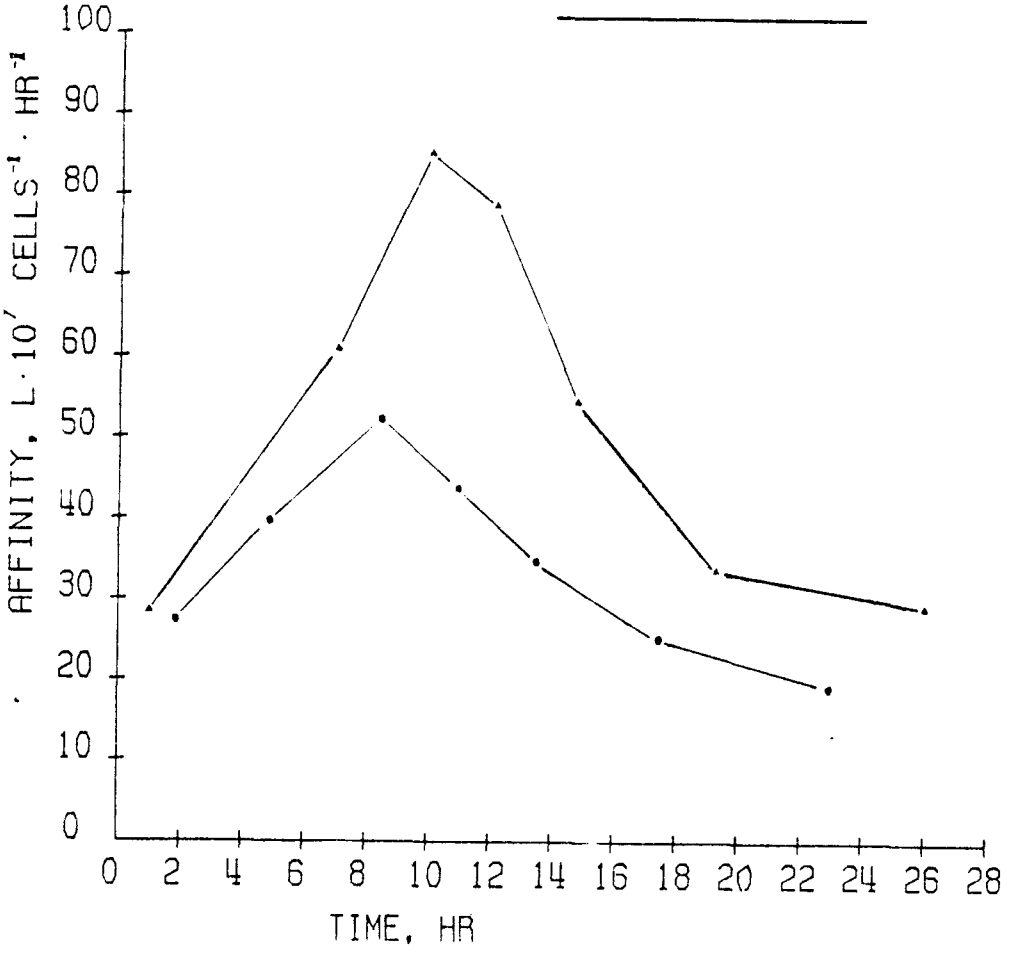

Figure 1. Affinity versus time for *Euglena gracilis* grown in LD, P-limited continuous culture with D = 0.028 hr^{-1} and S_o = 5.28 µM P. A threshold (S_T) of 10 nM Pi was assumed. Symbols: (▲) V_{MAX}/K_V; (●) a_T; shaded bar represents the dark period. Data reworked from Gotham (1977)

Equation (6) has been used to calculate diel changes in nitrogen transport rates in continuous culture (Caperon and Ziemann 1976, Eppley et al. 1971) but changes in V may have been due to changes in S as well as biological regulation. Normalization of V with $S-S_T$ [equation (7)] facilitates investigation of biological regulation of transport by emphasizing potential changes in part of the V versus S curve - the inital slope, provided the substrate in question is growth-limiting. Conway et al. (1976) used equation (6) to monitor long-term changes in the net transport rates of several limiting and non-limiting substrates in nutrient-perturbed continuous culture. However, their interpretation of the changes in transport rates did not account for the possibilities of feedback inhibition or diel changes in V due to LD cycles.

The affinity is only one kinetic parameter and cannot be used as an index of competitive ability by itself. If a system is not perturbed with nutrients, has continually low S and is devoid of allelopathic (excreted chemical) interactions and high grazing rates (see Keating 1977, Ryther and Sanders 1980), dominance, as determined by the highest transport rate, can probably be predicted with a_S, S and S_T. If a system is perturbed with nutrients, transport rates at saturating substrate concentrations and feedback effects would be required. Storage capacities would also be important (Lang 1980). Kinetic parameter values are also affected by physical and chemical environmental factors such as light, temperature and pH (Chen 1974, Fuhs et al. 1972, Goldman 1977, 1979, Reshkin and Knauer 1979, Rosenberg et al. 1969, Senft 1978, Zevenboom et al. 1980). Circadian rhythms in transport rates caused by LD cycles may also influence the outcome of competition (Williams 1971).

The organism which is the most efficient sequesterer of a limiting nutrient should dominate a system, regardless of its ensuing growth rate. Relative population biomass is important (Kayser 1979) but this should not be confused with μ. In steady state, $V = \mu Q$ but the growth rate of all species approaches the same value which is the dilution rate, D, in continuous culture. In perturbed systems, $V \neq \mu Q$. This follows from a consideration of the rate of change of Q in continuous flow growth vessels,

$$\frac{dQ}{dt} = V - DQ \quad , \tag{8}$$

which is the difference between the net transport and washout rates. Equation (8) is a summation of rates and is not based on physiological processes. At steady state, $dQ/dt = 0$, $\mu = D$ and therefore $V = \mu Q$. When $dQ/dt \neq 0$, $V = dQ/dt + DQ$ and $\mu \neq D$. In batch culture, $D = 0$ and $V = dQ/dt$. Studies with non-steady state populations have shown that μ is affected by Q, or some fraction of Q, and that Q affects V (Brown et al. 1978, Droop 1975, Fuhs et al. 1972, Rosenberg et al. 1969, 1977, Vaccaro and Jannasch 1966) but equation (8) does not provide an adequate description of this relationship.

No attempt will be made to relate a to μ in this study. Transport and growth rates are considered loosely coupled processes for the reasons stated above although a_S appears to be independent of μ because a_S is derived from measurements made at several steady state dilution (growth) rates. The apparent independence of a_S and μ could be due to the severe nutrient limitation inherent in P-limited continuous cultures.

One further point is that the affinity model does not account for increases in S at low D observed with some microorganisms (see the review by Rhee 1980). Explanations of this 'hookback' phenomenon will undoubtedly come from biochemical studies.

In summary, the affinity, a, for a nutrient by a microorganism is the initial slope of a V versus S curve and is measured in a non-rhythmic population starved for that nutrient and at very low nutrient concentrations. The affinity will have a constant value within these restricted conditions. It is postulated that the steady state affinity, a_s, approximates a. Equation (7) can be used to detect rhythms in affinity in entrained populations.

OBJECTIVES

This study examines Pi transport and cell division rhythms in three pelagic species of freshwater algae grown in LL and LD, P-limited systems. The affinity parameter, a_T, is used to investigate the kinetics of biological regulation of transport ability at very low Pi concentrations in perturbed continuous cultures. This study also provides comparative information on the effects of LD cycles on eucaryotes (possessing circadian clocks) and procaryotes (without circadian clocks).

MATERIALS AND METHODS

MICROORGANISMS

Microorganisms used were the green algae *Selenastrum capricornutum* Printz, described by Brown and Button (1979) and *Scenedesmus quadricauda* (UTEX 76, University of Texas Culture Collection, Austin, Texas), and the blue-green alga *Synechococcus* Nageli (ATCC 27146, American Type Culture Collection, Rockville, Maryland). All cultures were maintained bacteria free.

Stock cultures were maintained at 4°C in liquid Provisional Algal Assay Procedure (PAAP) medium (Toerien et al. 1971) described below. Transfers were made every 3 weeks and these cultures were grown for 1 week at 25 ± 2°C on a rotary shaker before being stored. 'Cool-White' fluorescent light provided continuous incident radiation of 8×10^{15} quanta$\cdot cm^{-2} \cdot sec^{-1}$. A small amount of each stock culture was routinely spread on nutrient or plate count agar to check for bacterial and/or fungal contamination.

MEDIA

The organisms were grown in PAAP culture medium prepared from the stock solutions listed in Table 2. Stock solutions were stored in the dark at 4°C and were made fresh every 6 months. Unmodified PAAP contains 20 μM K_2HPO_4 (batch strength). Continuous culture and serially diluted batch culture (see below) media were buffered with 12.1 g Tris (hydro-

TABLE 2. Composition of culture media.

	Compound	Concentration of stock solution ($g \cdot l^{-1}$)	Volume added to media ($ml \cdot l^{-1}$)	
			Continuous	Batch
1.	$FeCl_3 \cdot 6H_2O$[a]	0.053	2.5	10
	Na_2EDTA[a]	0.111		
2.	$NaHCO_3$	5.0	---	10
		100.0[c]	1	--
3.	$MgCl_2 \cdot 6H_2O$[b]	4.05		
	$MgSO_4 \cdot 7H_2O$[b]	4.99	2	10
	$CaCl_2 \cdot 2H_2O$[b]	1.179		
4.	$NaNO_3$	8.50	2	10
5.	K_2HPO_4[c]	0.1679	Variable	Variable
6.	KCl	0.70	2	10
7.	$MnCl_2 \cdot 4H_2O$[d]	1.385		
	$ZnCl_2$[d]	0.109		
	$CoSO_4 \cdot 7H_2O$[d]	0.0065	0.25	1
	$CuCl_2 \cdot 2H_2O$[d]	0.00004		
8.	$Na_2B_2O_4 \cdot 8H_2O$[e]	1.045		
	$Na_2MoO_4 \cdot 2H_2O$[e]	0.024	0.25	1
	$Na_2EDTA \cdot 2H_2O$[e]	0.00744		

[a] Stored together.

[b, d, e] Mixed separately but stored together.

[c] Autoclaved in ampules.

xymethyl)-aminomethane dissolved in 100 ml of water and 6.5 ml of concentrated hydrochloric acid per 20 l of medium. The pH was 7.8 after autoclaving. Water for the stock solutions and media was de-ionized and then filtered through a 0.22 μm membrane filter.

RADIOPHOSPHATE LABELLING

Phosphorus was labelled by adding carrier-free radiophosphorus (^{32}P) to the culture media. The ^{32}P was obtained as orthophosphate in dilute hydrochloric acid, pH 2-3. Greater than 98% of the ^{32}P was present as orthophosphate and less than 1% as pyrophosphate or polyphosphate. Radioactivity was measured by placing samples into a 2:1 toluene/Triton x-100 cocktail (v/v) and counting in a liquid scintillation counter.

CONTINUOUS CULTURE

The continuous culture apparatus (Fig. 2) was a single phase, single stage system (Brown and Button, 1979). Medium was pumped from a 20 l carboy with a variable speed peristaltic pump into a silicone stoppered, 500 ml, flat bottom boiling flask containing a magnetic stir bar. The dilution rate for all experiments ranged from 0.027 to 0.030 hr^{-1}. The incident radiation was 3×10^{16} quanta$\cdot cm^{-2} \cdot$sec. When required, a light/dark cycle of 14 hours of light and 10 hours of dark (LD 14:10) was maintained with a programmable timer. Each culture was started by inoculating 7 ml of exponentially growing cells into a half-full growth vessel and allowing the cells to grow undiluted (batch) for 1-2 days after which

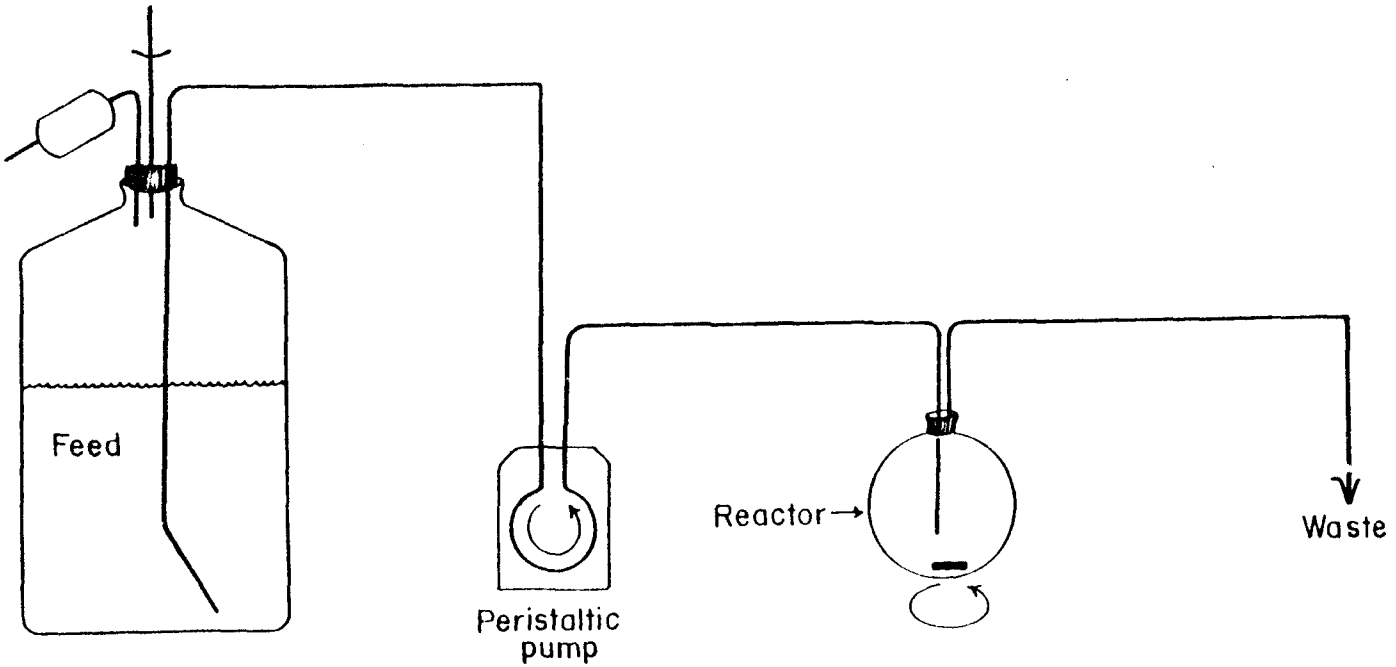

Figure 2. Schematic diagram of single stage, single phase continuous culture apparatus.

the pump was started for continuous flow culture. All experiments were conducted at 25 ± 2°C.

Samples of *Selenastrum* and *Synechococcus* cultures from the growth vessel were obtained by clamping off the effluent line and inserting a sterile, 20 gauge, syringe needle through the stopper. Within the needle was a 0.25 mm (inside diameter), stainless steel capillary tube which was inserted through the needle into the vessel. The needle was withdrawn so that the vessel contents flowed through the capillary tube (Robertson and Button 1979). The effluent was continuously filtered at 330 mm Hg through a 0.45 µm membrane filter or collected unfiltered. Samples of *Scenedesmus* culture were continuously collected in a 50 ml syringe fitted with an 18 gauge needle. When sampling during the dark period, the cultures were covered with foil and light was provided by a dim, incandescent bulb.

SERIALLY DILUTED BATCH (SDB) CULTURE

Serially diluted batch cultures were diluted 2:1 with fresh medium daily (at the same time) by removing half of the culture contents and adding an equal volume of fresh medium or by doubling the culture content volume with fresh medium. Daily dilutions were begun after each starter culture reached the late exponential phase of growth. Cultures were considered stable after 6 dilutions. Culture flasks were shaken continuously on a rotary shaker or stirred with a magnetic stir bar. Incident radiation was 6×10^{15} quanta$\cdot$cm$^{-2}\cdot$sec.

BIOMASS MEASUREMENTS

Total cell number, size distributions and cell volume measurements were determined with an electronic particle counter and pulse height analyzer.

Fifty ml of culture were filtered at 330 mm Hg through a rinsed, dried (60°C for 24 hours) and pre-weighed 0.45 µm membrane filter. The filter was dried again for 24 hours at 60°C and weighed. Cell dry weights were then determined from the difference between the weighings and the total cellular volume filtered.

GLASSWARE CONDITIONING

All new glassware was soaked for one week in strong hydrochloric acid, rinsed several times, filled with de-ionized water, autoclaved and left standing for several days filled with water before final rinsing. For use as a growth vessel, the glass was further conditioned by filling with a stock culture of algae and left standing for several days before use. For reuse, growth vessels were thoroughly rinsed, filled with deionized water and left standing overnight.

