# THE EFFECTS OF IRRADIANCE AND PHOSPHORUS ON BATCH CULTURES OF PAVLOVA (= MONOCHRYSIS) LUTHERI

A Thesis

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by

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## ABSTRACT

Pavlova (= Monochrysis) lutheri was grown in axenic batch cultures at 5 levels of irradiance ranging from 140 to 9 W $m^{-2}$ (experiments were replicated at three irradiances). The experiments were designed so that phosphorus limited the yield and the final biomass was not greater than 2.5 x $10^6$ cells $ml^{-1}$. Growth parameters were obtained from the nutrient-saturated, exponential phase and the phosphorus-controlled, transitional phase of each experiment. The nutrient saturated, maximum growth rate, $\mu_m$, varied from .979 to .247 $day^{-1}$ as a function of irradiance. Growth rates were "inhibited" at the highest irradiance. The differential form of Droop's cell-quota model was fitted to the time-series of cell numbers, dissolved phosphorus, and cellular phosphorus (cell quota) obtained from the phosphorus-controlled phase of each experiment. A comparison amongst experiments of the estimates of $\mu_m'$, $k_Q$ (the parameters in Droop's model) and $Q_m'$ (the cell quota at the transition point between constant, exponential growth and phosphorus-controlled growth) disproved the hypothesis that phosphorus-controlled growth rate is the same at all irradiances. The results of the experiments are described by a simple modification of Droop's cell quota model. The changes in chlorophyll-a $cell^{-1}$ within an experiment and amongst light levels, is discussed. One experiment was run with a light/dark cycle (16 hr on and 8 hr off) and the within-day dynamics of uptake, growth rate, cell size, and changes in chlorophyll-a are discussed. In measuring irradiance, it was decided to make no allowance for self-shading.

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## DEFINITIONS

### State Variables

$X$ number of cells ($10^6$ cells $ml^{-1}$)
$S$ dissolved nutrient (nM P $ml^{-1}$)
$Q$ cell quota: nutrient in the cell (nM P ($10^6$ cells)$^{-1}$)

### Measured Rates

$u$ specific rates of uptake, $-\frac{1}{X}\frac{dS}{dt}$ (nM P ($10^6$ cells)$^{-1}$ $hr^{-1}$)

$\mu$ specific rate of growth, $\frac{1}{X}\frac{dX}{dt}$ ($hr^{-1}$)

$\mu_m$ exponential, nutrient-saturated rate of growth at a given illumination ($hr^{-1}$)

### Empirically Derived or Estimated Rates

$u_m$ maximum rate of uptake (mM P ($10^6$ cells)$^{-1}$ $hr^{-1}$)

$\mu'_m$ maximum rate of nutrient-controlled growth - $\mu$ would equal $\mu'_m$ if Q equalled infinity - at a given illumination ($hr^{-1}$)

$\mu_{opt}$ exponential rate of growth, at an optimal level of irradiance ($hr^{-1}$)

$\mu'_{opt}$ maximum nutrient-controlled rate of growth, at an optimal level of irradiance ($hr^{-1}$)

$r$ "rate of respiration" - this constant causes the predicted growth rate to equal zero at the compensation level of irradiance ($hr^{-1}$)

### Empirical Constants

$S_o$ the concentration of dissolved nutrient at which uptake ceases (nM P $ml^{-1}$)

$Q_o$, $k_Q$ the minimum cell quota, or the subsistence quota: the concentration of nutrient/cell at which growth ceases (nM P ($10^6$ cells)$^{-1}$)

$I_o$, $k_I$ the compensation level of irradiance: the level of irradiance at which growth ceases (W $m^{-2}$)

$K_S$ the half-rate constant for uptake as a function of dissolved nutrient (nM P $ml^{-1}$)

$K_Q$ the half-rate constant for growth as a function of cell quota (nM P ($10^6$ cells)$^{-1}$)

$K_I$ the half-rate constant for maximum growth rate as a function of illumination (W $m^{-2}$)

$\alpha$ a parameter in Steele's equation for photosynthesis vs light ($m^2$ $W^{-1}$ $day^{-1}$)

$\beta$ a parameter in Steele's equation for photosynthesis vs light ($m^2$ $W^{-1}$)

### Observed Variables

$I$ irradiance, measured as photosynthetically active radiation, PAR (W $m^{-2}$)

$t$ time measured from the beginning of the experiment ($t_o$)

$\Delta t$ an increment of time between two samples

Observed Variables (cont.)

$X_o$ initial concentration of cells in a batch culture: cells at time = 0 ($10^6$ cells $ml^{-1}$)

$X_t$ the concentration of cells at time = t ($10^6$ cells $ml^{-1}$)

$Q_m$ maximum cell-quota when dissolved nutrient is in excess (nM P ($10^6$ cells)$^{-1}$)

$Q_m'$ the highest level of cell quota for the controlling nutrient - the growth rate is unaffected by changes in the cell quota above this level.

*Note: at $Q_m'$, $\mu = \mu_m$. The empirically derived value of the maximum nutrient-controlled growth rate, $\mu_m'$, is always larger than $\mu_m$ because, while $\mu_m$ is an observed growth rate, $\mu_m'$ is an empirical constant representing an asymtoptic value. The value $\mu_m'$ can never be observed and should not be confused or equated with the maximum, observed growth-rate.

# INTRODUCTION

## A. General

Aquatic ecologists need models to describe and predict the growth of phytoplankton. Experimental work, mainly with cultured algae, has supplied models for photosynthesis and for nutrient-controlled growth. No model has however been satisfactorily validated for the interaction between light and nutrients in controlling growth. The primary aim of the work described in this thesis was to examine this interaction. A secondary purpose was methodological. Most recent work on nutrient-growth relationships has involved chemostat cultures and model parameterization using linearly transformed data. Batch cultures, however, are also useful for model development and parameterization, and it is desirable that nonlinear methods be used for the latter. I also investigated this aspect of modelling the light/nutrient interaction.

An important aspect of models for phytoplankton growth is the way in which they handle the concept of limitation. According to Liebig's law of the minimum (Liebig, 1840), limitation refers to the limitation of final yield by that nutrient in shortest supply relative to the needs of a plant. Blackman (1905) extended the concept to include rate-limiting factors. This can lead to confusion, and it is therefore useful to distinguish between limitation of yield and control of a rate.

Smith (1936) developed one of the earliest models for the photosynthesis-light relationship. It was not for twenty years, however, that Talling (1957) applied it to algae. The science of modelling algal growth processes can

be said to have begun properly with the work of Monod (1942) on substrate-limited growth in micro-organisms. An important development was the conceptual separation of the processes of nutrient uptake and nutrient-controlled growth, begun by Mackereth (1953) and formalized by Caperon (1968) and Droop (1968). Caperon and Droop related growth rate to the cells internal store of controlling nutrient, a quantity termed the cell quota (Droop, 1968). Cell-quota models have been successfully used to describe growth in a number of algae controlled by a variety of nutrients (Fuhs, 1969; Paasche, 1973) and have been extended to describe the relationship between growth rate and uptake of controlling and noncontrolling nutrients (Droop, 1974, 1977; Nyholm, 1975; Rhee, 1974). Another development of the model, one that is especially important to ecologists and at the same time harks back to Liebig, is the discovery that nutrients do not "co-limit" growth. Instead there is a threshold response. Only one nutrient controls growth at any moment (Droop, 1974; Rhee, 1974, 1978).