PHOSPHORUS ANALYSES

The procedure for analyses of intracellular P and solution Pi have been described by Brown and Button (1979). Reagent stock solutions are

listed in Table 3. The reagent mixture for analysis of Pi consisted of 2 ml of ammonium molybdate, 5 ml of H_2SO_4, 1 ml of potassium antimonyl tartrate and 2 ml of ascorbic acid. Two ml of culture filtrate were mixed with 0.2 ml of the reagent mixture in a test tube and incubated at room temperature for 20-30 minutes. One-half ml of isoamyl alcohol was then added to extract the phosphomolybdate complex. After addition of the isoamyl alcohol, the sample was mixed and the alcohol fraction removed and placed into a scintillation vial. The alcohol extraction was repeated twice more and the fractions were combined. The aqueous fraction was then pipetted into a separate vial. The test tube and pipet used to remove the alcohol were rinsed with 1 ml of ethanol which was then poured into a third scintillation vial. Ten ml of toluene/Triton-x-100 scintillation cocktail were added to each vial. Each sample was analyzed in duplicate. Pi in the culture filtrate was determined by multiplying the fraction of the total ^{32}P in the combined isoamyl alcohol extracts and ethanol rinse by the total ^{31}P concentration in the culture filtrate.

TRANSPORT RATE MEASUREMENTS

Duplicate 50 ml samples were taken from non-radiolabelled SDB cultures and placed into sterile, 125 ml Erlenmeyer flasks. Sterile phosphate (final concentration of 3.25 μM K_2HPO_4) was added to one flask and enough ^{32}P to make 10^5 cpm/ml was added to both flasks. Five ml were withdrawn 0.5, 1, 2, 5, 10, 20 and 45 minutes after addition of the K_2HPO_4 and ^{32}P, filtered through 0.45 μm membrane filters (which were

TABLE 3. Phosphorus assay reagents.

Reagent	Concentration	Storage
1. Ammonium molybdate	30 $g \cdot l^{-1}$	In plastic bottle
2. H_2SO_4	4.85 N	In glass bottle
3. Potassium antimonyl tartrate	1.36 $g \cdot l^{-1}$	Refrigerate in plastic bottle
4. Ascorbic acid	54 $g \cdot l^{-1}$	Freeze in aliquots

pre-rinsed with 5 ml of water), rinsed again with 5 ml of water and placed in scintillation vials containing 10 ml of scintillation cocktail.

Transport kinetics were first order, i.e. $dS^*/dt = KS^*$ where S^* is the amount of ^{32}P in solution and K is the rate constant. Therefore, K was the slope of ln % S^* versus time. Data were not used after more than 5% of the ^{32}P was taken up by the algae. Orthophosphate transport rates were then calculated as KS/N where S and N are the added unlabelled orthophosphate and biomass concentrations respectively (Brown et al. 1978).

RESULTS

SELENASTRUM CAPRICORNUTUM

Continuous Culture

The results for *Selenastrum capricornutum* are based on four P-limited (0.96 μM P) continuous cultures with a dilution rate (D) of 0.028 ± 0.002 hr^{-1} under LD, free run and steady state (LL) conditions (Figs. 3-6). The first 24 hours of constant light after repeated LD cycles is referred to as the free run and the 'subjective' dark in the free run is the time during which the lights are normally off.

Cell division patterns in LD and free run conditions are similar to steady state (Fig. 3a) and are therefore asynchronous. The relative increases in cell concentration ([maximum - minimum]/minimum) were 11, 20 and 9% for LD, free run and steady state conditions respectively. The coefficient of variation for cell concentration for any given sampling (reported cell concentrations are means of three replicate counts of each sample) was typically less than 3%.

Rhythms with periods of approximately 24 hours were observed in mean cell size, $\bar{W}$, during LD and free run conditions (Fig. 3b) and Q during LD conditions (Fig. 4a). Q in free run continued to decrease in the 'subjective' dark. Rhythms in $\bar{W}$ are probably circadian, according to the criteria of Sweeney (1976a, see page 4) although temperature compensation and phase shifting were not studied. The relative increases in Q during LD, free run and steady state conditions were 54, 21 and 12% respectively.

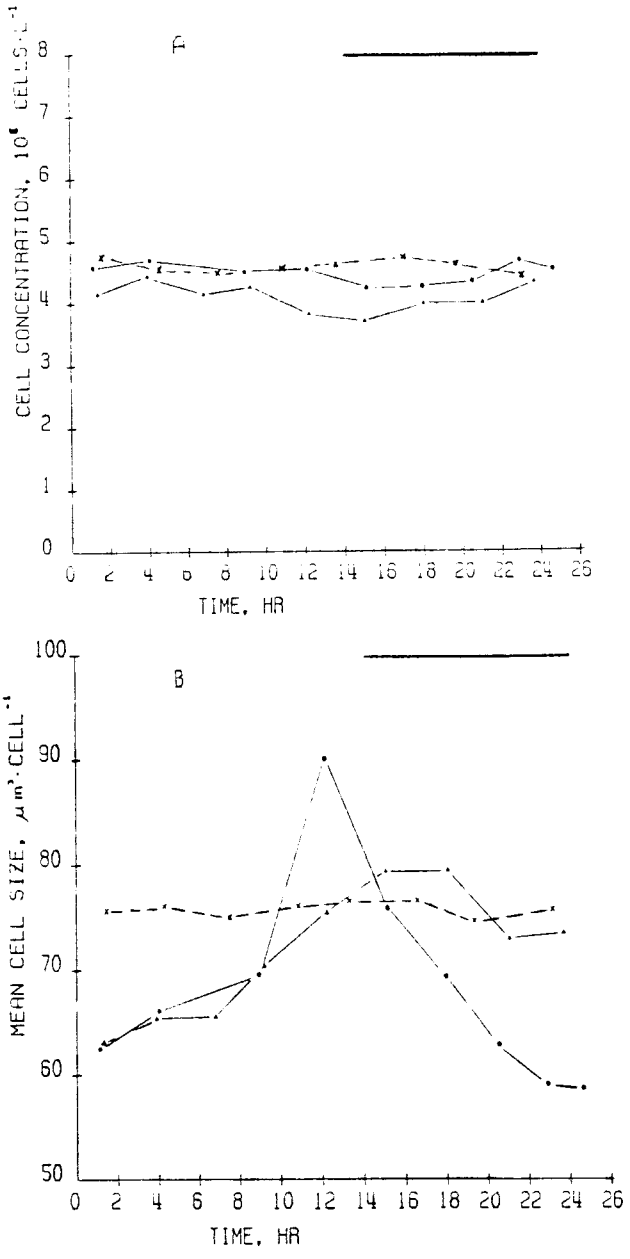

Figure 3. (a) Cell concentration versus time and (b) mean cell size versus time for *Selenastrum capricornutum* grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; (x) LL; shaded bar represents dark period in LD and 'subjective' dark in free run.

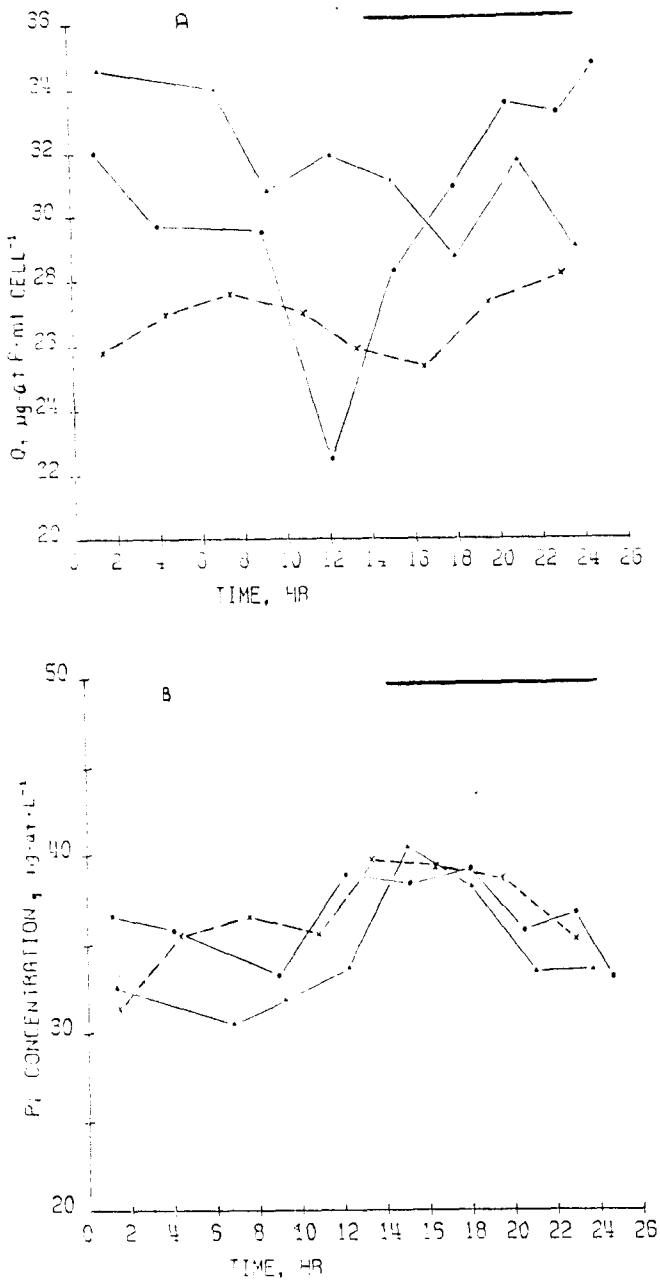

Figure 4. (a) Intracellular P concentration, Q, versus time and (b) dissolved Pi concentration versus time for *Selenastrum capricornutum* grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; (✗) LL; shaded bar represents dark period in LD and 'subjective' dark in free run.

Pi data for three experiments are shown in Fig. 4b. The concentrations ranged from 30 to 41 ng-at $P \cdot l^{-1}$ with a slight increase at 1400 hours (the lights were turned on at 0000 hours).

Pi affinities, a_T, for LD, free run and LL conditions are calculated from equation (7) using S_T = 10 nM Pi (Fig. 5). The relative changes for LD, free run and steady state conditions were 21, 26, and 22% respectively and the mean a_T values with 95% confidence limits were 0.21 ± 0.02, 0.29 ± 0.03 and 0.22 ± 0.02 $1 \cdot 10^8$ $cells^{-1} \cdot hr^{-1}$ respectively. Light conditions appear to have a negligible effect on a_T. The steady state affinity, a_S, with 95% confidence limits obtained from a geometric mean regression (Ricker 1973) of DQ versus Pi (E. J. Brown, unpublished data) for six dilution rates was 0.24 ± 0.06 $1 \cdot 10^8 cells^{-1} \cdot hr^{-1}$, indicating that a_T compares well to a_S on a per cell basis for *Selenastrum*. Since the conditions of low Q and low S are met in the continuous cultures, a_T in steady state is probably a good estimate of a_S and therefore of a.

The affinities expressed on a cell volume basis are shown in Fig. 6a. There is now a pronounced 24 hour rhythm for a_T in LD and free run conditions compared to the variability observed in steady state a_T, with maxima occurring shortly after the lights are turned on. The relative changes were 68, 52, and 22% for LD, free run and steady state conditions respectively compared to 21, 26 and 22% when expressed on a per cell basis. The mean a_T in steady state with 95% confidence limits was 28.8 ± 1.8 $l \cdot ml\ cell^{-1} \cdot hr^{-1}$. A dry weight conversion factor (Lang 1980) and a ratio of 1.07 for mean to median cell size was used to convert the LL a_S value with 95% confidence limits of 0.104 ± 0.021 $l \cdot mg\ dry\ wgt^{-1} \cdot hr^{-1}$

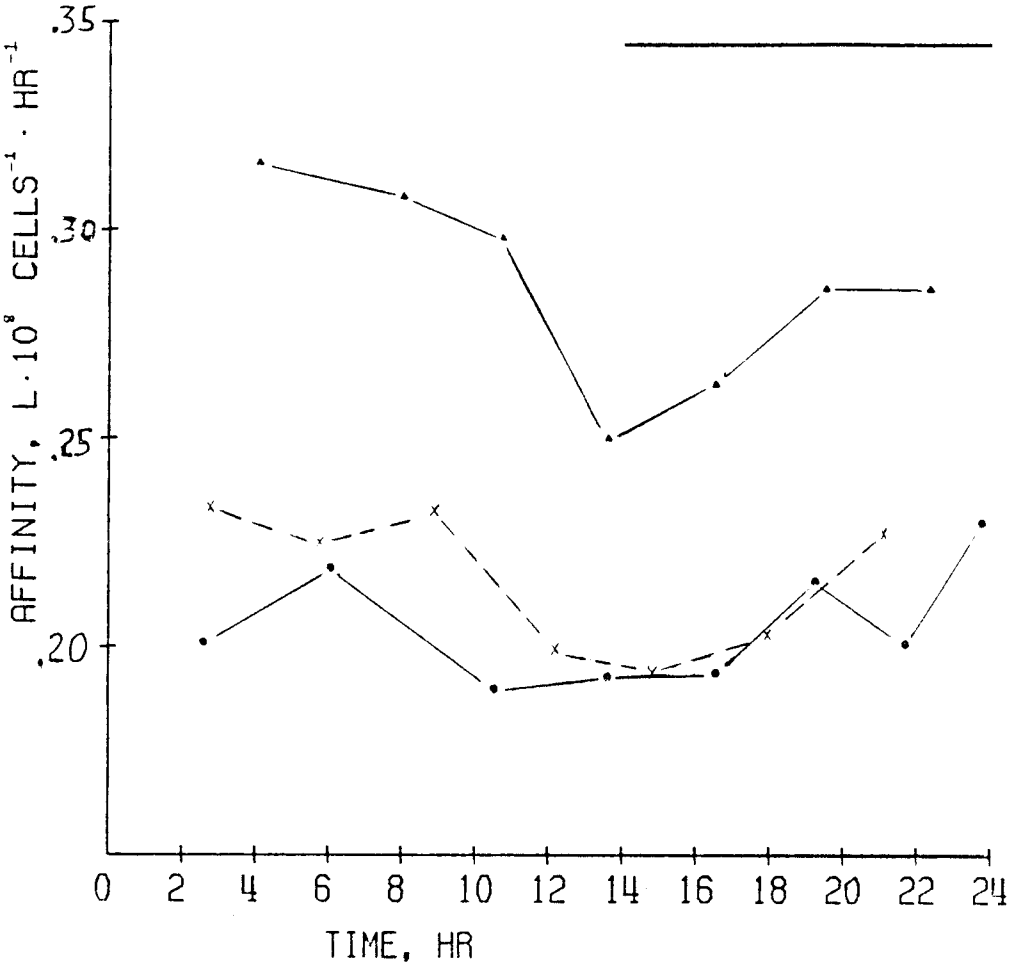

Figure 5. Affinity, a_T, versus time for *Selenastrum capricornutum* grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; (✗) LL; shaded bar represents dark period in LD and 'subjective' dark in free run.

(Brown and Button unpublished data; their dry weight measurements were based on median cell size) to 33.7 ± 6.7 $l \cdot ml\ cells^{-1} \cdot hr^{-1}$. Although most of the values for a_T (Fig. 6a; LD, free run and LL) are within the 95% confidence limits for a_S, rhythms in LD and free run a_T are still apparent when compared to LL a_T. Similar results were obtained for LD, free run and LL a_T expressed on a cell dry weight basis (Fig. 6b). The dry weight conversion factor was time dependent in LD and the conversion factors used to convert LD and free run cell volume to cell dry weight were derived from LD SDB culture (Fig. 8). The mean value with 95% confidence limits for LL a_T was 0.083 ± 0.005 $l \cdot mg\ dry\ wgt^{-1} \cdot hr^{-1}$.

Although the precision of the affinity measurements is good, the accuracy cannot be estimated because of possible complexities in the correlation of Pi concentrations with true dissolved inorganic orthophosphate concentrations. It is known that the low pH of the phosphate assay reaction mixture hydrolyzes some organic P compounds (Leloir and Cardini 1957) which would lead to overestimates of the true dissolved inorganic orthophosphate concentrations if these hydrolyzable compounds are present in culture filtrates.

Serially Diluted Batch (SDB) Culture

The data shown in Figs. 7-9 are taken from one SDB culture of *Selenastrum* grown in medium identical to continuous culture medium (0.96 µM P) except that it did not have ^{32}P. The culture was diluted daily for 6

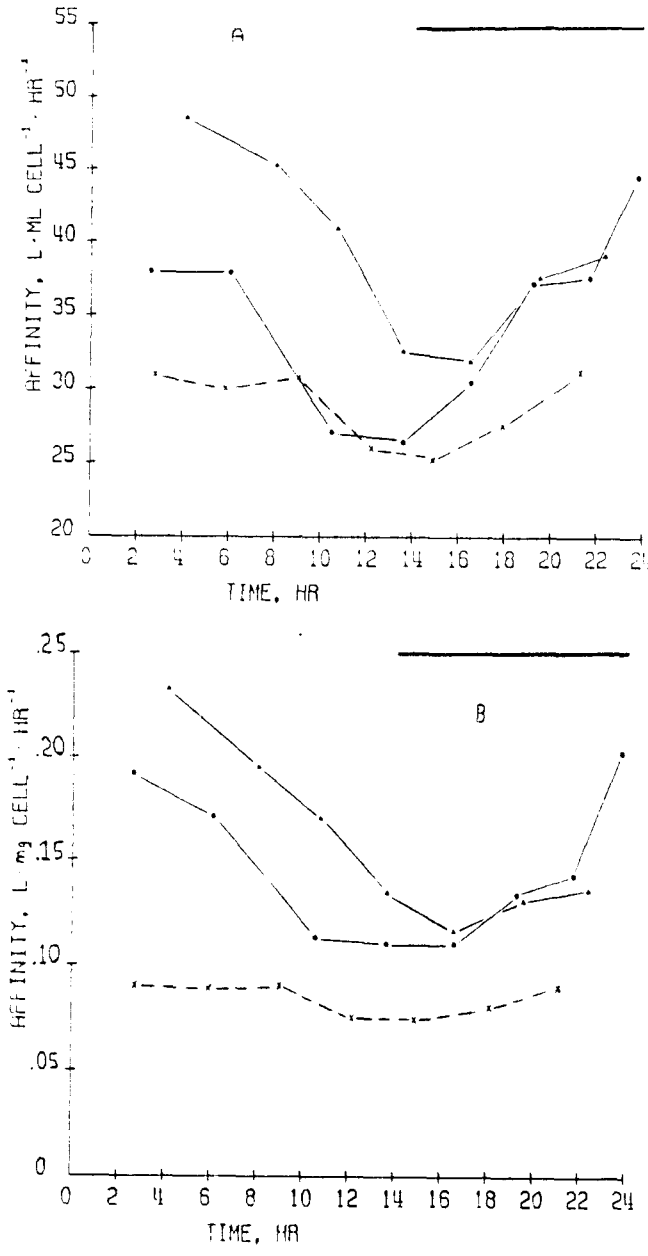

Figure 6. Affinity, a_T, versus time for *Selenastrum capricornutum* grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; (x) LL; shaded bar represents dark period in LD and 'subjective' dark in free run.

days and sampling began 24 hours after the final dilution. Division rate data for a duplicate experiment sampled the day of the final dilution is also shown in Fig. 7b. Division rates between two consecutive samples were calculated from $C_{t2} = C_{t1}e^{rt}$ where C_{t2} is the cell concentration at time t2, C_{t1} is the cell concentration at time t1, r is the division rate and t = t2 - t1.

Selenastrum exhibited rhythms with periods of approximately 24 hours in r and $\bar{W}$ (Fig. 7). Division rate was maximal in the latter part of the dark phase. Ideally, perfect synchronization (all cells dividing at exactly the same time) would result in a step increase of n-fold in cell concentration where n is the average number of autospores or daughter cells produced per parent. The cell concentration does not decrease after division unless dilutions are continued. Cell concentration increased 75% during the first 24 hours which is equivalent to an average division rate of 0.023 hr^{-1}. The average dilution rate was 0.029 hr^{-1}. This is a good degree of synchrony if only two autospores are produced. However, if four autospores are produced, only 25% of the population divided. Therefore the population doubling time would be 30.1 hours and the average cell generation time would be four days. If four autospores are produced, perfect synchrony would require a growth rate of 1.39 day^{-1} (0.06 hr^{-1}) which is greater than the reported maximum of 1.2 day^{-1} at 20°C (Brown and Button 1979). Imperfect synchrony is called phased cell division (Chisholm et al. 1975).

Dry weights per cell volume are shown in Fig. 8. The maximum occurred towards the middle of the dark period. This data was used to determine

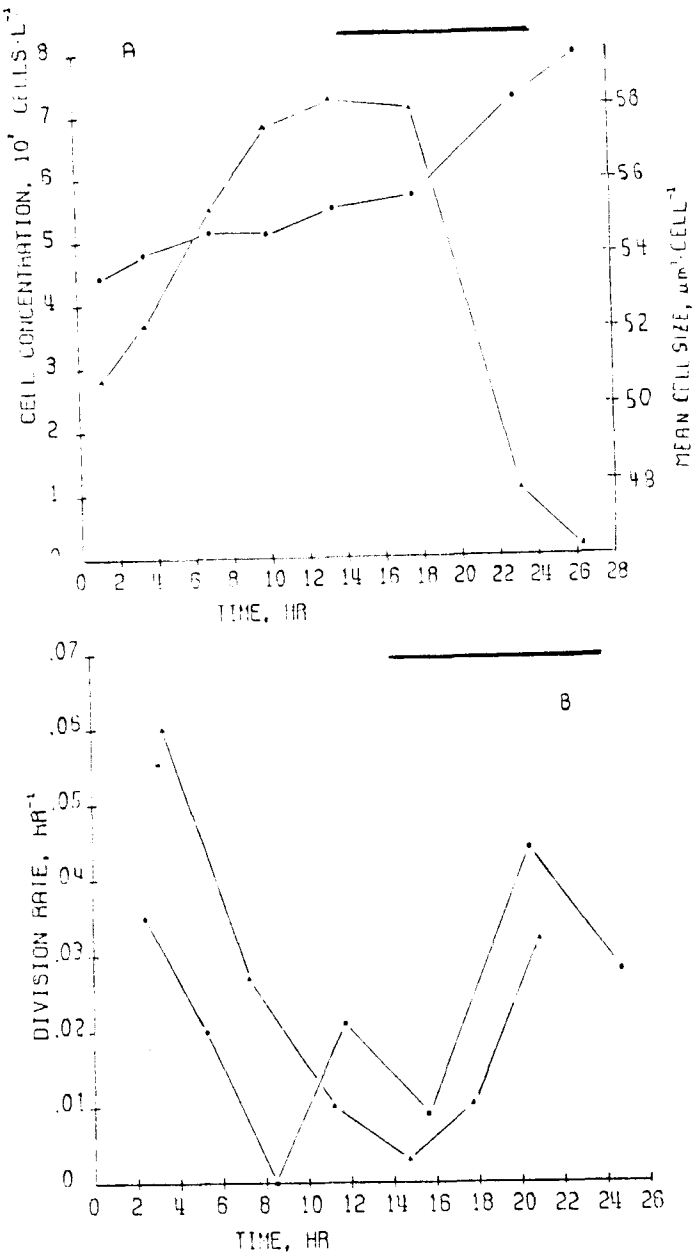

Figure 7. (a) Cell concentration and mean cell size versus time and (b) division rate versus time for *Selenastrum capricornutum* grown in P-limited, LD SDB culture. Symbols: (a) (●) cell concentration; (▲) mean cell size; (b) (▲) division rate; (●) division rate for a duplicate experiment. Measurements were made during the 24 hours following the final dilution for the duplicate experiment; shaded bar represents dark period.

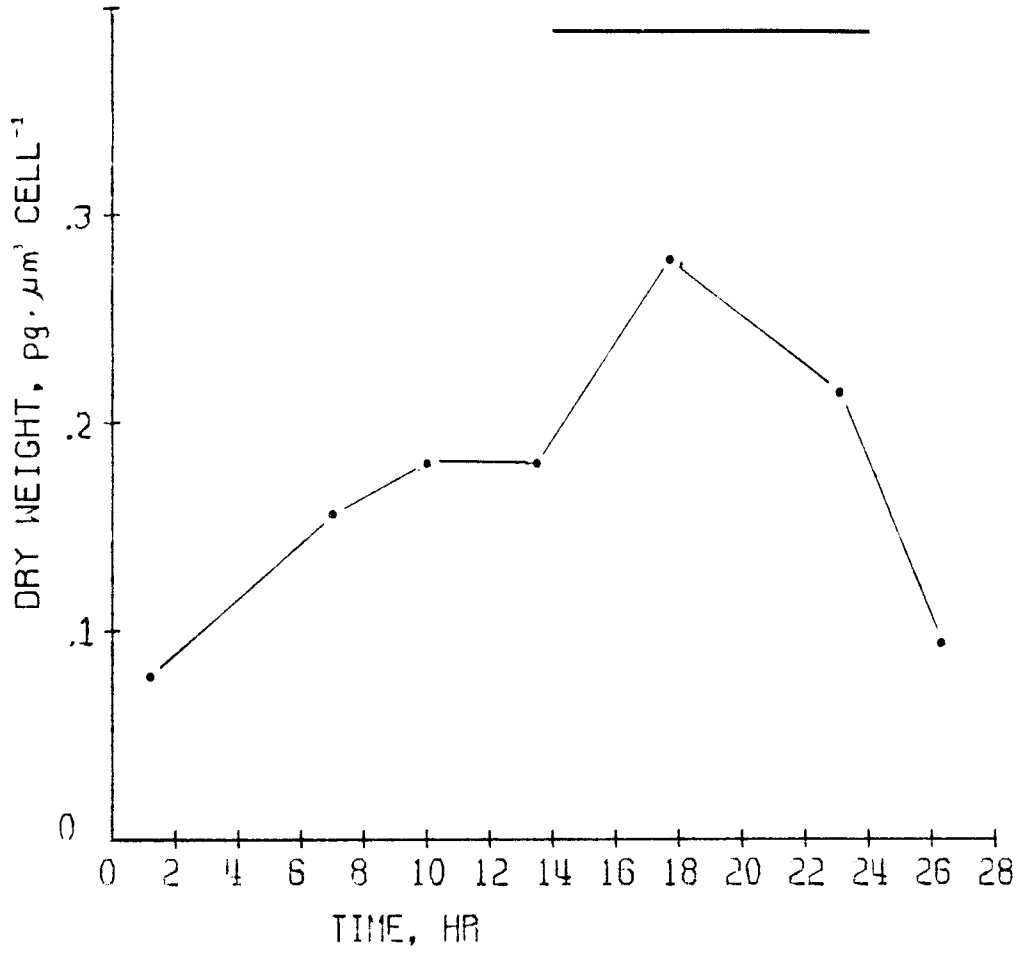

Figure 8. Dry weight versus time for *Selenastrum capricornutum* grown in LD, P-limited SDB culture. Shaded bar represents dark period.

cell volume to dry weight conversion factors for LD and free run continuous cultures (see page 41).

Transport rate measurements were begun 24 hours after the final dilution (Fig. 9) so that Q would be low in order to minimize feedback inhibition of transport (see page 14). In a 2.89 µM P SDB culture radiolabelled with ^{32}P, Q was 106 µg-at P•ml cells^{-1} 23 hours after the final dilution. This is a low Q since it is less than 6% of the 1900 µg-at P•ml cell^{-1} observed early in a 5 µM P, continuous light batch culture. Therefore, when initial phosphate transport rates (V) were measured in a 0.96 µM P, LD SDB culture, Q (although not measured) was likely to be very low. The phosphate transport rates were measured by adding 3.25 µM K_2HPO_4 labelled with ^{32}P (Fig. 9a) and also by adding carrier-free ^{32}P (Fig. 9b). The rhythms in V when 3.25 µM K_2HPO_4 was added differ, depending on which biomass units were used to normalize the data, although maximum rates are only separated by several hours. Rhythms in the carrier-free rate constants (K/N) are more difficult to interpret because actual transport rates could not be measured since the Pi concentrations were not known (K/N could not be multiplied by S to yield V; see page 35). Even small fluctuations in S of 5 ng-at P•l^{-1} would cause relatively large changes in V and initial values of S (S decreases with time due to transport) must be constant to facilitate comparison of V measured at different times. However, it appears that the carrier-free transport rate constant, K/N, peaks early in the light period, at least when expressed on a dry weight basis (Fig. 9b). Controls containing 40 mg•l^{-1} $HgCl_2$ and autoclaved treated cells did not result in significant adsorption of ^{33}P (^{33}P was obtained from New England Nuclear for phosphate transport rate experi-

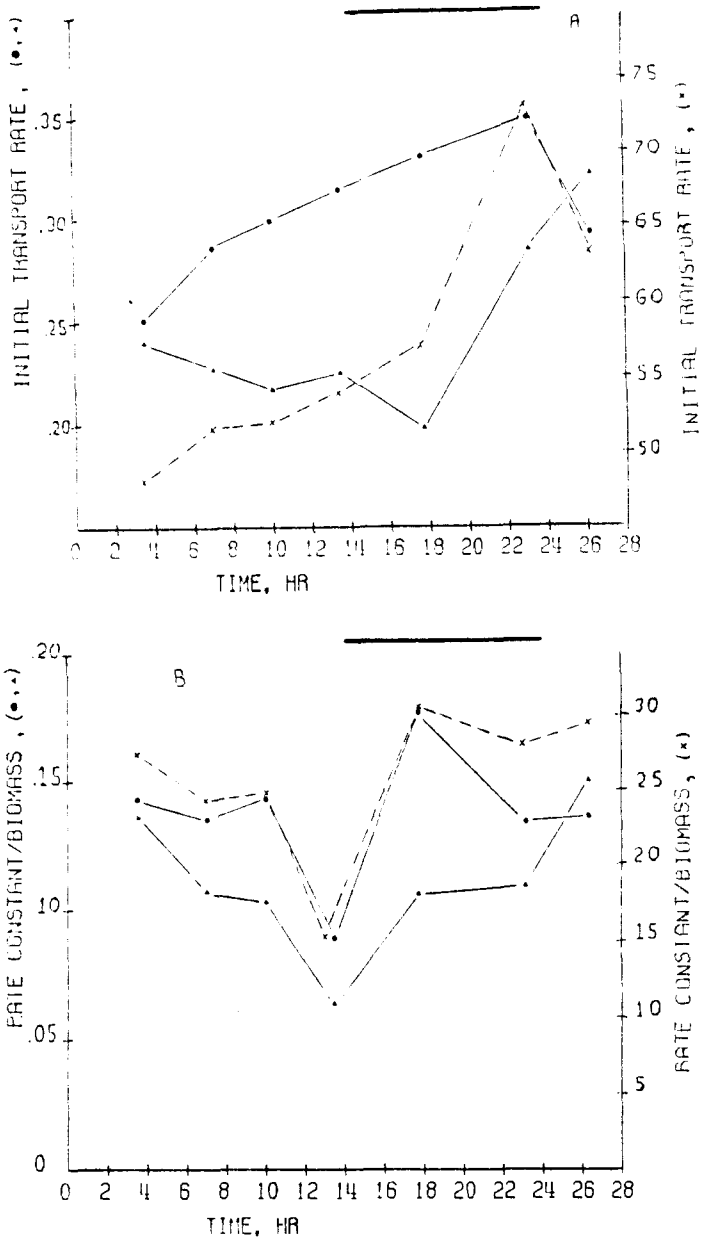

Figure 9. (a) Initial transport rate (μg-at P·biomass^{-1}·hr^{-1}) versus time and (b) rate constant/biomass (l·biomass^{-1}·hr^{-1}) versus time for *Selenastrum capricornutum* grown in P-limited, LD SDB culture. Symbols: (●) per 10^8 cells; (▲) per mg cells, (x) per ml cells. Shaded bar represents dark period.

ments not shown here) to glassware, filters or cells at concentrations of 10^9 cells·l^{-1}.

Selenastrum was also grown in 2.84 μM P SDB culture under continuous light to determine whether daily nutrient perturbations alone could synchronize cell division. The results for duplicate experiments are shown in Figs. 10 and 11. Measurements began 18 hours after the final dilution. Rhythms were not evident for cell volume but division rates are more difficult to interpret. The average division rates were 0.042 and 0.031 hr^{-1} for experiments A and B (Fig. 11), compared to the average dilution rate of 0.029 hr^{-1}. Comparison of LL SDB division rates (Fig. 11) with LD SDB division rates (Fig. 7b) indicates a much weaker phasing of cell division in LL SDB culture. Division rates were asynchronous in LD continuous culture with a constant rate of nutrient supply (Fig. 3a). Therefore, nutrient perturbations appear to affect phasing of cell division in *Selenastrum* more strongly than LD cycling but a combination of both types of perturbations produces strong phasing and thus is a synergistic interaction.