Meanwhile, models of the relationship between illumination, photosynthesis and growth developed separately. Jassby and Platt (1976) summarize a number of models for photosynthesis. Gross carbon fixation in photosynthesis has been relatively easy to model; the difficulty appears to lie in relating photosynthesis to growth and in handling the effects of respiration. Attempts to link nutrient uptake with photosynthesis (MacIsaac and Dugdale, 1972), to use carbon:chlorophyll ratios to mediate between photosynthesis and nutrient controlled growth (Tett et al., 1975) and to design a grand model for light and nutrients have not proven

satisfactory. The interaction between light and nutrients in the control of phytoplankton growth is clearly important to ecologists. A major unresolved question in respect of this interaction is whether variations in illumination and nutrient quota cause a threshold (either-or) or combined (additive or multiplicative) type of response. My experiments were designed to answer this question in relation to phosphorus-controlled growth of batch cultures of the Haptophycean alga Pavlova (= Monochrysis) lutheri Green (Droop).

B. Culture Techniques

A simple and elegant method of determining the relationships between various environmental and physiological factors and growth of algae is the technique of continuous culture, especially the chemostat. In a chemostat at steady state all the rates of change are equal to zero (see Herbert et al., 1956) and the growth rate is equal to the dilution rate. It is possible to determine the relationship between growth rate and the corresponding physiological variable (such as the concentration of the nutrient controlling growth) by running the chemostat at various dilution rates.

Because a chemostat is a steady-state device, it is not always easy to distinguish cause and effect. In addition, chemostat results might not explain transient changes in the growth of algae. It is thus desirable to use batch as well as continuous culture when investigating algal growth. Droop (1975) has shown, however, that the chemostat-derived model of nutrient-controlled growth adequately described the dynamics of several batch-culture experiments. Moreover, it is theoretically possible to use batch-culture data to quantify the relationship between variables (Lederman, 1974). For reasons to be discussed more fully later I decided to use the batch-culture technique, and carried out batch experiments at several illuminations on phosphorus-controlled growth.

A theoretical curve of biomass throughout the course of a batch cultures is given in Figure 1. The dynamics of algal growth in a batch culture can be categorized into four phases (stages): lag, exponential (logarithmic), transitional (nutrient controlled), and stationary (Spencer,

1954). These phases, excluding the lag, are marked in Figure 1. An experiment starts when algae are inoculated into a medium which has been formulated so that all nutrients are available in excess of initial needs. During the lag phase the behaviour of the algae is unexplained; however, there is an initial rapid uptake of nutrients. Once the algae have adjusted to the experimental conditions, they grow at a constant, exponential rate and the rate is controlled by illumination or carbon fixation. The exponential phase continues until a nutrient in the cell decreases to the point where its concentration takes over the control of growth rate (labelled as transition point in Figure 1). The rate of growth steadily decreases as the cellular concentration of the contrclling nutrient decreases. Growth ceases when there is no more of the controlling nutrient available within the cell than the minimum necessary to sustain life.

The "transition point" of growth in a batch culture takes place at a particular (and measurable) cell quota. If there is a threshold transition, the transition point should be at a lower cell quota for lower illuminations. If there is a combined effect during the transition, the transition point should be the same at various light levels and the phosphorus-controlled growth rates should be lower as the illumination decreased. This argument is explored more fully, later( pages 14 and 15).

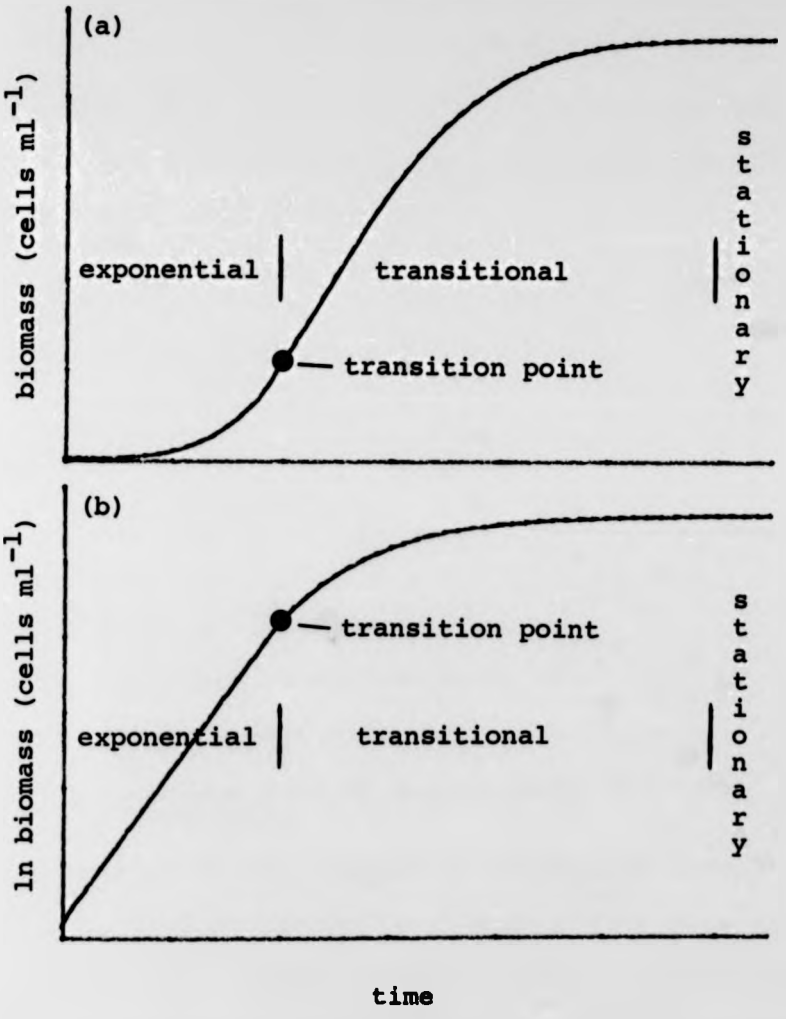



Figure 1. Theoretical curve of biomass (a) and ln biomass (b) throughout a batch culture. Three phases are labelled and a dot is placed at the transition point.

C. Models of Algal Growth

It is possible to quantify the dynamics of the light-controlled and phosphorus-controlled phases of growth. This is accomplished through the use of simple mathematical models. The growth rate during the exponential phase ($\mu_m$) is a constant for each light level:

$$\frac{dX}{dt} = \mu_m X; \tag{1a}$$

integrating between $X = X_o$ and $X = X_t$,

$$X_t = X_o e^{\mu_m t}; \tag{1b}$$

which can be logarithmically transformed,

$$\ln X_t = \mu_m t + \ln X_o. \tag{1c}$$

where:

$X_o$ = initial biomass;

$X_t$ = biomass at a given time "t";

$t$ = time from t = 0; and,

$\mu_m$ = maximum rate of growth given the light conditions

The situation during phosphorus-controlled growth is more complex. Various mathematical models have been presented (Nyholm, 1977 reviews several models) to describe the relationship between growth rate ($\mu$; or, $\frac{1}{X}\frac{dX}{dt}$) and the cellular concentration of controlling nutrient, cell quota (Q). An algebraic form of each model is suitable for chemostat experiments where time is not a variable, but batch-culture experiments require the general form in which each model is written as differential equations. The two most widely used cell-quota models are Droop's (1968) and Caperon's (1968) and in some respects they are similar. Both models may be written as:

$$\frac{dX}{dt} = \mu X \tag{2}$$

$$\frac{dQ}{dt} = u - \mu Q \tag{3}$$

$$\frac{dS}{dt} = -uX \tag{4}$$

The state variables and rates are defined as:

X = biomass ($10^6$ cells $ml^{-1}$);

Q = cell quota ($\frac{\text{particulate nutrient } ml^{-1}}{10^6 \text{ cells } ml^{-1}}$ = nutrient ($10^6$ cells)$^{-1}$);

S = dissolved nutrient (nutrient $ml^{-1}$);

$\mu$ = specific growth rate = $\frac{1}{X}\frac{dX}{dt}$ ($hr^{-1}$); and,

u = specific uptake rate = $-\frac{1}{X}\frac{dS}{dt}$ (nutrient ($10^6$ cells)$^{-1}$ $hr^{-1}$).