SCENEDESMUS QUADRICAUDA

Cell division in populations of *Scenedesmus quadricauda* grown at D = 0.03 hr^{-1} in LD and free run, P-limited (0.96 μM P), continuous culture was rhythmic (Fig. 12). The maximum division rate (r + D) occurred about the middle of the light period. Approximately 56% of the colonies divided during the first 6 hours of the free run. Changes in $\bar{W}$ also occurred (Fig. 13) but the rhythm is probably not circadian because $\bar{W}$ continued to

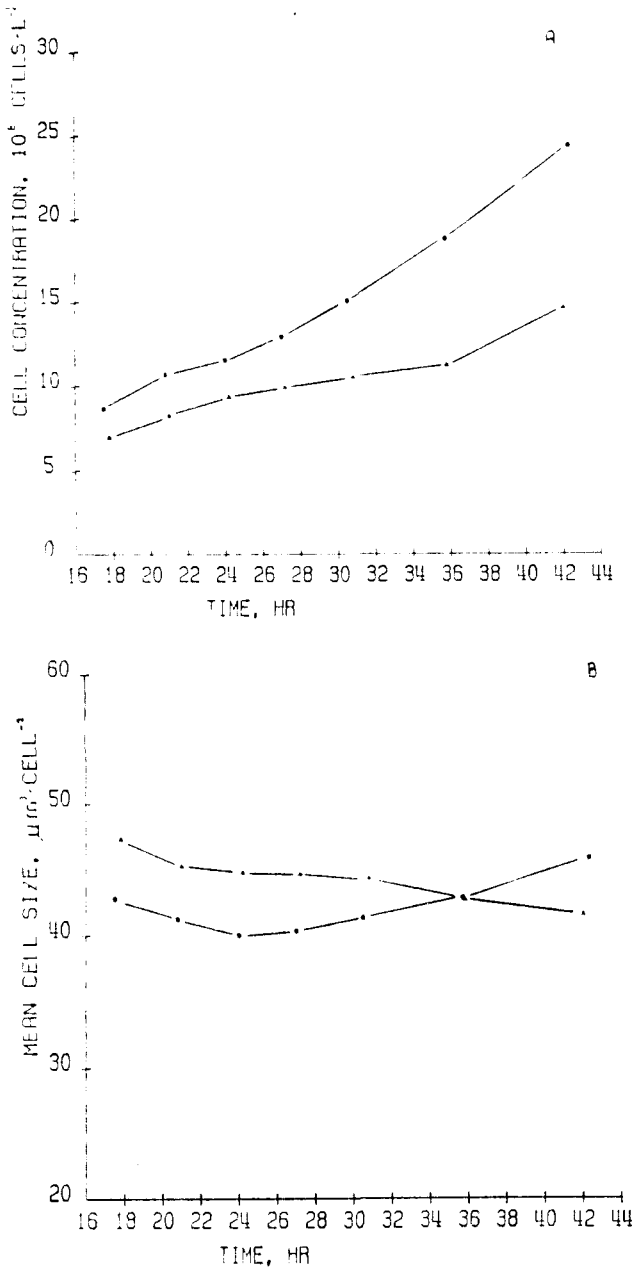

Figure 10. (a) Cell concentration versus time and (b) mean cell size versus time for duplicate experiments of *Selenastrum capricornutum* grown in P-limited, LL SDB culture. Symbols: (●) experiment A; (▲) experiment B.

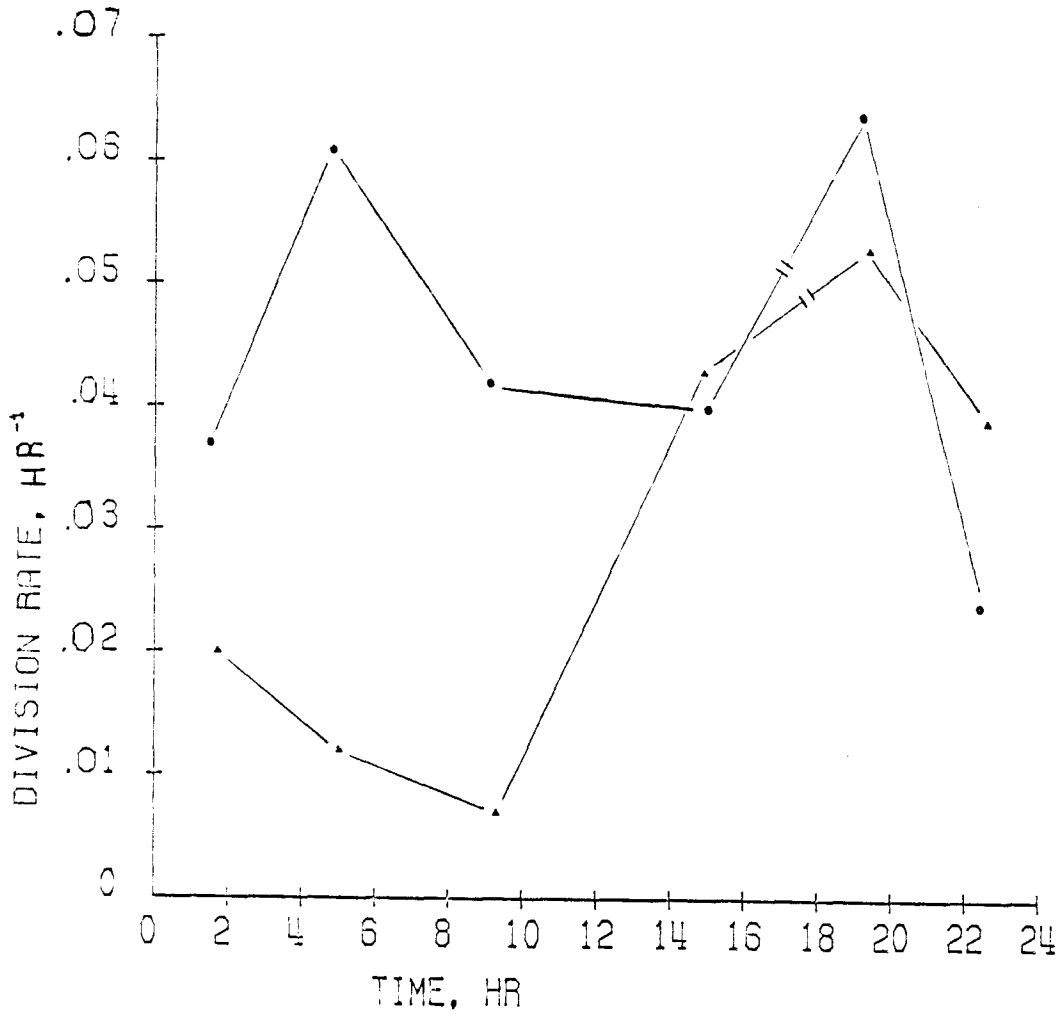

Figure 11. Division rate versus time for duplicate experiments of *Selenastrum capricornutum* grown in P-limited, LL SDB culture. Symbols: (●) experiment A; (▲) experiment B. The data to the right of the break in the lines were collected before the data to the left of the break but are plotted to the right to facilitate comparison with Figure 7b.

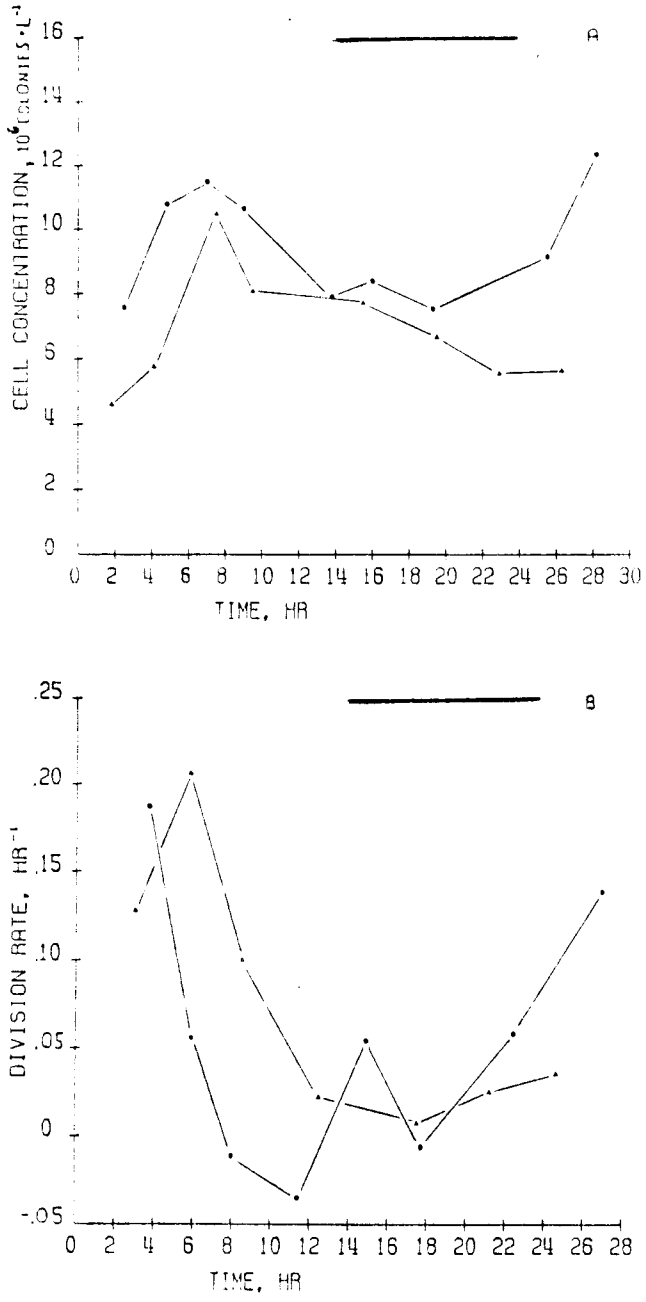

Figure 12. (a) Cell concentration versus time and (b) division rate versus time for *Scenedesmus quadricauda* grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; shaded bar represents dark period in LD and 'subjective' dark in free run.

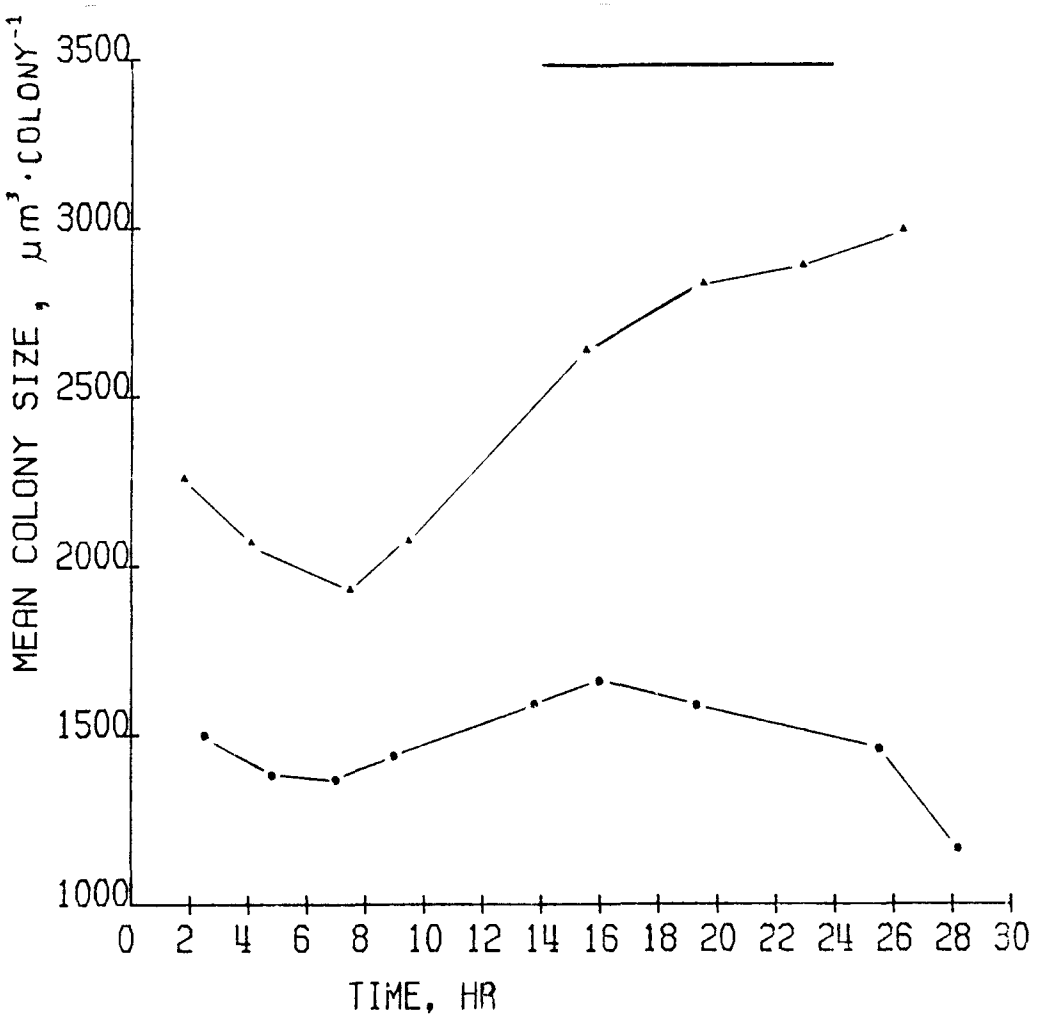

Figure 13. Mean colony size versus time for *Scenedesmus quadricauda* grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; shaded bar represents dark period in LD and 'subjective' dark in free run.

increase during the 'subjective' dark period rather than decrease as occurred during the dark period in LD. The patterns in cell division and $\bar{W}$ are very different from those of *Selenastrum* (Fig. 3). Rhythms in Q and Pi are shown in Fig. 14.

The affinity, a_T, using S_T = 36 ng-at P·l^{-1} (Lang and Brown 1981) is rhythmic (Fig. 15). The shape of the curve is not greatly affected by the choice of biomass parameter. The maximum a_T occurred at 14.9 hours and the relative change was 397% when expressed on a per colony basis and 338% when expressed on a total cell volume basis. A dry weight conversion factor (Lang 1980) and a ratio of 1.1 for mean to median cell size was used to convert the LL a_S values with 95% confidence limits of 0.194 ± 0.085 l·mg dry wgt^{-1}·hr^{-1} (Lang and Brown, unpublished data; their dry weight measurements were based on median cell size) to 61 ± 27 l·ml cell^{-1} ·hr^{-1}. The value of a_T based on colony number is 89.7 ± 36 l·10^9 colonies^{-1}·hr^{-1} (Lang and Brown, unpublished data).

If the increase in a_T were due to a decrease in Q, one would expect a negative correlation between a_T and Q. However, the correlation is not negative and therefore changes in a_T are probably not due to feedback inhibition (Fig. 16). The closed loop in Fig. 16 is indicative of a repeated, but not completely reproducible, rhythm. Variability (drift) in values for cell concentration, $\bar{W}$ and a_T at time t and at t + 24 hours occurred in *Scenedesmus* (Figs. 12, 13 and 15) as well as *Selenastrum* (Figs. 3 and 6).

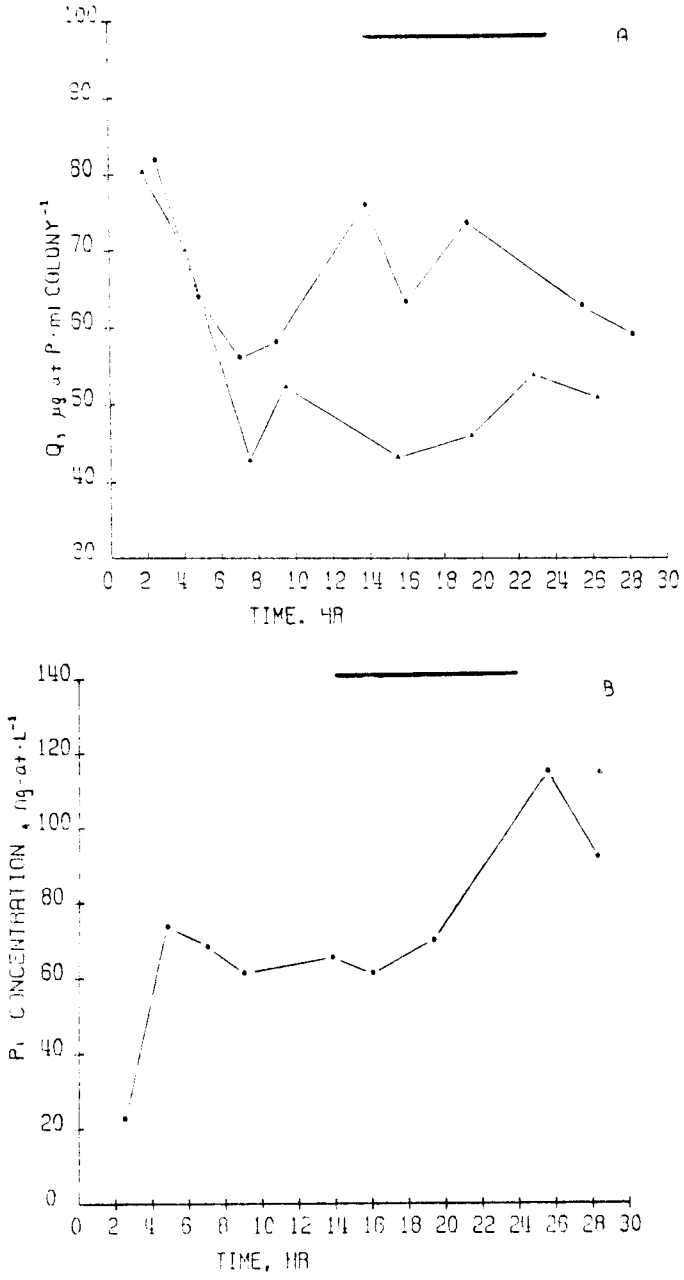

Figure 14. (a) Intracellular P concentration, Q, versus time and (b) dissolved Pi concentration versus time for *Scenedesmus quadricauda* grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; shaded bar represents dark period in LD and 'subjective' dark in free run.

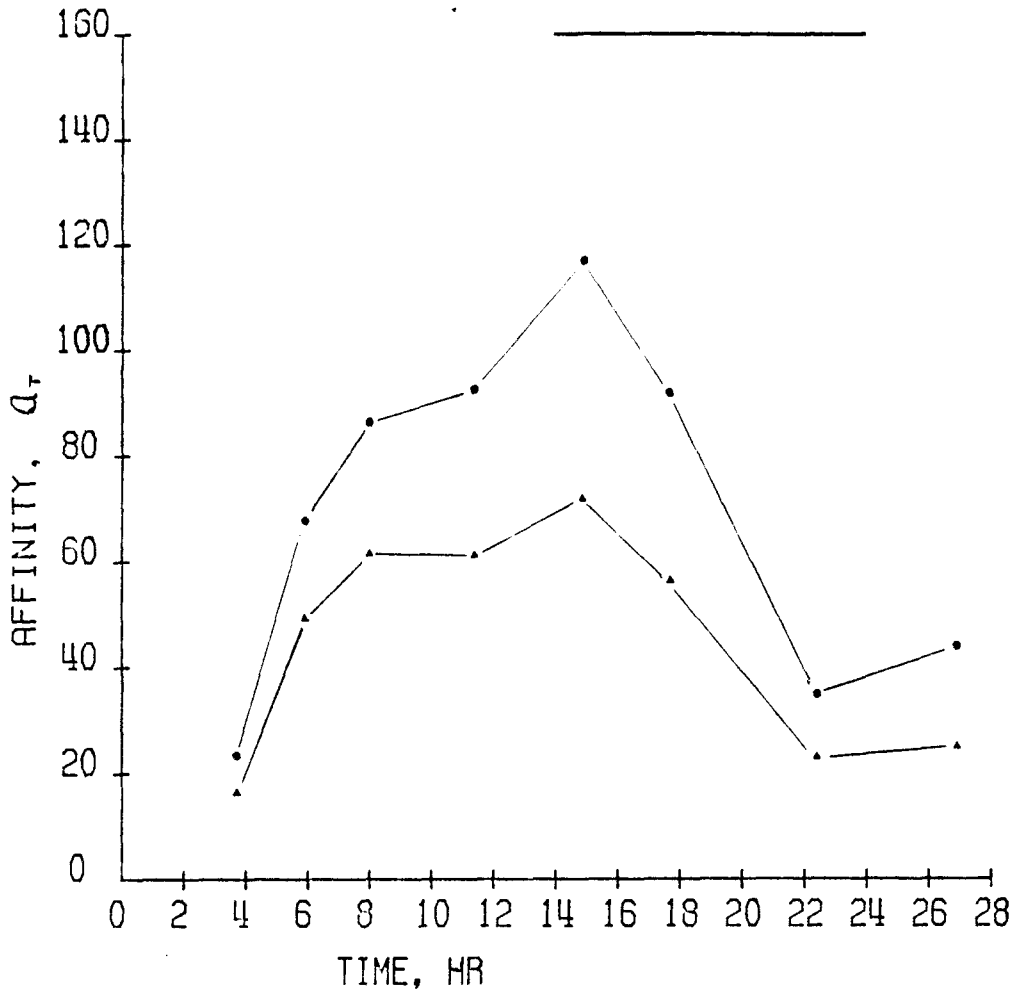

Figure 15. Affinity, a_T, versus time for *Scenedesmus quadricauda* grown in P-limited continuous culture. Symbols: (●) LD ($1 \cdot 10^9$ colonies^{-1}·hr^{-1}); (▲) LD (1·ml colony^{-1}·hr^{-1}); shaded bar represents dark period in LD.

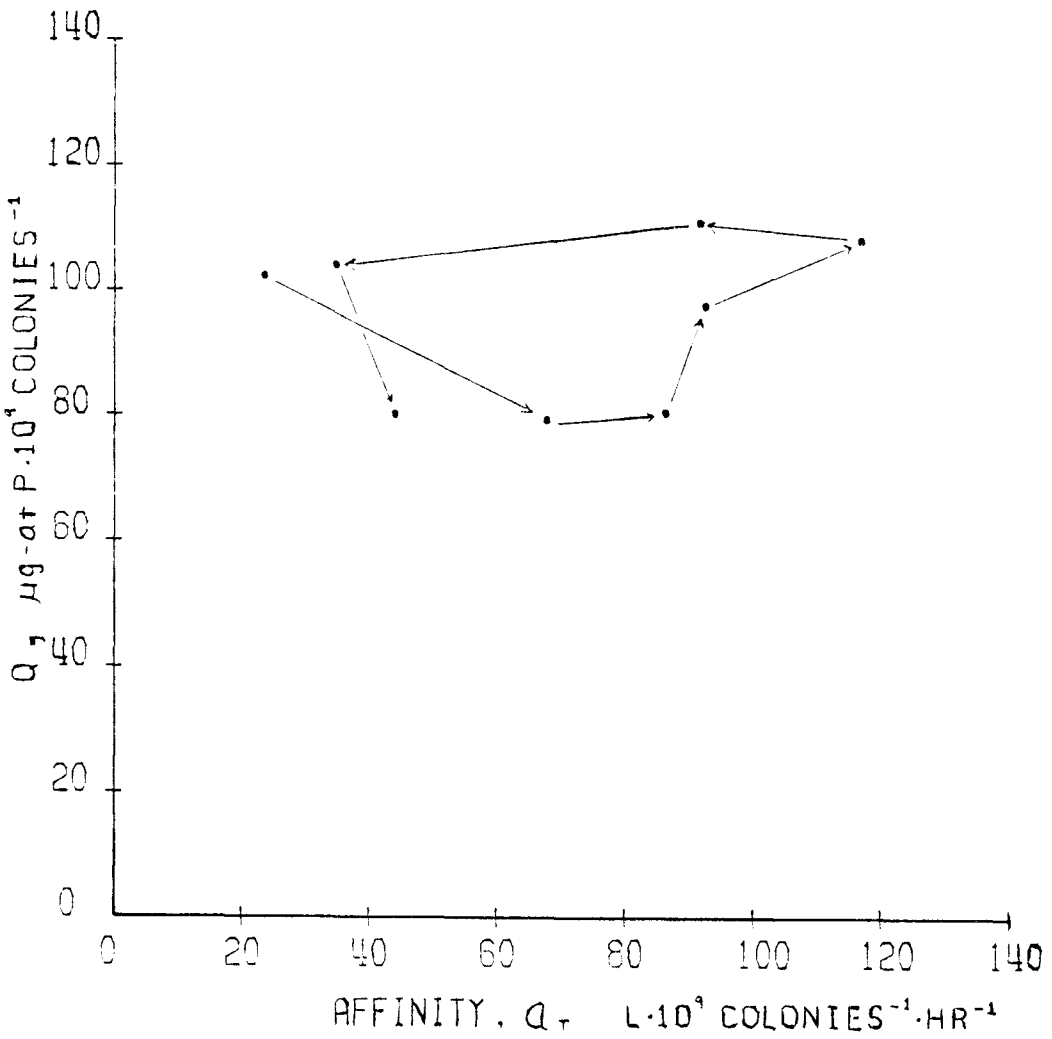

Figure 16. Intracellular P concentration, Q, versus a_T for *Scenedesmus quadricauda* grown in LD, P-limited continuous culture. Arrows refer to time sequence.

SYNECHOCOCCUS NÄGELI

The results for the procaryotic alga *Synechococcus* are based on two P-limited (0.96 µM P) continuous cultures with a dilution rate of 0.03 hr^{-1} under LD, free run and continuous light conditions (Figs. 17-20). Cell division rates (r + D) were rhythmic in LD 14:10 but cell concentrations continued to increase during the 'subjective' dark period of the free run (Fig. 17). It is unlikely, therefore, that cell division rhythms are coupled to a circadian clock. The 24 hour rhythms in cell concentrations were not completely reproducible from cycle to cycle. However, the average division rate during the 14 hour light period was 0.042 hr^{-1} which means that 80% of the cells divided during that 14 hour period (Fig. 17).

$\bar{W}$ was rhythmic in the LD and free run experiments with the cells increasing in size during the light and decreasing during the dark and 'subjective' dark (Fig. 18). Overall, $\bar{W}$ ranged from 2.3 to 2.8 $\mu m^3 \cdot cell^{-1}$.

Q decreased during the light period in the LD and free run experiments but failed to increase during the 'subjective' dark. Q increased in the dark in LD (Fig. 19a). Pi values are shown in Fig. 18b. Values were very low and ranged from 0.6 to 2.3 ng-at $P \cdot l^{-1}$ without any apparent 24 hour rhythms.

Affinities were calculated using a threshold value of 0 and are shown in Fig. 20. The dry weight conversion factor of Lang (1980) and a ratio of 1.1 for mean to median cell size was used to convert the LL a_S values of Lang and Brown (1981) to 1351 ± 621 $l \cdot ml\ cells^{-1} \cdot hr^{-1}$ (limits

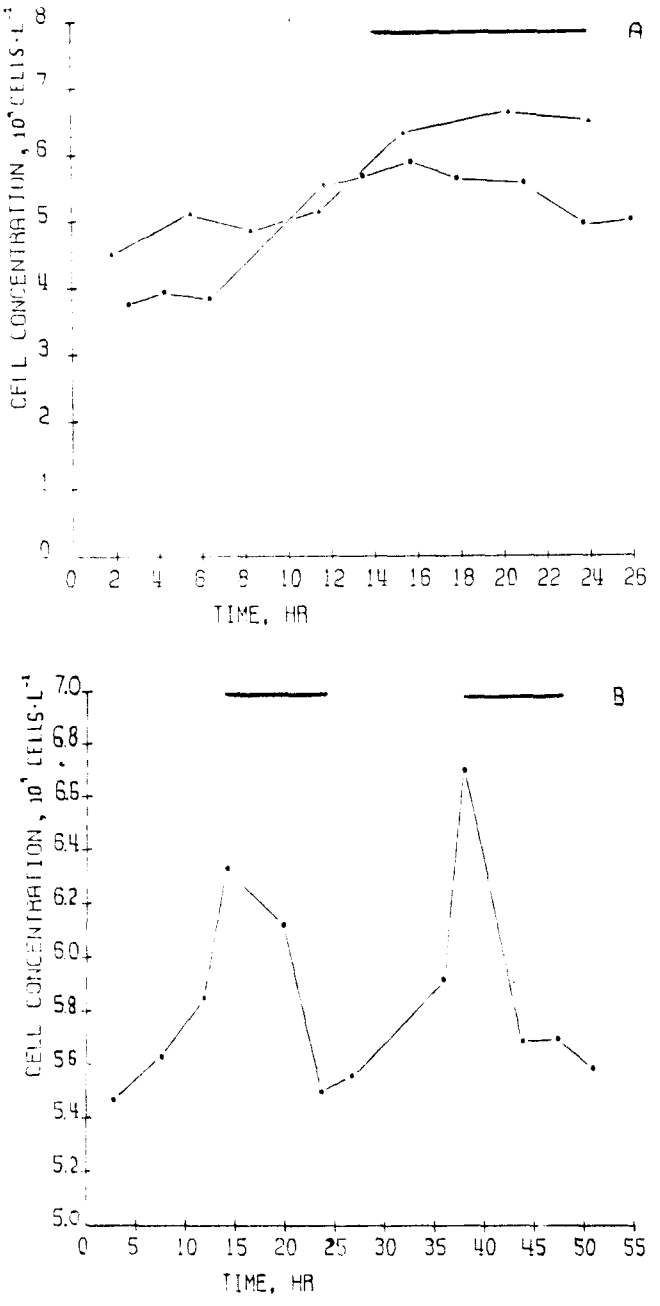

Figure 17. Cell concentration versus time. (a) 24 hour experiment and (b) 48 hour experiment for *Synechococcus* Nageli grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; shaded bar represents dark period in LD and 'subjective' dark in free run.

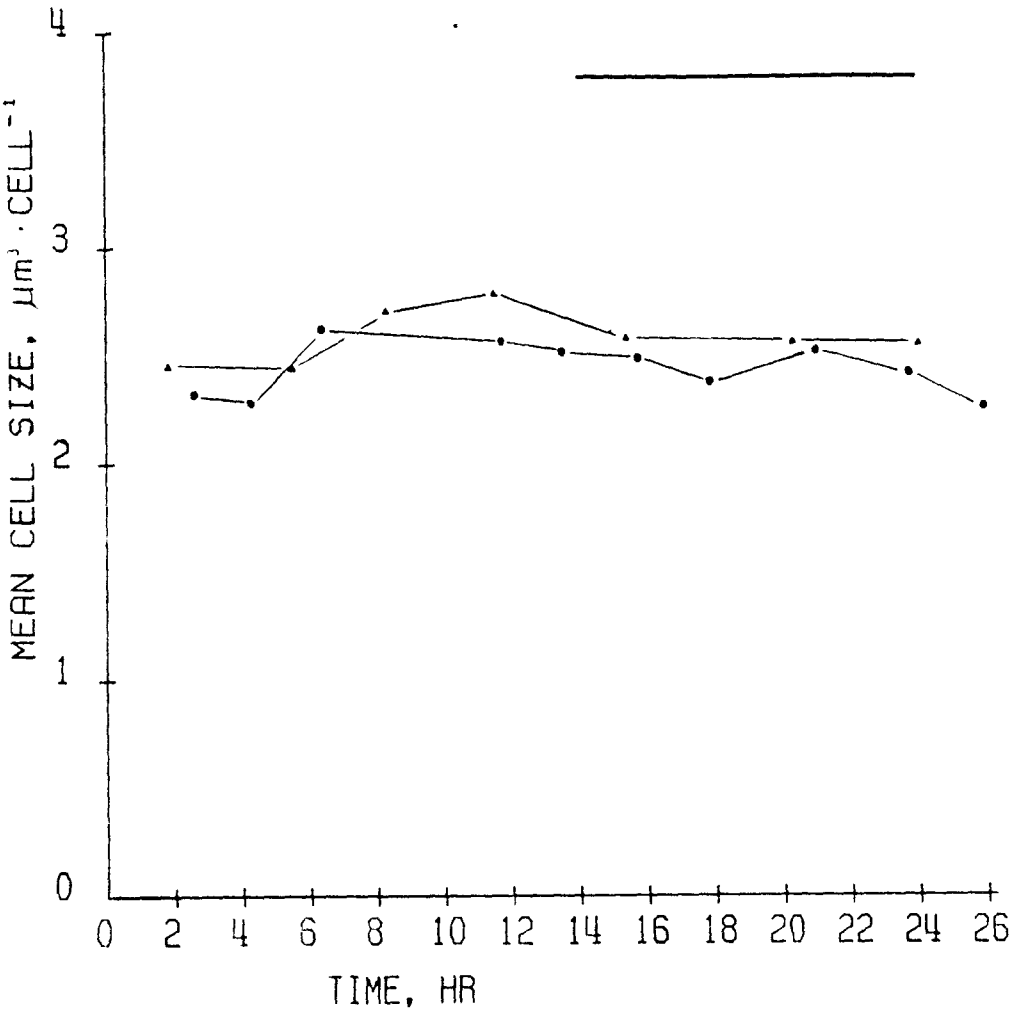

Figure 18. Mean cell size versus time for *Synechococcus* Nageli grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; shaded bar represents dark period in LD and 'subjective' dark in free run.