The two models differ in how they describe specific growth rate and are presented below for comparison:

Droop's Model

$$\frac{dX}{dt} = \mu_m' \left(1 - \frac{k_Q}{Q}\right) X, \tag{5}$$

$$\frac{dQ}{dt} = u_m \left(\frac{S - S_o}{K_S + S - S_o}\right) - \mu_m' \left(1 - \frac{k_Q}{Q}\right) Q, \tag{6}$$

$$\frac{dS}{dt} = -u_m \left(\frac{S - S_o}{K_S + S - S_o}\right) X. \tag{7}$$

Caperon's Model

$$\frac{dX}{dt} = \mu_m' \left(\frac{Q - Q_o}{K_Q + Q - Q_o}\right) X, \tag{8}$$

$$\frac{dQ}{dt} = u_m \left(\frac{S - S_o}{K_S + S - S_o}\right) - \mu_m' \left(\frac{Q - Q_o}{K_Q + Q - Q_o}\right) Q \tag{9}$$

$$\frac{dS}{dt} = -u_m \left(\frac{S - S_o}{K_S + S - S_o}\right) X. \tag{10}$$

The constants and rate coefficients (the parameters of the models) are defined as:

$\mu_m'$ = maximum specific growth rate: value of $\mu$ when $Q = \infty$ ($hr^{-1}$);

$u_m$ = maximum specific uptake rate: value of u when $S = \infty$ (nutrient $(10^6$ cells$)^{-1}$ $hr^{-1}$);

$k_Q = Q_o$ = subsistence level of cellular nutrient: level of Q at which $\mu = 0$ (nutrient $(10^6$ cells$)^{-1}$);

$K_Q$ = half-rate constant for growth: level at which $\mu = 1/2\ \mu_m'$ (nutrient $(10^6$ cells$)^{-1}$);

$S_o$ = level of dissolved nutrient at which u = 0 (nutrient $ml^{-1}$); and

$K_S$ = half-rate constant for uptake: level of S at which $u = 1/2\ u_m$ (nutrient $ml^{-1}$).

The expressions for uptake and growth, in both models, are similar in form (although with the addition of $S_o$ or $Q_o$ terms) to the equation for a rectangular hyperbola as used by Monod (1942, 1949), which has regularly been applied to describe adsorption, uptake, and growth. The equations (of Droop and Caperon) for growth are equivalent when $K_Q = Q_o$, as shown below:

$$\frac{\mu}{\mu_m'} = \left(\frac{Q - Q_o}{K_Q + Q - Q_o}\right) = \left(\frac{Q - Q_o}{Q}\right) = \frac{Q - k_Q}{Q} = \left(1 - \frac{k_Q}{Q}\right);$$

if $K_Q = Q_o$.

Thus, the only difference between the two models is the increased complexity of Caperon's model which has six parameters, whereas Droop's has five.

Note: all variables and parameters are listed in DEFINITIONS.

D. Parameter Estimation

An equation or group of equations that describe a functional relationship between two or more experimentally measured quantities - the observed or state variables - is generally called a mathematical model, or simply a model. Models are made up of at least one independent and one dependent variable, and various constants, called parameters. Parameters may be dimensionless or they may have dimensions of units that are mathematically consistent with the dependent and independent variables.

Using an equation for a line as an example, the parameters in the equation, $Y = mX + b$, are m (the slope) and b (the intercept). In this model the parameters are linear since only one parameter is present in each term of the equation. Likewise each variable occurs in only one term. Due to its simplicity, a linear model is a very useful description of relationships between various biological variables; however, natural phenomena are often more complicated and many relationships between variables are nonlinear with respect to both the variables and the parameters. Nonlinear relationships can sometimes be linearized by transforming one or more of the variables (Barnes, 1952 and Sokal and Rohlf, 1969). Thus Monod's model for substrate-limited growth can be linearized in a variety of ways (Dowd and Riggs, 1965). Nonlinear models have the drawback that it is often difficult to determine unbiased (in a statistical sense) estimates of nonlinear parameters. Transformation is no help in this - the estimates it enables are often biased.

Given a model and a set of data, it is necessary to

choose a value for each parameter so that the model gives the best possible fit to the data. Parameter estimation is the general name given to procedures that calculate the best values for the set of parameters in a model. All estimation techniques attempt to select the values for the parameters which result in the predictions of the model being closer to the observations than would those predictions resulting from any other combination of parameter values.

Least squares linear regression is the estimation technique that calculates values for the two parameters, slope and intercept, using a model for a straight line. The parameters are estimated by the exact solution of an algebraic equation, making the linear model one of the simplest models to parameterize. Equation 5 can be rewritten so that it can be treated as a linear model. This is especially suitable for the special case of chemostat data; where $\mu = D$ (growth rate equals the dilution rate of the chemostat, see Herbert et al., 1956). Equation 5 for growth rate:

$$\frac{1}{X}\frac{dX}{dt} = \mu = D = \mu_m' \left(1 - \frac{k_Q}{Q}\right) \text{ becomes}$$

$$D = \mu_m' - \frac{\mu_m' k_Q}{Q} = \mu_m' - \frac{1}{Q}\left(\mu_m' k_Q\right). \qquad (11)$$

Equation 11 has the same form as the equation for a straight line, but it should be used with caution as there are problems associated with transformed equations (Dowd and Riggs, 1965). Furthermore, the transformed equations cannot be directly applied to batch-culture data because the rates are constantly changing. $\mu$ cannot be estimated from D, but it is possible, with batch-culture data, to make approximations

of the rates ($\mu$ and u) by finite difference:

$$\mu = \frac{1}{X}\frac{dX}{dt} \simeq \frac{\Delta X}{X}\frac{1}{\Delta t} \simeq \frac{\ln X_{t+1} - \ln X_t}{(t+1) - t}, \quad (12)$$

and Q is then calculated as the average of the Q's at t and t + 1. The error which is inherent in the finite-difference approximation decreases as $\Delta t$ decreases. There are two major criticisms of the use of finite differences: many closely spaced measurements must be taken or the errors in the estimated rates might be large; and, when the values are closely spaced due to taking many measurements, the true differences in state variables might be small relative to measurement errors and the estimated rate might therefore be unrecognizable (that is, not distinguishable from zero).

In order to fit nonlinear models to data it is necessary to use a nonlinear parameter estimation procedure. These procedures are iterative solutions that minimize an objective function based (usually) on the sum of squared residual error. Each "residual" is the difference between an observed value of the dependent variable and the value predicted by the model, given a set of values for the parameters. In each iteration a slightly different set of parameter values is used, until the residual error is at a minimum. In the case of models in the form of differential equations, the sum of squared error is calculated by comparing integrals of the equations with observed values of the state variables.

Various estimation procedures are available as program packages for digital computers. These packages include commonly available programs based on the technique of Marquardt (1963), and the less widely distributed but com-

prehensive and sophisticated package by Bard (1967b). With certain "well-formed" problems any technique may be satisfactory - the major differences being the time taken to converge on a solution and the amount of computer memory needed for the program. I have chosen to use the program package by Bard (1967b), which has large space requirements but is well documented and relatively easy to implement, and reliable at finding "true" solutions. A full presentation of various estimation techniques is given by Bard in Nonlinear Parameter Estimation (1974).