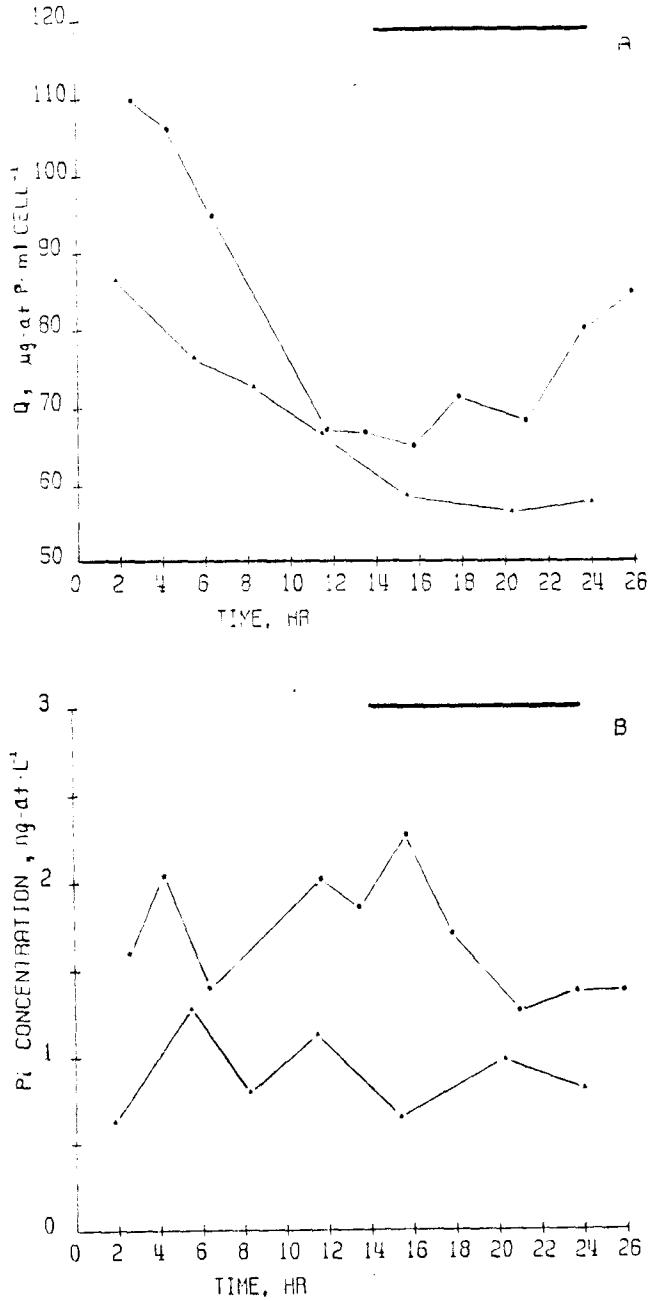

Figure 19. (a) Intracellular P concentration versus time and (b) dissolved Pi concentration versus time for *Synechococcus* Nägeli, grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run; shaded bar represents dark period in LD and 'subjective' dark in free run.

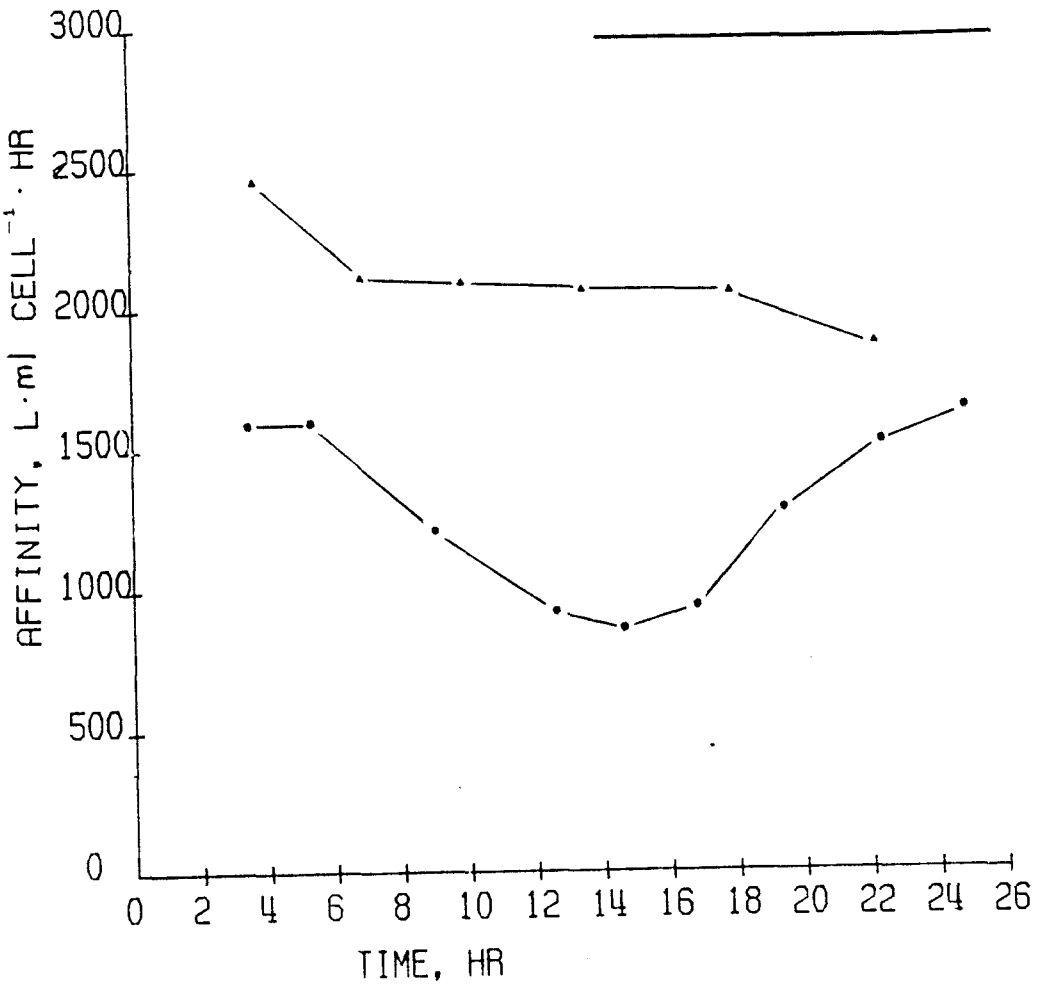

Figure 20. Affinity, a_T, versus time for *Synechococcus* Nageli grown in P-limited continuous culture. Symbols: (●) LD; (▲) free run, shaded bar represents dark period in LD and 'subjective' dark in free run.

are 95% confidence limits). It is doubtful whether equation (7) can be used to calculate a_T for efficient microorganisms such as *Synechococcus*. Changes of 1 or 2 ng-at $P \cdot l^{-1}$ will result in large changes in a_T and it is unlikely that the methodology for measurement of Pi employed in this study can resolve such small differences accurately. Regardless of the actual values for a_T, *Synechococcus* can utilize extremely low Pi concentrations since the total dissolved P never exceeded 11 ng-at $P \cdot l^{-1}$, compared to a minimum of 38 for *Selenastrum* and 31 for *Scenedesmus*.

DISCUSSION

SELENASTRUM CAPRICORNUTUM

The results show that rhythms with periods of approximately 24 hours in $\bar{W}$ and a_T (expressed per cell volume or dry weight) are induced by exposure to LD cycles (Figs. 3b and 6) and rhythms with periods of approximately 24 hours in cell concentration, $\bar{W}$, dry weight to cell volume ratio and V are induced by exposure to a combination of LD cycles and nutrient perturbations (Figs. 7-9). Although some of the time series values for a_T in LD, free run and LL fall within the 95% confidence limits for LL a_S, rhythms in LD and free run a_T (expressed per cell volume and dry weight) are apparent when compared to time series values of LL a_T. Furthermore, the maximum a_T (per cell volume and dry weight) and maximum V occur at approximately the same time in LD. Therefore calculation of the initial slope of a V versus S plot with equation (7)(a_T) is possible.

Interpretation of rhythms in transport activity, however, may be obscured because standardization of activity with different population biomass parameters leads to different results. This is clearly shown for *Selenastrum* (Figs. 5, 6 and 9). On the other hand, rhythms in a_T for *Scenedesmus* were only slightly dependent upon choice of biomass unit (Fig. 15). Therefore, it may not be possible from kinetic evidence alone to determine whether apparent rhythms in transport activity are caused by rhythms in biomass parameters or by biological control of transport mechanisms.

The following model illustrates the limitations of transport data when attempting to interpret rhythms in transport ability. It is a simplified model involving several assumptions and is offered as an aid in a qualitative, rather than a quantitative, sense.

Let T_i be the concentration of available transporters of system i in a growth vessel. Assume that the substrate in question can only be transported by system i. Then,

$$\frac{dT_i}{dt} = b_i T_i - DT_i \tag{9}$$

where b_i is a rate constant reflecting biological control of T_i and may include synthesis, degradation, energy limitation and switching on and off of transporters. At steady state, $dT_i/dt = 0$ and $b_i = D$. At non-steady state,

$$T_{i,t} = T_{i,o} \exp \left[\int b_i dt - Dt\right] \tag{10}$$

where $T_{i,t}$ is T_i at time t and $T_{i,o}$ is T_i at time 0.

The transport rate of a nutrient (VN) is assumed to be a function of S, T_i and the shear velocity of the fluid (Pasciak and Gavis 1975) when the transporters are not saturated with substrate. The shear velocity affects the size of the laminar layer surrounding individual cells and thus influences the diffusion rate of substrate across the layer to the transporters. When initial S and shear velocity are constant and change in S is minimized by terminating a transport rate measurement after 5%

of the substrate is transported (see page 35), VN is a function only of T_i, but no information about the individual components of b_i can be determined. In the experimental design used in this study, S was not constant in continuous culture so $a_T N$ is considered instead of VN. In *Selenastrum*, LD $a_T N$ versus time is relatively constant compared to *Scenedesmus* (Fig. 21a). Therefore T_i is relatively constant in LD continuous culture for *Selenastrum* and $b_i = D$. The change in LD a_T when expressed per cell volume or per dry weight probably reflects changes in cell volume or dry weight (Fig. 21b). In this model, changes in V or a_T will reflect circadian control of T_i only if Q is low, D is constant and all components of b_i are constant with the exception of the circadian component. The criteria of low Q and constant D are met in both LD continuous and SDB cultures employed in this study, but there is no way of evaluating whether the last criterion is met. Biochemical studies are probably required to elucidate the nature of the individual components of b_i.

The need for two entraining variables, LD cycles and nutrient perturbations, to strongly phase cell division in *Selenastrum* is surprising although asynchronous division in continuous culture exposed to LD cycles is known. Perry (1976) found that division in the P-limited diatom, *Thalassiosira pseudonana*, was asynchronous with LD 15:9. She did not detect a rhythm in Pi transport rate regardless of whether the rates were expressed per culture volume, per cell or per weight of chlorophyll a. Division was also asynchronous for the diatom, *Lauderia borealis*, with LD 11:13 (Marra 1980). Since cell division rate in continuous culture and a_T (per cell volume or dry weight) were not in phase (uncoupled) in

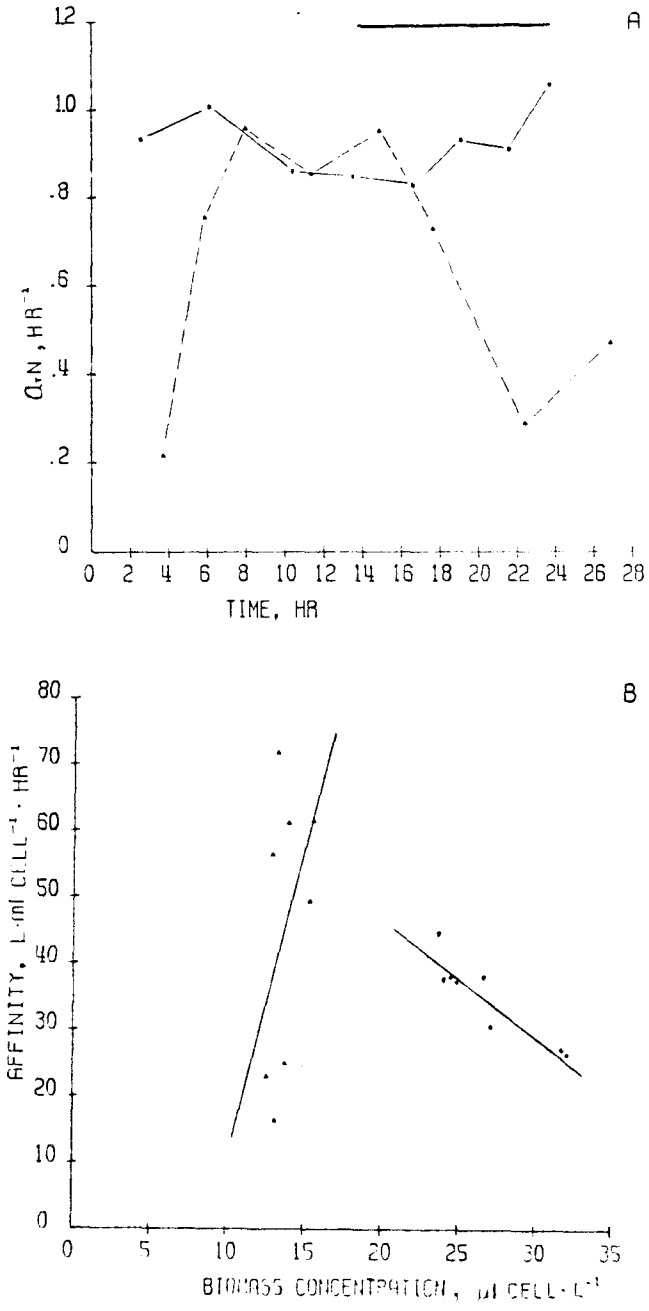

Figure 21. (a) a_TN versus time in LD and (b) a_T versus biomass concentration in LD continuous culture. Symbols: (●) *Selenastrum capricornutum*; (▲) *Scenedesmus quadricauda*; shaded bar represents dark period.

LD, division rate, which has been suggested as a marker of in situ transport activity (Weiler and Chisholm 1976), may not be generally useful.

SCENEDESMUS QUADRICAUDA

Rhythms in cell division rate and $\bar{W}$ were observed in LD continuous culture (Figs 12 and 13). The division rate rhythm is similar in free run and is therefore circadian in nature but the $\bar{W}$ rhythm is apparently not circadian because $\bar{W}$ continues to increase in the free run 'subjective' dark period. This is opposite of what was observed for *Selenastrum* (Fig. 3).

The rhythm in LD a_T (Fig. 15), unlike *Selenastrum*, is only slightly dependent upon choice of biomass parameter which suggests that the rhythm might be circadian in nature. Fig. 21a shows that there is a 24 hour rhythm in LD $a_T N$ and hence in T_i, the concentration of transporters assumed to be responsible for the transport of Pi. Fig. 21b shows that there is not a strong correlation between LD a_T and biomass (cell volume/l).

SYNECHOCOCCUS NÄGELI

Synechococcus is able to remove lower concentrations of Pi than can either *Selenastrum* or *Scenedesmus*. If the kinetics of these three algae are typical of their taxonomic groups, we would expect to find small, unicellular, blue-green algae widespread in oligotrophic systems which are frequently P-limited if it is assumed that laboratory kinetics reflect in situ kinetics. Reports of *Synechococcus* occurring in natural systems

have been rare but *Synechococcus* may have been overlooked due to their extremely small size and bacterial morphology. Recently, there have been reports of small, unicellular cyanophytes in the North Atlantic and Sargasso Sea (Johnson and Sieburth 1979, Waterbury et al. 1981). Waterbury et al. (1981) estimate that *Synechococcus* spp. may be responsible for 15-40% of the total primary productivity in the Sargasso Sea and 10% in the more productive waters of Woods Hole, Mass. Small plankton less than 3 μm in diameter account for between 20 and 70% of the primary productivity in a number of New Zealand and North American lakes (unpublished data cited in Paerl and Mackenzie 1977).

Ability to compete for phosphate may be a general feature of most blue-green algae. The maximum V_{MAX}/K_v (see page 19) for *Anabaena flos-aquae*, a species commonly found in eutrophic systems, grown in LL batch culture is 15.2 l·mg dry wgt^{-1}·day^{-1} (Nalewajko and Lean 1978) which is four to five times higher than the a_s values for *Selenastrum* (Brown and Button 1979) and *Scenedesmus* (Lang and Brown 1981) and higher than the V_{MAX}/K_v values of 0.7 for the diatom *Navicula pelliculosa* and 2.6 for *Scenedesmus quadricauda* (experiment 2)(Nalewajko and Lean 1978). However, the maximum V_{MAX}/K_v value for *Anabaena* is less than the a_s value for *Synechococcus* (Lang and Brown 1981). If other filamentous blue-green algal species commonly associated with eutrophic systems are found to have high affinities for phosphate, mechanisms other than inability to compete for phosphate must be postulated to account for their absence from oligotrophic systems. These filamentous species are certainly large enough not to have been overlooked in floristic studies. Perhaps

eutrophic cyanophytes have unusually low yields (or high Q_o) and/or their phosphate transport ability is relatively inefficient in LD systems. The reason for their absence may be related to pH, total dissolved inorganic carbon concentration or inorganic carbon speciation. Shapiro (1973) found that the species composition of a small, shallow lake shifted from dominance by blue-green algae to dominance by green algae when the pH was lowered from 9.8 to 5 or 6, or CO_2 was bubbled through the water column resulting in a lowered pH and therefore a higher concentration of dissolved inorganic carbon with a different relative speciation.