Since the behaviour of a nonlinear problem is rarely predictable, it is necessary to test an estimation technique with the model and a simulated data set before attempting to fit the model to experimental data. It has been demonstrated (Lederman, 1974) that Bard's estimation technique can fit various phytoplankton growth models to simulated batch-culture data. In a similar manner, all the models presented in this study were fit to simulated data in order to test the applicability of Bard's technique. Although this test seems obvious, it is not always carried out. Erroneous conclusions can result from using unsuitable estimation techniques without prior testing with simulated data.

E. Hypothesis for Light/Phosphorus Interactions

Now that models of nutrient-controlled growth have been presented, it is possible to demonstrate mathematically the difference between the threshold- and combined-effect hypotheses of light/phosphorus interaction. First, however, it is necessary to assume that $\mu_m$ (the exponential or nutrient-saturated rate of growth) is different at different illuminations. If the threshold hypothesis is correct, $\mu_m'$ should be the same at both light levels, and the exponential phase should continue for a longer time at the lower light levels until the effect of phosphorus causes the growth rate to be smaller than the exponential growth rate. Given the equation:

$$\mu = \mu_m' \left(1 - \frac{k_Q}{Q}\right),$$

and the fact that phosphorus-controlled growth rate ($\mu$) equals $\mu_m$ at the transition point, the value of Q at this transition ($Q_m'$) can be predicted from the new equation:

$$\mu_m = \mu_m' \left(1 - \frac{k_Q}{Q_m'}\right), \text{ or,}$$

$$Q_m' = k_Q \frac{\mu_m'}{\mu_m' - \mu_m} . \qquad (13a)$$

Thus, if $\mu_m$ differs with illumination, $Q_m'$ should vary at different illuminations. Hypothetical values are given for two light levels in Table 1, under "threshold hypothesis".

The alternative hypothesis is that illumination and phosphorus cell-quota simultaneously affect growth rate when the cell quota becomes lower than $Q_m'$. Under this hypothesis, $Q_m'$ is constant for all light levels. Instead, $\mu_m'$ (as well as $\mu_m$) vary as a function of illumination. The alternative to equation 13a is:

$$\mu_m' = \left(\frac{\mu_m}{1 - k_Q/Q_m}\right). \qquad (13b)$$

Values are given for two light levels in Table 1, under "interaction hypothesis".

In the present study, data from batch cultures grown at five light levels were analyzed to determine whether the effects of phosphorus control were the same at all illuminations. The two growth models, equations 5 - 10, were fit to the data sets using a variety of parameter estimation techniques. The growth parameters from the different light conditions were then compared as a test of the two opposing hypotheses of light/phosphorus interaction.

TABLE 1. Comparison of predicted growth rates based on two hypotheses of light/nutrient interactions. The values are for growth in batch cultures at two hypothetical illuminations.

| | relative irradiance | $\mu_m$ | $\mu_m'$ | $Q_m'$ |
|---|---|---|---|---|
| Threshold hypothesis | 100% | 1.0 | 1.33 | 2.0 |
| | 50% | .5 | 1.33 | .8 |
| Interaction hypothesis | 100% | 1.0 | 1.33 | 2.0 |
| | 50% | .5 | .66 | 2.0 |

The following equation describes the relationship between $\mu_m$ and $\mu_m'$ at the transition between nutrient-saturated and nutrient-controlled growth:

$$\mu_m = \mu_m' \left(1 - \frac{k_Q}{Q_m'}\right),$$

where: $\mu_m$ = nutrient-saturated growth rate

$\mu_m'$ = maximum, nutrient-controlled growth rate

$k_Q$ = subsistence quota for controlling nutrient

$Q_m'$ = cell quota at which control of growth changes from nutrient-saturated to nutrient-controlled

F. Models of Growth Rate vs Irradiance

Since this study resulted in estimates of $\mu_m$ at different illuminations, it was possible to parameterize models that predict exponential growth rate as a function of illumination. A simple model was presented by Eppley and Dyer (1965) and is analogous to Monod's (1942) model for substrate-limited growth:

$$\mu_m = \mu_{opt} \left(\frac{I}{I + K_I}\right), \qquad (14)$$

where: $\mu_{opt}$ = exponential growth rate at the optimum irradiance ($hr^{-1}$);

$\mu_m$ = exponential growth rate at a given irradiance ($hr^{-1}$);

$I$ = irradiance (watt $m^{-2}$); and,

$K_I$ = half-rate constant, for irradiance (watt $m^{-2}$).

There are a variety of similar, empirical functions (reviewed by Jassby and Platt, 1976), but the above is shown because of its simplicity.

The simple model may be expanded to include respiration, thus describing the way in which growth ceases at or below a certain (compensation) illumination. There are two general ways of modelling respiration:

1) Use a constant respiration rate -

$$\mu_m = \mu_{opt} \left(\frac{I}{I + K_I}\right) - r, \qquad (15)$$

where: $r$ = respiration rate ($hr^{-1}$).

2) Cause growth rate to equal zero at a 'minimum' illumination (compensation illumination), which results in growth rate becoming negative at illuminations lower

than the mimimum -

$$\mu_m = \mu_{opt} \left(\frac{I - I_o}{I - I_o + K_I}\right), \qquad (16)$$

where: $I_o$ = subsistence (compensation) level for light (watt $m^{-2}$).

This expression simplifies if it is found that $K_I = I_o$ (as is the case for nutrients in Pavlova, Droop, 1968):

$$\mu = \mu_{opt} \left(\frac{I - I_o}{I}\right) = \mu_{opt} \left(1 - \frac{I_o}{I}\right). \qquad (17)$$

Another model, which may be useful, is one presented by Steele (1962) that describes inhibition of photosynthesis at high irradiances and is here used to describe growth rate vs irradiance. The equation, with the addition of a respiration term, may be written:

$$\mu_m = \alpha I e^{-\beta I} - r, \qquad (18)$$

where: $\alpha$ = empirical constant ($m^2$ $watt^{-1}$ $hr^{-1}$);

$\beta$ = empirical constant ($m^2$ $watt^{-1}$); and,

$r$ = respiration rate ($hr^{-1}$).

## MATERIALS

A clonal culture (strain 261) of brackish-water, rock-pool, haptophyte, Pavlova lutheri (Droop) Green (= Monochrysis lutheri Droop) was obtained from the culture collection at the Scottish Marine Biological Association. Batch "starter cultures" were maintained in 100 ml erlenmyer flasks fitted with an air lock through a bung in the top and an extraction line through a side arm. Five to 25 ml of the starter culture were used as an inoculum for the chemostats and for the last two batch-culture experiments. The volume of the starter cultures were replenished by aseptic addition of fresh medium. The starter and experimental batch cultures were grown in the same medium as the chemostats, an artificial, half-strength seawater, S104 (Appendix ID, similar to S88 in Droop, 1966). All cultures of the alga were axenic and sterility tests were regularly carried out.

One to three chemostats were maintained in 250 ml spherical flasks held to a frame in a perspex water tank (Appendix IA). A single, two-liter, spherical, batch-culture vessel was clamped to the opposite side of the frame and was used for all experiments. Each culture assembly could be easily removed to be cleaned and autoclaved. Two sides of the water tank were clear and a bank of fluorescent lamps (Appendix IB) was placed against each of these sides. Light intensity was adjusted to five levels ranging from 141 to 9 watts/$m^2$ (Photosynthetically Active Radiation, PAR - see Appendix IB) by placing layers of tracing paper over the clear sides of the tank. Water-saturated air was continuously pumped through the cultures,

and in chemostats the movement of the air provided enough agitation to keep the cultures well mixed (details of the chemostats are given in Appendix IC). In the larger batch vessel, an anchor-shaped stirring rod, motor and belt driven at two revolutions per second, kept the cells in suspension (Figure 2). All cultures were grown at 20.5°C.

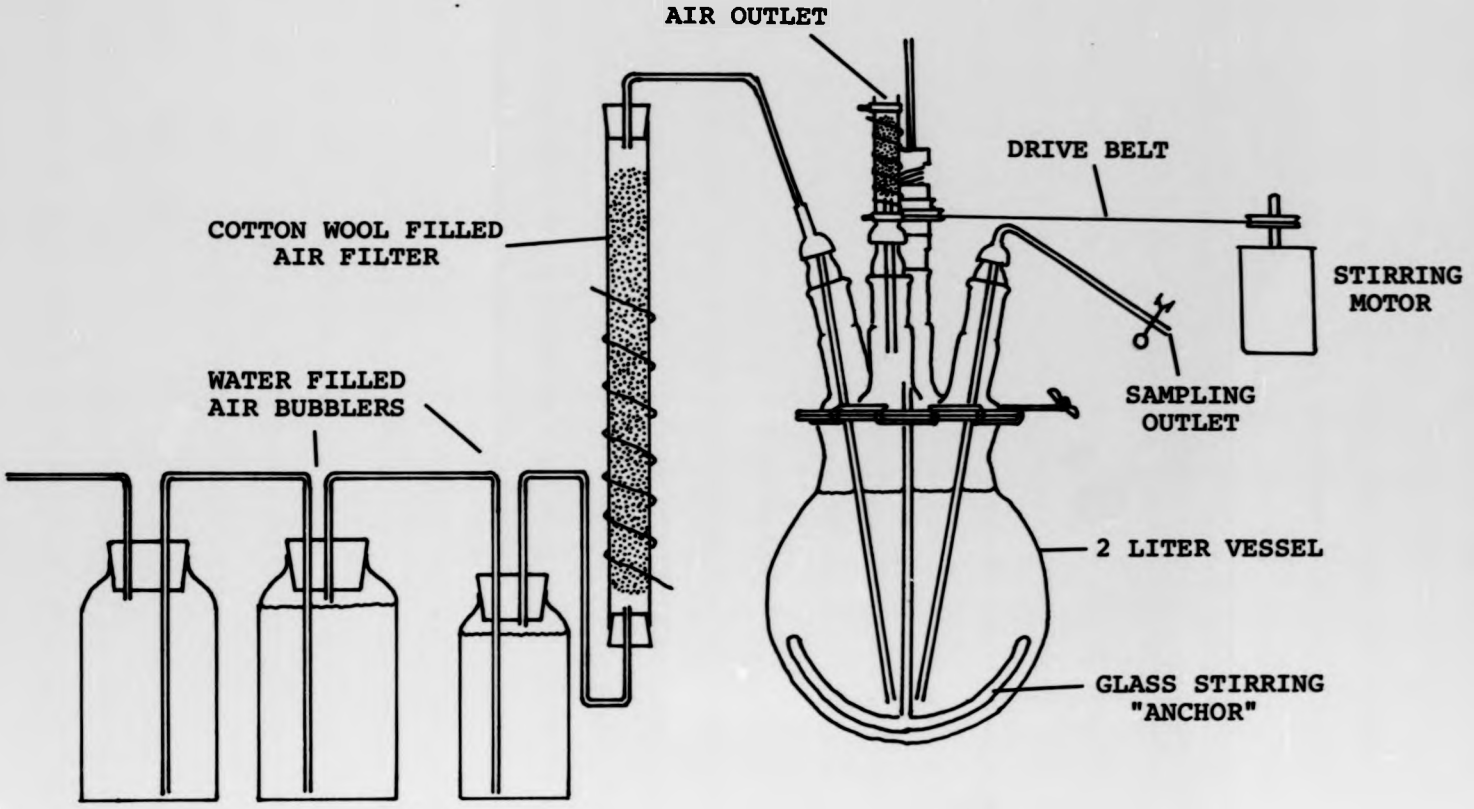



Figure 2. Diagram of the batch-culture apparatus.

# METHODS

## A. Batch-Culture Experiments

The first six, batch-culture experiments were started by aseptically transferring 50-75 ml from a chemostat. The full start-up procedure is given in Appendix IIA. The dilution rates of the chemostats were between .3 and .4 $day^{-1}$ and the chemostats were sampled only when a batch culture was started. The purpose of the chemostats was to provide a means to bring the $^{32}P$ tracer into equilibrium with the non-labelled P in the cells - this took about two weeks after a new supply of $^{32}P$ was obtained. Experiments 7 and 8 were started with 8-10 ml aseptically transferred starter culture. Because the medium in the starter cultures was $PO_4$ enriched, the values of total nutrient and initial nutrient/cell in experiments 7 and 8 were appropriately adjusted for all calculations.

The batch cultures were first sampled five minutes after inoculation and sampling was continued until the cultures had reached the stationary phase and were in steady state, with growth rate of zero, for at least three days. The sampling intervals varied from 15 minutes during the first two hours to two days at the end of an experiment. More frequent measurements would have excessively diminished the culture volume (in none of the experiments was the volume depleted to half the original volume) and were not necessary for the analysis of the results.

A sample was obtained by blocking the air outlet and unclamping the sampling line. First, 8 ml was taken to rinse the line and the 25 ml sampling cylinder. The 8 ml rinse was saved in another cylinder and a 8-15 ml sample

was taken. Each sample was subsampled using precision automatic pipettes into the following subsamples for analysis (Appendix IIB):

1) cell count: 2.5 or 5.0 ml was diluted with half-strength seawater to 50 ml and counted in a Model B Coulter Electronics Particle Counter.

2) chlorophyll-a: one ml was diluted with buffered 100% acetone to 10 ml for fluorometric measurement.

3) total phosphorus: one or two ml were put in a liquid scintillation vial and 10 ml of scintillation solution added.

4) particulate phosphorus: 2.5, 5, or 10 ml were filtered through a .45 μ, 25 mm diameter, membrane filter under a vacuum of 2-3 psi (.14-.21 kg $cm^{-2}$) and rinsed with 2.5 or 5 ml of half-strength seawater. The filter was put in a liquid scintillation vial and 10 ml of scintillation solution added.

The samples provided a time-series of measures of the state variables: cell concentration, phosphorus/cell (particulate phosphorus concentration/cell concentration), dissolved-phosphorus concentration (total-phosphorus concentration minus particulate-phosphorus concentration), and chlorophyll-a/cell (chlorophyll-a concentration/cell concentration).

B. Nonlinear Parameter Estimation Technique

The model equations (5-10) that must be fitted to the various measured and derived data sets are nonlinear with respect to their parameters. Nonlinear parameter estimation problems are often difficult to solve, as nonlinear equations are not always predictable. It is often useful to employ various estimation techniques if any one proves unsatisfactory. A very thorough explanation of nonlinear parameter estimation has been given by Bard (1974). A digital computer program package by Bard (written in FORTRAN and including documentation, Bard, 1967b) has been shown to be useful with simulated batch-culture data and models similar to the ones used in this study (Lederman, 1974). Only a general description will be given here - enough to understand the implementation with the various models used in this study.

Given an initial guess for each parameter, the procedure iterates towards the solution of an objective function. During each iteration a new value for each parameter is systematically chosen, and the procedure is stopped when the changes in the parameter values are very small. Bard's technique uses a maximum-likelihood objective function which, in this study, was equivalent to weighted least squares with equal weights. Restrictions are placed on the distribution of the residuals (the predicted values, given a set of parameters, minus the corresponding measured values): the residuals must

1. have zero mean,
2. be uncorrelated between samples, and
3. be normally distributed.

In general, the smallest number of samples to which a model may be fitted is the number of parameters plus one.