The role of 24 hour rhythms in the metabolism of procaryotic microorganisms is not well understood although rhythms in several processes have been observed. Daily rhythms in nitrogen and CO_2 fixation and division rates have been reported for blue-green algae (Keller and Paerl 1980, Lindsey et al. 1971, Paerl 1979, Paerl and Keller 1979, Paerl and MacKenzie 1977, Saino and Hattori 1978). Paerl and his colleagues argue that CO_2 and nitrogen fixation rates have evolved out of phase with each other in *Anabaena* to minimize competition for light-generated reductant. In this study, division was rhythmic in LD (Fig. 17) and the percentage of dividing cells per day was 80% at 25°C. The percentage of dividing cells in *Anacystis nidulans* (now known as *Synechococcus*) grown at 39°C with LD 9:15 was 100% per day (Lindsey et al. 1971).

It was observed from measurements of cell number during two sucessive cycles in a continuous culture of *Synechococcus* (Fig. 7b), as well as other rhythms in *Selenastrum* and *Scenedesmus*, that the rhythms were not completely reproducible. Chisholm and Costello (1980) have also found that

division rate patterns were dependent upon the physiological state of the inoculum. Future studies of a_T should incorporate a greater number of cycles for statistical treatment and should also investigate the effects of the physiological state of the inoculum.

COEXISTENCE AND THE 'PARADOX OF THE PLANKTON'

A number of explanations can be invoked to account for coexistence of microorganisms in aquatic systems including temporal or spatial separation of nutrient requirements (Williams 1971, Goldman et al. 1979), multiple resource limitation (Tilman 1977), temporal distribution of nutrient perturbations and allelopathy (stimulation or inhibition of growth via excreted metabolites). Keating (1977) demonstrated that allelopathy was also responsible for species succession rather than co-existence in a community dominated by blue-green algae.

The maximum affinity, a_T^{MAX}, expressed on a cell volume basis occurred early in the light period for *Selenastrum* and at the end of the light period for LD *Scenedesmus* (Figs. 6a and 15). The initial transport rate for *Selenastrum* at 3.25 μM K_2HPO_4 in SDB culture was maximal at the end of the dark period (Fig. 9a) which agrees well with the timing of a_T^{MAX} in LD and free run continuous culture. The occurrence of the a_T^{MAX}'s at 0000 (*Selenastrum*) and 1454 hours (*Scenedesmus*) makes these two microorganisms potentially useful in a test of Williams' (1971) hypothesis of temporal separation of limiting nutrient requirements as a solution to the paradox of the plankton (see Literature Review). In a competition experiment, an

inoculum of *Scenedesmus* injected into a LD continuous culture of *Selenastrum* could not compete and washed out. Filtrate experiments to look for allelopathic effects were not done.

Comparisons of Pi, biomass and a_T in LD single species continuous cultures for *Selenastrum* and *Scenedesmus* suggest that conditions for coexistence with one limiting resource in a well-mixed continuous culture are rather restrictive. Although the a_T^{MAX} values (per cell volume) are well separated temporally and *Scenedesmus'* a_T is higher than a_T in *Selenastrum* about half of the LD cycle, *Selenastrum* was able to remove Pi at lower concentrations of Pi and the *Selenastrum* biomass (cell volume per liter) remained twice that of *Scenedesmus*. Hence, while temporal separation of nutrient requirements may be important, transport thresholds of the growth-limiting nutrient and yields of cells on growth limiting nutrient are also important. Williams' (1971) hypothesis may still be valid for some species, but it is unlikely to account for the coexistence of large numbers of species in natural assemblages grown in continuous culture with LD cycles (Harrison and Davis 1979, Paul et al. 1979, Turpin and Harrison 1979, Thomas et al. 1980). The only report of coexistence of microbial species in axenic continuous culture with one limiting resource is by Van Gemerden (1974). He reported the coexistence of two sulfide-limited photosynthetic bacteria in LD. Tilman (1977) and Titman (1976) reported the coexistence of two diatoms, one of which was P-limited and the other silicate-limited, grown in SDB culture in LD. While multiple resource limitation may account for the coexistence of some species, it is an unlikely explanation of the coexistence of large numbers of species,

such as the 17 diatoms in the 2000 liter outdoor, continuous culture of Paul et al. (1979), because of the large number of different substrates which are necessary.

Species specific response to nutrient perturbations when growing in LD, such as the transport rhythm on a per cell basis in *Selenastrum* requiring two entraining variables, may play a role in the outcome of competition in nutrient limited systems. This has implications for aquaculture since proper timing of fertilizer applications may favor a species with high food value. Turpin and Harrison (1979, 1980) found that the species composition of 3 liter continuous and SDB cultures of natural assemblages with the same dilution rate depended on the application scheme. In one experiment (Turpin and Harrison 1980), fertilizer was applied continuously, every 3 days or weekly. In another experiment (Turpin and Harrison 1979), fertilizer was applied continuously, 8 times per day or once per day at the same average rate. The natural assemblages of the latter experiment were seeded with laboratory strains of algae. Their findings of differing species composition in each experiment can be interpreted on the basis of varying storage capacity (Lang and Brown 1981) as well as effects of nutrient perturbations on transport activity.

Large systems are probably not as well mixed as small continuous cultures and thus may contain spatially heterogeneous distributions of nutrients (Goldman et al. 1979, McCarthy and Goldman 1979). Even if the supply rate is constant, spatial distributions may behave like temporal distributions to a given subpopulation. Scaling up to large continuous culture could account for the unusual observations of Malone et al. (1975)

who found that division in the diatom, *Chaetoceros* sp., when grown in 2000 liter, outdoor continuous cultures was synchronized at a dilution rate of 2.0 day^{-1} but not at lower rates of 0.5, 1.0 and 1.5 day^{-1}.

Coexistence in natural assemblages grown in small scale, LD continuous cultures (Harrison and Davis 1979, Thomas et al. 1980) can be accounted for by the effects of LD cycles and multiple resource limitation although these mechanisms may not account for large numbers of coexisting species for the reasons stated above. Perhaps mutually growth-stimulating, biochemical interactions (allelopathy) occur. Such interactions could evolve during the life span of the aquatic system and would not be likely to occur among laboratory strains of microorganisms.

CONCLUSIONS

It is unlikely that V and hence a can be measured with short term, phosphate perturbation experiments because of difficulties in accurately measuring phosphate transport rates at very low concentrations. Changes of only several ng-at $P \cdot l^{-1}$ will result in large changes in rate constants. Continuous culture methods, however, offer an alternative strategy for the investigation of rhythms and other changes in transport activity at low Pi concentrations. Changes in Pi concentration in non-steady state continuous cultures do, however, preclude the use of V as calculated from equation (6) because changes in substrate concentrations at non-saturating concentrations result in changes in V. Instead, the use of initial slopes as measured by equation (7)(a_T) can be used for nutrient-limited, non-steady state continuous cultures. Measurements of a_T may not be possible for efficient exploiters of very low limiting nutrient concentrations such as *Synechococcus*. Rhythms in transport activity may be biased by the choice of biomass unit so comparison of rhythms between species should include a comparison of rhythms in biomass.

This study has shown that cell division rates may not be a useful marker of in situ transport activity because rhythms in cell division and transport activity are not necessarily coupled. It was shown that a_T on a per cell basis is constant and cell division in *Selenastrum* is not phased when exposed only to LD cycles and only slightly phased when exposed to cyclic nutrient perturbations in LL. However, the combination of LD cycles and cyclic nutrient perturbations is synergistic resulting

in strong phasing of cell division as well as a rhythm in V on a per cell basis in *Selenastrum*. Thus, in situ cyclic nutrient perturbations may be another possible mechanism for coexistence and/or species succession.

REFERENCES CITED

Argast, M. and W. Boos. 1980. Co-regulation in *Escherichia coli* of a novel transport system for sn-glycerol-3-phosphate and outer membrane protein Ic (e, E) with alkaline phosphatase and phosphate-binding protein. J. Bacteriol. 143:142-150.

Beever, R. E. and D. J. W. Burns. 1977. Adaptive changes in phosphate uptake by the fungus *Neurospora crassa* in response to phosphate supply. J. Bacteriol. 132:520-525.

Brinkmann, K. 1973. Respiration dependent types of temperature compensation in the circadian rhythm of *Euglena gracilis*. *In* B. Chance, E. K. Page, A. K. Ghosh and B. Hess (eds.), Biological and biochemical oscillators, Academic Press, New York, 534 pp.

Brody, S. and L. Forman. 1980. Interactions between exogenous unsaturated fatty acids and mitochondria in *Neurospora crassa*. Abstracts of the Annual Meeting of the American Society for Microbiology, P157, K185.

_____ and S. A. Martins. 1976. Circadian rhythms in *Neurospora*: the role of unsaturated fatty acids (Abstract). *In* J. W. Hastings and H.-G. Schweiger (eds.), The molecular basis of circadian rhythms, Dahlem Konferenzen, Abakon, Berlin.

_____ and _____. 1979. Ciradian rhythms in *Neurospora crassa*: effects of unsaturated fatty acids. J. Bacteriol. 137:912-915.

Brown, E. J. and D. K. Button. 1979. Phosphate-limited growth kinetics of *Selenastrum capricornutum* (Chlorophyceae). J. Phycol. 15:305-311.

_____ and R. F. Harris. 1978. Algal transient phosphate uptake kinetics and the cell quota concept. Limnol. Oceanogr. 23:35-40.

_____, _____ and J. F. Koonce. 1978. Kinetics of phosphate uptake by aquatic microorganisms: deviations from a simple Michaelis-Menten equation. Limnol. Oceanogr. 23:26-34.

Bunning, E. 1973. The physiological clock. 3rd edition, Springer-Verlag, New York, 258 pp.

Burns, D. J. W. and R. E. Beever. 1977. Kinetic characterization of the two phosphate uptake systems in the fungus *Neurospora crassa*. J. Bacteriol. 132:511-519.

Button, D. K. 1978. On the theory of control of microbial growth kinetics by limiting nutrient concentrations. Deep-sea Res. 25:1163-1177.

Caperon, J. and J. Meyer. 1972. Nitrogen-limited growth of marine phytoplankton - II. Uptake kinetics and their role in nutrient limited growth of phytoplankton. Deep-Sea Res. 19:619-632.

_____ and D. A. Ziemann. 1976. Synergistic effects of nitrate and ammonium ion on the growth and uptake kinetics of *Monochrysis lutheri* in continuous culture. Mar. Biol. 36:73-84.

Chen, M. 1974. Kinetics of phosphorus absorption by *Corynebacterium bovis*. Microb. Ecol. 1:164-175.

Chisholm, S. W. and J. C. Costello. 1980. Influence of environmental factors and population composition on the timing of cell division in *Thalassiosira fluviatilis* (Bacillariophyceae) grown in light/dark cycles. J. Phycol. 16:375-383.

_____ and P. A. Nobbs. 1976. Simulation of algal growth and competition in a phosphate-limited cyclostat. *In* R. P. Canale (ed.), Modeling biochemical processes in aquatic ecosystems. Ann Arbor Science.

_____ and R. G. Stross. 1976a. Phosphate uptake kinetics in *Euglena gracilis* (Z)(Euglenophyceae) grown on light/dark cycles. I. Synchronized batch cultures. J. Phycol. 12:210-217.

_____ and _____. 1976b. Phosphate uptake kinetics in *Euglena gracilis* (Z)(Euglenophyceae) grown in light/dark cycles. II. Phased PO_4-limited cultures. J. Phycol. 12:217-222.

_____, _____ and P. A. Nobbs. 1975. Light/dark phased cell division in *Euglena gracilis* (Z)(Euglenophyceae) in PO_4-limited continuous culture. J. Phycol. 11:367-373.

Conway, H. L., P. J. Harrison and C. O. Davis. 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. Mar. Biol. 35:187-199.

Cook J. R. and T. W. James. 1960. Light-induced division synchrony in *Euglena gracilis* var. *bacillaris*. Exptl. Cell. Research. 21:583-589.

Crabeel, M. and M. Grenson. 1970. Regulation of histidine uptake by specific feedback inhibition of two histidine permeases in *Saccharomyces cerevisiae*. Eur. J. Biochem. 14:197-204.

Dieckmann, C. and S. Brody. 1979. Circadian rhythms in *Neurospora crassa*: oligomycin-resistant mutations affect periodicity. Science 207:896-898.

Dohler, G. and H.-J. Rosslenbroich. 1979. Diurnal variation in photosynthetic CO_2 fixation of marine phytoplankton populations. Z. Pflanzenphysiol. Bd. 94, S. 417-425.

Doyle, R. and R. V. Poore. 1974. Nutrient competition and division synchrony in phytoplankton. J. Exp. Mar. Biol. Ecol. 14:201-210.

Droop, M. R. 1968. Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. J. Mar. Biol. Ass. U.K. 48:689-733.

_____. 1973. Some thoughts on nutrient limiation in algae. J. Phycol. 9:264-272.

_____. 1974. The nutrient status of algal cells in continuous culture. J. Mar. Biol. Ass. U.K. 54:825-855.

_____. 1975. The nutrient status of algal cells in batch culture. J. Mar. Biol. Ass. U.K. 55:541-555.

Edmunds, L. N., Jr. 1965. Studies on synchronously dividing cultures of *Euglena gracilis Klebs* (strain Z). II. Patterns of biosynthesis during the cell cycle. J. Cell. Comp. Physiol. 66:159-182.

_____ and K. J. Adams. 1981. Clocked cell cycle clocks. Science 211: 1002-1013.

Ehret, C. F., J. J. Wille and E. Trucco. 1973. The circadian oscillation: an integral and undissociable property of eucaryotic gene-action systems. *In* B. Chance, E. K. Pye, A. K. Ghosh and B. Hess (eds.), Biological and biochemical oscillators, Academic Press, New York, 534 pp.

Engel, P. C. 1977. Enzyme kinetics. Halsted Press, New York, 96 pp.

Eppley, R. W., T. T. Packard and J. J. MacIsaac. 1970. Nitrate reductase in Peru current phytoplankton. Mar. Biol. 6:195-199.

_____, J. N. Rogers, J. J. McCarthy and A. Sournia. 1971. Light/dark periodicity in nitrogen assimilation of the marine phytoplankters *Skeletonema costatum* and *Coccolithus huxleyi* in N-limited chemostat culture. J. Phycol. 7:150-154.

Feldman, J. F. 1968. Circadian rhythmicity in amino acid incorporation in *Euglena gracilis*. Science 160:1454-1456.

_____, G. Gardner and R. Denison. 1979. Genetic analysis of the circadian clock of *Neurospora*. *In* M. Suda, O. Hayaishi and H. Nakagawa (eds.), Biological rhythms and their central mechanism, The Naito Foundation, Elsevier/North-Holland Biomedical Press.

Fuhs, G. W., S. D. Demmerle, E. Canelli and M. Chen. 1972. Characterization of phosphorus-limited plankton algae. *In* G. E. Likens (ed.), Nutrients and eutrophication: the limiting nutrient controversy. Am. Soc. Limnol. Oceanogr. Spec. Symp. 1:113-133.

Gargas, E., I. Hare, P. Martens and L. Edler. 1979. Diel changes in phytoplankton photosynthetic efficiency in brackish waters. Mar. Biol. 52:113-122.

Geesey, G. G. and R. Y. Morita. 1979. Capture of arginine at low concentrations by a marine psychrophilic bacterium. Appl. Environ. Microbiol. 38:1092-1097.

Gerdes, R. G., K. P. Strickland and H. Rosenberg. 1977. Restoration of phosphate transport by the phosphate-binding protein in spheroplasts of *Escherchia coli*. J. Bacteriol. 131:512-518.

Goldman, J. C. 1977. Temperature effects on phytoplankton growth in continuous culture. Limnol. Oceanogr. 22:932-936.

_____. 1979. Temperature effects on steady-state growth, phosphorus uptake and the chemical composition of a marine phytoplankter. Microb. Ecol. 5:153-166.

_____ and R. Mann. 1980. Temperature-influenced variations in speciation and chemical composition of marine phytoplankton in outdoor mass cultures. J. Exp. Mar. Biol. Ecol. 46:29-39.

_____, J. J. Mc Carthy and D. G. Peavey. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature 279:210-215.

Goodwin, B. C. 1969. Synchronization of *Escherichia coli* in a chemostat by periodic phosphate feeting. Eur. J. Biochem. 10:511-514.

Gotham, I. J., III. 1977. Nutrient limited cyclostat growth: a theoretical and physiological analysis. Ph.D. thesis, State University of New York at Albany.

_____ and G.-Y. Rhee. 1980. Comparative studies of nutrient-limited growth and nutrient uptake in phytoplankton growing under a light/dark cycle. Abstracts of papers submitted for the 43rd annual meeting, American Society of Limnology and Oceanography, Inc., June 1980.

Grant, D., D. C. Swinton and K.-S. Chiang. 1978. Differential patterns of mitochondrial, chloroplastic and nuclear DNA synthesis in the synchronous cell cycle of *Chlamydomonas reinhardtii*. Planta 141: 259-267.