Constraints can be placed on the estimates of the parameters. In this study, upper and lower bounds were set for each parameter (Table 2). The bounds were generous and were chosen to deter the procedure from choosing negative or very large estimates of the parameters. A penalty function, based on the upper and lower bounds, acts to "discourage" the procedure choosing estimates near the bounds. If the procedure cannot meet the constraints the program indicates that the algorithm failed to converge.

The least-squares objective function is maximized (a minimum, sum-of-squared residuals is sought) by a modified Gauss-Newton method (Eisenpress and Greenstadt, 1966; Bard, 1967a; and Carroll, 1961). This method differs from more standard methods, such as Marquardt's (1963), through the choice of directions and changes made in the parameter values during each iteration. Bard's modification is usually more stable than other techniques and is more likely to converge on a global maximum (Bard, 1970; and Greenstadt, 1967). Marquardt's method, for example, may converge on an (erroneous) local minimum of the sum-of-squared residuals.

Special subroutines were written for each model and are given in Appendix III. The program package is especially versatile as both algebraic and differential equations can be accommodated through the use of different program configurations. Also in Appendix III are the program configuration, initial guesses, and a sample data set for each model.

TABLE 2. Upper and lower bounds for the parameters.

**Algebraic Equations**

| | lower bounds | upper bounds |
|---|---|---|
| $\mu_m'$ $(hr^{-1})$ | 0.0 | 10.0 |
| $k_Q = Q_o$ $(nM\ P(10^6\ cells)^{-1})$ | 0.0 | 10.0 |
| $K_Q$ $(nM\ P(10^6\ cells)^{-1})$ | 0.0 - .5 | 10.0 - 100.0 |

**Differential Equations**

| | lower bounds | upper bounds |
|---|---|---|
| $\mu_m$ $(nM\ P(10^6\ cells)^{-1} hr^{-1})$ | 0.0 | 1.0 - 10.0 |
| $K_S$ $(nM\ P\,ml^{-1})$ | 0.0 | 10.0 - 100.0 |
| $S_o$ $(nM\ P\,ml^{-1})$ | 0.0 | 10.0 |
| $\mu_m'$ $(hr^{-1})$ | 0.0 | 10.0 |
| $k_Q = Q_o$ $(nM\ P(10^6\ cells)^{-1})$ | 0.0 | 10.0 |
| $K_Q$ $(nM\ P(10^6\ cells)^{-1})$ | 0.0 - .5 | 10.0 - 100.0 |

# RESULTS

## A. Time-Series of Measured Variables from the Batch Cultures

The data from the eight, batch-culture experiments are tabulated in Appendix IV A. All of the experiments were grown under constant illumination except experiment 8 which was grown with a daily photoperiod (this will be referred to as L/D) of 16 hours of constant illumination, 0800-2400, and 8 hours of total darkness, 0000-0800. Experiments 7 and 8 were started with inocula from the "starter culture" stock. This change in procedure was made because the chemostats did not maintain a steady state and, with the cell concentration slowly diminishing, this necessitated either an increase in the size of the inoculum for the batch culture or emptying, cleaning, and restarting a chemostat and waiting for a steady state (this problem is discussed in Appendix IV B).

Figure 3 is a graph of biomass vs time for experiment 1 and is given as a comparison to the idealized curves in Figure 1. Four distinct regions are defined in the figure legend: lag, exponential, transitional, and stationary phases.

All of the data from experiment 1 are plotted in Figures 4 and 5. The graphs are especially confusing during the initial stages of the experiment. The general trends of the different variables after the lag phase were:

1) Cell numbers (X) and particulate phosphorus (PP) increased with time.
2) Cell quota of phosphorus (Q = particulate phosphorus/cell) initially increased and

then decreased with time.

3) Chlorophyll/cell slightly increased, then decreased with time. Chlorophyll/ml increased then decreased with time.

4) Dissolved phosphorus (S) decreased with time.

The results of the remaining experiments will not be presented in such detail because a large part of the data analysis was concerned only with cell counts in the exponential and phosphorus-controlled phases and cell quota in the phosphorus-controlled phase.

Figures 6-13 are graphs of ln X (cell numbers) vs time from the beginning of the exponential phase and Q (cell quota of phosphorus) vs time from the beginning of the transitional or nutrient-controlled phase. In all of the experiments, the exponential phase was light controlled and the transitional phase was phosphorus controlled. Due to diel variations in the data from experiment 8, L/D, only data from 24 hour intervals, at 1000 hours, are shown in Figure 13 and only these points were used in the parameter estimation. The natural logarithms of the cell counts are plotted in Figures 6-13 so that the exponential phase, a straight line, can be clearly distinguished from the phosphorus-controlled phase of each experiment. For the sake of continuity, the first point in the phosphorus-controlled phase is also the last point in the exponential phase.

Irradiance was measured at the surface of the culture. As the cultures developed, however, self shading began to occur. Calculations showed that the maximum decrease in average illumination experienced by the algae, in culture 7 which had the greatest final chlorophyll concentration, was about 15%. It is however, unclear if algae respond to average illumination or the maximum experienced when mixing brings them to the surface of the culture. It was therefore decided to use external irradiance in presenting results.

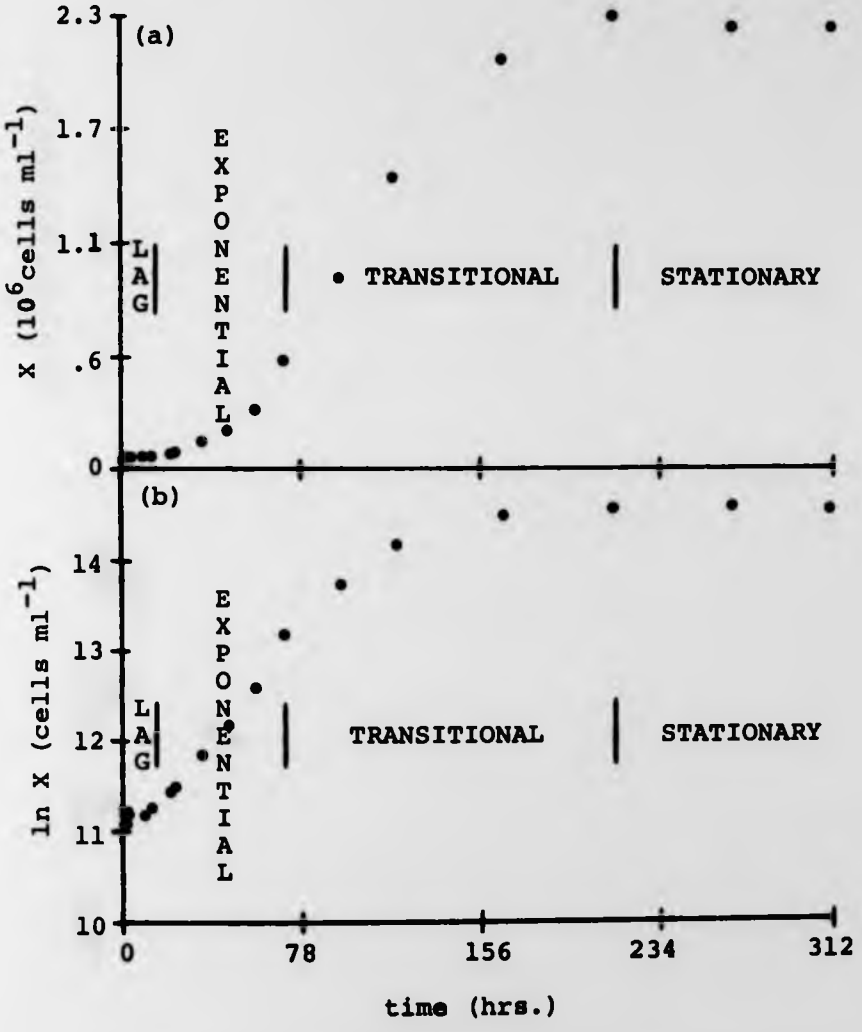



Figure 3. Biomass (a) and ln biomass (b) vs time for experiment 1.

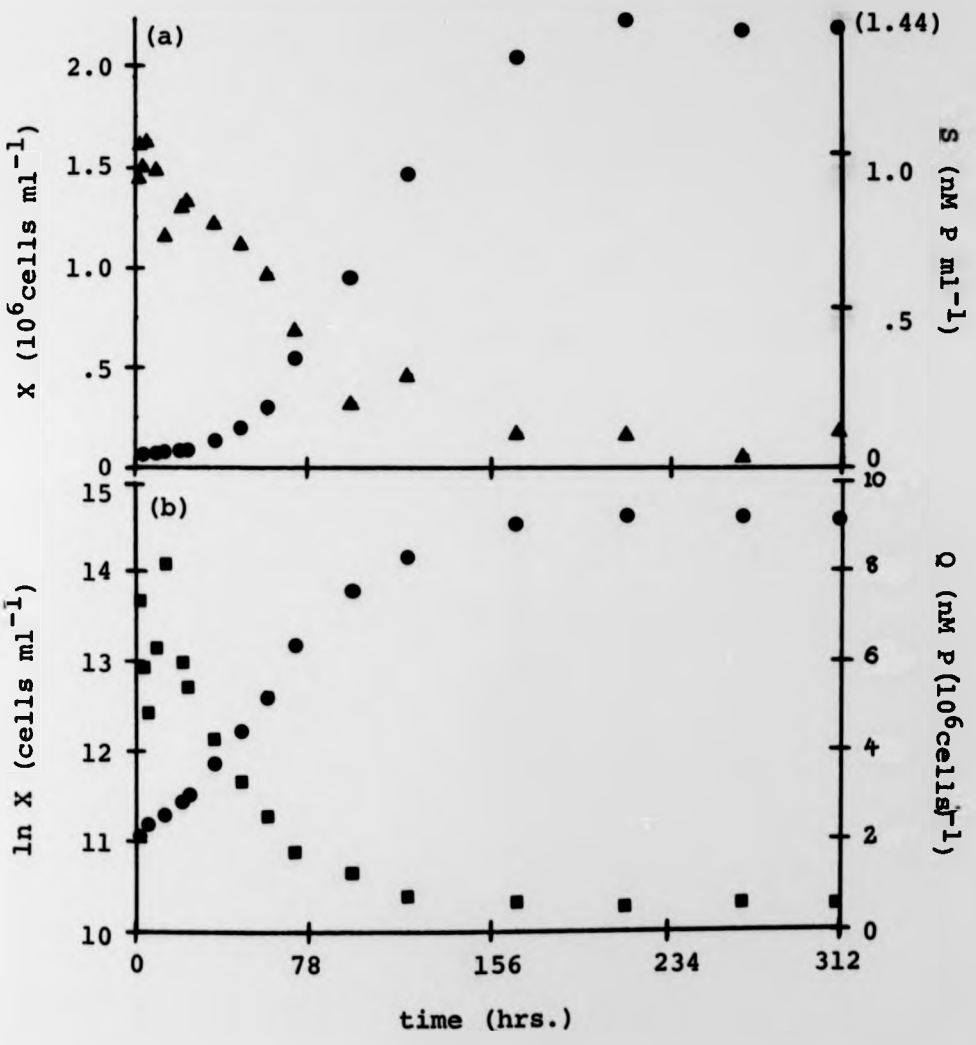



Figure 4. (a) Biomass - ● , dissolved phosphorus - ▲ , and (b) ln biomass - ● , and phosphorus cell quota - ■ vs time for experiment 1.

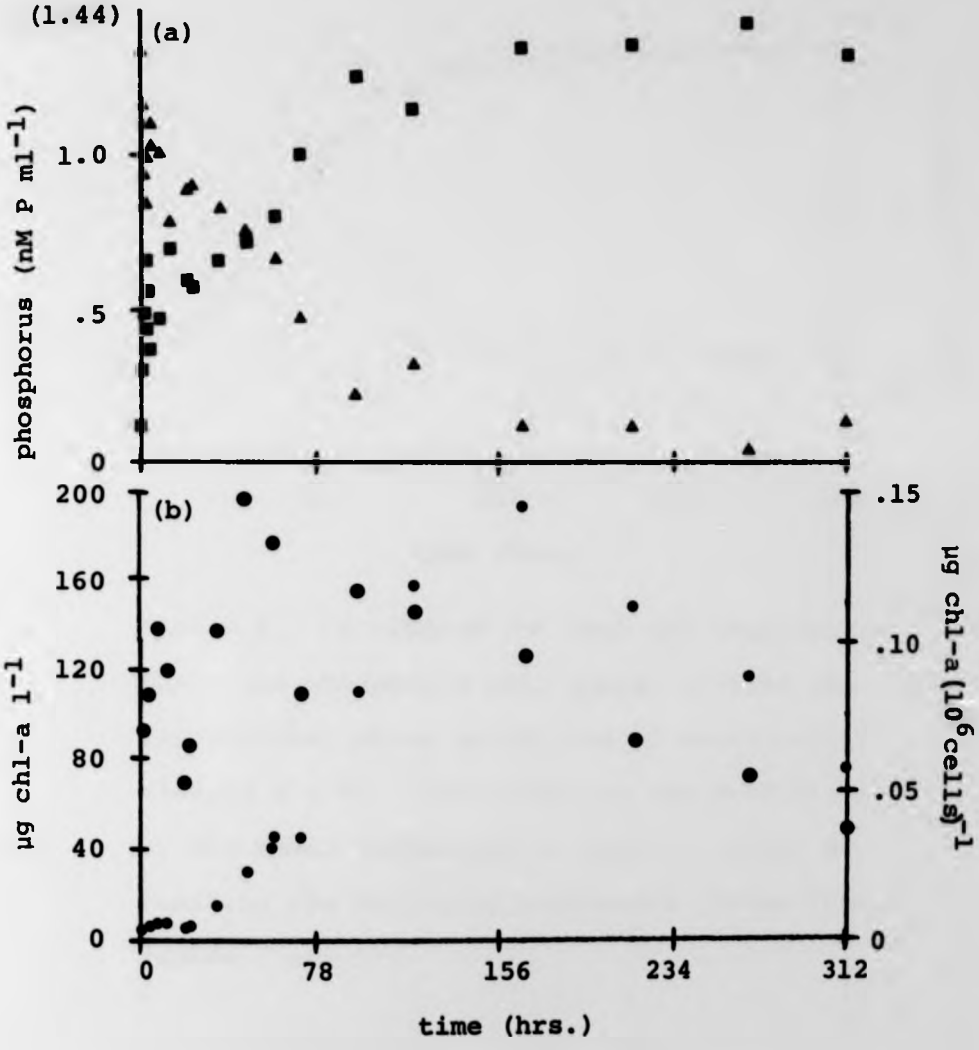



Figure 5. (a) Particulate phosphorus - ■ , dissolved phosphorus - ▲ , and (b) chlorophyll-a/l - ● , and chlorophyll-a/cell - ● vs time for experiment 1.