Hansche, P. E. 1969. A theoretical basis for the entrainment of chemostat populations. J. Theoret. Biol. 24:335-350.

Hansen, S. R. and S. P. Hubbell. 1980. Single-nutrient microbial competition: qualitative agreement between experimental and theoretically forecast outcomes. Science 207:1491-1493.

Harris, G. P. 1973. Diel and annual cycles of net plankton photosynthesis in Lake Ontario. J. Fish. Res. Board Can. 30:1779-1787.

Harrison, P. J. and C. O. Davis. 1979. The use of outdoor phytoplankton continuous cultures to analyse factors influencing species selection. J. Exp. Mar. Biol. Ecol. 41:9-23.

Hastings, J. W. and H.-G. Schweiger (eds.). 1976. The molecular basis of circadian rhythms, Dahlem Konferenzen, Abakon, Berlin.

Healey, F. P. 1980. Slope of the Monod equation as an indicator of advantage in nutrient competition. Microb. Ecol. 5:281-286.

Hecky, R. E. and P. Kilham. 1974. Environmental control of phytoplankton cell size. Limnol. Oceanogr. 19:361-366.

Herrmann, E. C. and R. R. Schmidt. 1965. Synthesis of phosphorus-containing macromolecules during synchronous growth of *Chlorella pyrenoidosa*. Biochim. Biophys. Acta. 95:63-75.

Hitchcock, G. L. 1980. Influence of temperature on the growth rate of *Skeletonema costatum* in response to variations in daily light intensity. Mar. Biol. 57:261-269.

Hobson, L. A. 1974. Effects of interactions of irradiance, daylength, and temperature on division rates of three species of marine unicellular algae. J. Fish. Res. Board Can. 31:391-395.

Hutchinson, G. E. 1961. The paradox of the plankton. Am. Naturalist 95:137-145.

Jacklett, J. W. 1980. Protein synthesis requirement of the *Aplysia* circadian clock tested by active and inactive derivatives of the inhibitor anisomycin. J. Exp. Biol. 85:33-42.

Jassby, A. D. and T. Platt. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol Oceanogr. 21:540-547.

Jeanjean, R. 1973. The relationship between the rate of phosphate absorption and protein synthesis during phosphate starvation in *Chlorella pyrenoidosa*. FEBS Letters 32:149-151.

Johnson, P. W. and J. M. Sieburth. 1979. Chroococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass. Limnol. Oceanogr. 24:928-935.

Karakashian, M. W. and H. G. Schweiger. 1976a. Temperature dependence of cycloheximide-sensitive phase of circadian cycle in *Acetabularia mediterranea*. Proc. Natl. Acad. Sci. USA 73:3216-3219.

_____ and _____. 1976b. Evidence for a cycloheximide-sensitive component in the biological clock of *Acetabularia*. Exptl. Cell Res. 98:303-312.

Kayser, H. 1979. Growth interactions between marine dinoflagellates in multispecies culture experiments. Mar. Biol. 52:357-369.

Keating, K. I. Allelopathic influence on blue-green bloom sequence in a eutrophic lake. Science 196:885-887.

Kellar, P. E. and H. W. Paerl. 1980. Physiological adaptations in response to environmental stress during an N_2-fixing *Anabaena* bloom. Appl. Environ. Microbiol. 40:587-595.

Kilham, P. and D. Tilman. 1979. The importance of resource competition and nutrient gradients for phytoplankton ecology. Arch. Hydrobiol. Beih. Ergebn. Limnol. 13:110-119.

Lang, D. S. 1980. Phosphorus metabolism of several aquatic microplankton. M. S. Thesis, University of Alaska.

Lang, D. S. and E. J. Brown. 1981. Phosphorus-limited growth of a green and a blue-green alga. In preparation.

Law, A. T. and D. K. Button. 1977. Multiple-carbon-source-limited growth kinetics of a marine Coryneform bacterium. J. Bacteriol. 129:115-123.

Leloir, L. F. and C. E. Cardini. 1957. Characterization of phosphorus compounds by acid lability. *In* S. P. Colowick and N. O. Kaplan (eds.), Methods in enzymology, Vol. III, Preparation and assay of substrates, pp. 840-849, Academic Press, New York.

Lewin, J. and V. N. R. Rao. 1975. Blooms of surf-zone diatoms along the coast of the Olympic Peninsula, Washington. VI. Daily periodcity phenomena associated with *Chaetoceros armatum* in its natural habitat. J. Phycol. 11:330-338.

Lindsey, J. K., B. D. Vance, J. S. Keefer and V. G. Scholes. 1971. Spheroplast formation and associated ultrastructural changes in a synchronous culture of *Anacystis nidulans* treated with lysozyme. J. Phycol. 7:65-71.

MacCaul, W. A. and T. Platt. 1977. Diel variations in the photosynthetic parameters of coastal marine phytoplankton. Limnol. Oceanogr. 22: 723-731.

Malone, T. C. 1971. Diurnal rhythms in netplankton and nannoplankton assimilation ratios. Mar. Biol. 10:285-289.

_____, C. Garside, K. C. Haines and D. A. Roels. 1975. Nitrate uptake and growth of *Chaetoceros* sp. in large outdoor continuous cultures. Limnol. Oceanogr. 20:9-19.

Marano, F., S. Amancio and A. M. Durrand. 1978. Synchronous growth and synthesis of macromolecules in a natural wall-less volvocale, *Dunaliella bioculata*. Protoplasma 95;135-144.

Marra, J. 1980. Time course of light intensity adaptation in a marine diatom. Mar. Biol. Letters 1:175-183.

McCarthy, J. J. and J. C. Goldman. 1979. Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. Science 203:670-672.

McMurry, L. and J. W. Hasting. 1972. No desynchronization among four circadian rhythms in the unicellular alga, *Gonyaulax polyedra*. Science 175:1137-1139.

Medveczky, N. and H. Rosenberg. 1971. Phosphate transport in *Escherichia coli*. Biochim. Biophys. Acta 241:494-506.

Monod, J. 1942. Recherches sur la croissance des cultures bacteriennes. 2nd edn. Hermann, Paris.

Nalewajko, C. and D. R. S. Lean. 1978. Phosphorus kinetics-algal growth relationships in batch cultures. Mitt. Internat. Verein. Limnol. 21:184-192.

Njus, D. 1976. Experimental approaches to membrane models. *In* J. W. Hastings and H.-G. Schweiger (eds.), The molecular basis of circadian rhythms, Dahlem Konferenzen, Abakon, Berlin.

Njus, D., F. M. Sulzman and J. W. Hastings. 1974. Membrane model for the circadian clock. Nature 248:116-120.

Paerl, H. W. 1979. Optimization of carbon dioxide and nitrogen fixation by the blue-green alga *Anabaena* in freshwater blooms. Oecologia 38:275-290.

_____ and P. E. Kellar. 1979. Nitrogen-fixing *Anabaena*: physiological adaptations instrumental in maintaining surface blooms. Science 204:620-622.

_____ and L. A. Mackenzie. 1977. A comparative study of the diurnal carbon fixation patterns of nannoplankton and net plankton. Limnol. Oceanogr. 22:732-738.

Pasciak, W. J. and J. Gavis. 1975. Transport limited nutrient uptake rates in *Ditylum brightwelli*. Limnol. Oceanogr. 20:604-617.

Perry, M. J. 1976. Phosphate utilization by an oceanic diatom in phosphorus-limited chemostat culture and in the oligotrophic waters of the central North Pacific. Limnol. Oceanogr. 21:88-107.

Prezelin, B. B. and A. C. Ley. 1980. Photosynthesis and chlorophyll *a* fluorescence rhythms of marine phytoplankton. Mar. Biol. 55:295-307.

Reshkin, S. J. and G. A. Knauer. 1979. Light stimulation of phosphate uptake in natural assemblages of phytoplankton. Limnol. Oceanogr. 24:1121-1124.

Rhee, G.-Y. 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. J. Phycol. 9:495-506.

_____. 1974. Phosphate uptake under nitrate limitation by *Scenedesmus* sp. and its ecological implications. J. Phycol. 10:470-475.

_____. 1980. Continuous culture in phytoplankton ecology. *In* M. R. Droop and H. W. Jannasch (eds.), Advances in Aquatic Microbiology, Vol. 2, Academic Press, New York.

Ricker, W. E. 1973. Linear regressions in fishery research. J. Fish. Res. Board Can. 30:409-434.

Robertson, B. R. and D. K. Button. 1979. The phosphate-limited continuous culture of *Rhodotorula rubra*: kinetics of transport, leakage and growth. J. Bacteriol. 138:884-895.

Rooney, D. W., J. L. Keene, K. A. Agit-Simh, R. G. Meier and D. M. Raben. 1977. Synchronization of *Chlamydomonas* division in chemostat cultures by periodic temperature reductions: effect on cellular ATP. J. Therm. Biol. 2:157-160.

Rosenberg, H., R. G. Gerdes and K. Chegwidden. 1977. Two systems for the uptake of phosphate in *Escherichia coli*. J. Bacteriol. 131: 505-511.

_____, N. Medveczky and J. M. LaNauze. 1969. Phosphate transport in *Bacillus cereus*. Biochim. Biophys. Acta 193:159-167.

Ryther, J. H. and J. G. Sanders. 1980. Experimental evidence of zooplankton control of the species composition and size distribution of marine phytoplankton. Mar. Ecol. Prog. Ser. 3:279-283.

Saino, T. and A. Hattori. 1978. Diel variation in nitrogen fixation by a marine blue-green alga, *Trichodesmium thiebautii*. Deep-sea Res. 25:1259-1263.

Senft, W. H. 1978. Dependence of light-saturated rates of algal photosynthesis on intracellular concentration of phosphorus. Limnol. Oceanogr. 23:709-718.

Shapiro, J. 1973. Blue-green algae: why they become dominant. Science 179:382-384.

Sharp, J. H., P. A. Underhill and D. J. Hughes. 1979. Interaction (allelopathy) between marine diatoms: *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*. J. Phycol. 15:353-362.

Smayda, T. J. 1975. Phased cell division in natural populations of the marine diatom *Ditylum brightwelli* and the potential significance of diel phytoplankton behavior in the sea. Deep-Sea Res. 22:151-165.

Staker, R. D. and S. F. Bruno. 1980. Diurnal vertical migration in marine phytoplankton. Bot. Mar. 23:167-172.

Staley, J. T. 1971. Growth rates of algae determined *in situ* using an immersed microscope. J. Phycol. 7:13-17.

Stross, R. G. 1975. Causes of daily rhythms in photosynthetic rates of phytoplankton. II. Phosphate control of expression in tundra ponds. Biol. Bull. 149:408-418.

_____. 1980. Growth cycles and nutrient-limited photosynthesis in phytoplankton. Limnol Oceanogr. 25:538-544.

_____, S. W. Chisholm and T. A. Downing. 1973. Causes of daily rhythms in photosynthetic rates of phytoplankton. Biol. Bull. 145:200-209.

_____ and S. M. Pemrick. 1974. Nutrient uptake kinetics in phytoplankton: a basis for niche separation. J. Phycol. 10:164-169.

Sullivan, C. W. 1977. Diatom mineralization of silicic acid. II. Regulation of $Si(OH)_4$ transport rates during the cell cycle of *Navicula pelliculosa*. J. Phycol. 13:86-94.

Sulzman, F. M. and L. N. Edmunds, Jr. 1972. Persisting circadian oscillations in enzyme activity in non-dividing cultures of *Euglena*. Biochem. Biophys. Res. Commun. 47:1338-1344.

Sundberg, I. and M. Nilshammar-Holmvall. 1975. The diurnal variation in phosphate uptake and ATP level in relation to deposition of starch, lipid, and polyphosphate in synchronized cells of *Scenedesmus*. Z. Pflanzenphysiol. Bd. 76.S:270-279.

Sweeney, B. M. 1976a. Circadian rhythms, definition and general characterization, *In* J. W. Hastings and H.-G. Schweiger (eds.), The molecular basis of circadian rhythms, Dahlem Konferenzen, Abakon, Berlin.

_____. 1976b. Evidence that membranes are components of circadian oscillators, *In* J. W. Hastings and H.-G. Schweiger (eds.), The molecular basis of circadian rhythms, Dahlem Konferenzen, Abakon, Berlin.

Takabayashi, A., T. Nishimura and T. Iwamura. 1976. Incorporation of radioactive tracers of some labelled compounds into DNA, RNA, and protein in *Chlorella* cells at various stages of the cell cycle in synchronous culture. J. Gen. Appl. Microbiol. 22:183-196.

Taylor, P. A. and P. J. LeB. Williams. 1975. Theoretical studies on the coexistence of competing species under continuous-flow conditions. Can. J. Microbiol. 21:90-98.

Thomas, W. H., M. Pollock and D. L. R. Seibert. 1980. Effects of simulated upwelling and oligotrophy on chemostat-grown natural marine phytoplankton assemblages. J. Exp. Mar. Biol. Ecol. 45:25-36.

Tilman, D. 1977. Resource competition between planktonic algae: an experimental and theoretical approach. Ecology 58:338-348.

Titman, D. 1976. Ecological competition between algae: experimental comfirmation of resource based competition theory. Science 192: 463-465.

Toerien, D. F., C. H. Huang, J. Radimsky, E. A. Pearson and J. Scherfig. 1971. Final Report. Provisional algal assay procedures. SERL report No. 71-6. Sanitary Engineering Research Laboratory, Universith of California, Berkeley.

Tommassen, J. and B. Lugtenberg. 1980. Outer membrane protein e of *Escherichia coli* K-12 is co-regulated with alkaline phosphatase. J. Bacteriol. 143:151-157.

Turpin, D. H. and P. J. Harrison. 1979. Limiting nutrient patchiness and its role in phytoplankton ecology. J. Exp. Mar. Biol. Ecol. 39:151-166.

_____ and _____. 1980. Cell size manipulation in natural marine, planktonic, diatom communities. Can. J. Fish. Aquat. Sci. 37:1193-1195.

Vaccaro, R. F. and H. W. Jannasch. 1966. Studies on heterotrophic activity in seawater based on glucose assimilation. Limnol. Oceanogr. 11:596-607.

Vanden Driessche, T. 1966. The role of the nucleus in the circadian rhythms of *Acetabularia mediterranea*. Biochim. Biophys. Acta 126: 456-470.

_____, S. Bonotto and J. Brachet. 1970. Inability of rifampicin to inhibit cicadian rhythmicity in *Acetabularia* despite inhibition of RNA synthesis. Biochim. Biophys. Acta. 224:631-634.

Van Gemerden, H. 1974. Coexistence of organisms competing for the same substrate: an example among the purple sulfur bacteria. Microb. Ecol. 1:104-119.

Waterbury, J. B., S. W. Watson and F. W. Valois. 1981. The role of *Synechococcus* spp. as oceanic primary producers. Abstracts of the annual meeting, American Society for Microbiology, P187, N87.

Weiler, C. S. and S. W. Chisholm. 1976. Phased cell division in natural populations of marine dinoflagellates from shipboard cultures. J. Exp. Mar. Biol. Ecol. 25:239-247.

Williams, F. M. 1971. Dynamics of microbial populations. *In* B. C. Patten (ed.), Systems analysis and simulation in ecology, Vol. I, Academic Press, New York, pp. 607.

Williamson, C. E. 1980. Phased cell division in natural and laboratory populations of marine planktonic diatoms. J. Exp. Mar. Biol. Ecol. 43:271-279.

Willsky, G. R., R. L. Bennett and M. H. Malamy. 1973. Inorganic phosphate transport in *Escherichia coli:* involvement of two genes which play a role in alkaline phosphatase regulation. J. Bacteriol. 113: 529-539.

_____ and M. H. Malamy. 1980a. Characterization of two genetically separable inorganic phosphate transport systems in *Escherichia coli*. J. Bacteriol. 144:356-365.

_____ and _____. 1980b. Effect of arsenate on inorganic phosphate transport in *Escherichia coli*. J. Bacteriol. 144:366-374.

Zevenboom, W. 1980. Growth and nutrient uptake kinetics of *Oscillatoria agardhii*. A comparative investigation of continuous cultures and natural populations of a cyanobacterium. Academisch Proefschrift, Universiteit van Amsterdam, Amsterdam.

_____ and L. Mur. 1979a. Influence of growth rate on short term and steady state nitrate uptake by nitrate-limited *Oscillatoria agardhii*. FEMS Microbiol. Letters 6:209-212.

_____ and _____. 1979b. Nitrogen-limited growth of *Oscillatoria agardhii* in chemostat cultures. *In* W. Zevenboom, Growth and nutrient uptake kinetics of *Oscillatoria agardhii*. A comparative investigation of continuous cultures and natural populations of a cyanobacterium. Academisch Proefschrift, Universiteit van Amsterdam, Amsterdam.