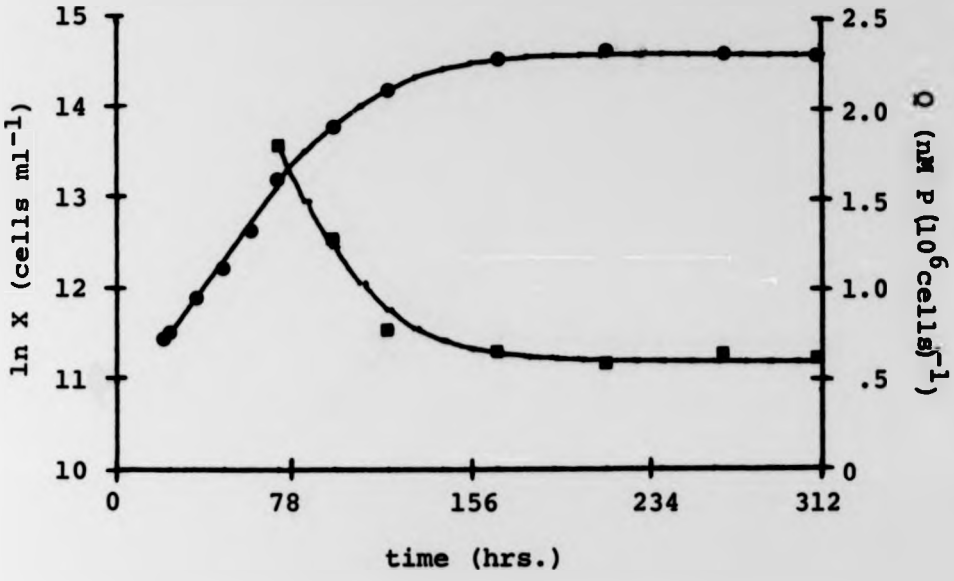



Figure 6. Ln biomass - ● from the exponential phase and phosphorus cell quota - ■ from the transitional phase to the end of experiment 1 (140.53 W m$^{-2}$). The lines are the predictions by the model (equations 1a and 5-7) after estimating the following parameters (taken from Tables 7 and 9):

| | | |
|---|---|---|
| $\mu_m$ | .814 ± .038 | (day$^{-1}$) |
| $u_m$ | .6 ± 151 | (nM P (10$^6$ cells)$^{-1}$ hr$^{-1}$) |
| $K_S$ | 9.9 ± 2800 | (nM P ml$^{-1}$) |
| $S_o$ | .12 ± .040 | (nM P ml$^{-1}$) |
| $\mu_m'$ | .96 ± .041 | (day$^{-1}$) |
| $k_Q$ | .594 ± .0169 | (nM P (10$^6$ cells)$^{-1}$) |

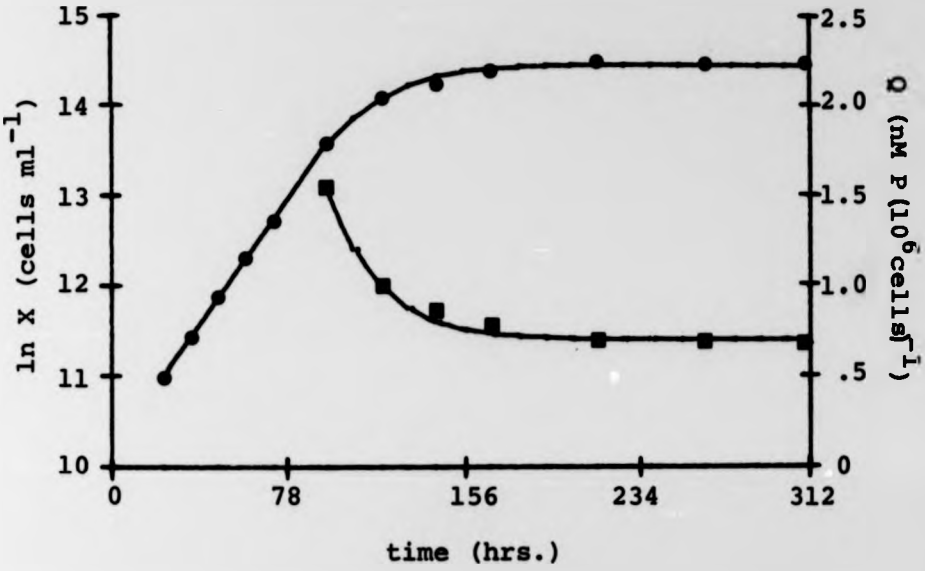



Figure 7. Ln biomass - ● from the exponential phase and phosphorus cell quota - ■ from the transitional phase to the end of experiment 2 (140.53 W m$^{-2}$). The lines are the predictions by the model (equations 1a and 5-7) after estimating the following parameters (taken from Tables 7 and 9):

| | | |
|---|---|---|
| $\mu_m$ | .852 ± .0096 | (day$^{-1}$) |
| $u_m$ | .5 ± .80 | (nM P (10$^6$ cells)$^{-1}$ hr$^{-1}$) |
| $K_S$ | 9.8 ± 1700 | (nM P ml$^{-1}$) |
| $S_o$ | .104 ± .0089 | (nM P ml$^{-1}$) |
| $\mu'_m$ | 1.22 ± .077 | (day$^{-1}$) |
| $k_Q$ | .704 ± .0093 | (nM P (10$^6$ cells)$^{-1}$) |

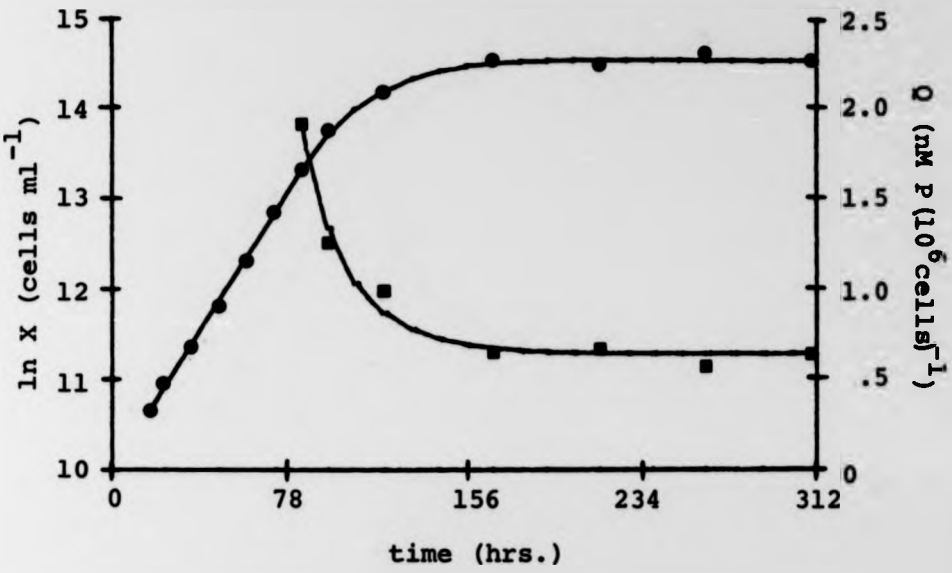



Figure 8. Ln biomass - ● from the exponential phase and phosphorus cell quota - ■ from the transitional phase to the end of experiment 3 (64.15 W m$^{-2}$). The lines are the predictions by the model (equations 1a and 5-7) after estimating the following parameters (taken from Tables 7 and 9):

| | | |
|---|---|---|
| $\mu_m$ | .958 ± .0144 | (day$^{-1}$) |
| $u_m$ | .005 ± .005 | (nM P(10$^6$ cells)$^{-1}$ hr$^{-1}$) |
| $K_S$ | .001 ± .018 | (nM P ml$^{-1}$) |
| $S_o$ | .098 ± .026 | (nM P ml$^{-1}$) |
| $\mu'_m$ | 1.28 ± .111 | (day$^{-1}$) |
| $k_Q$ | .64 ± .017 | (nM P(10$^6$ cells)$^{-1}$) |

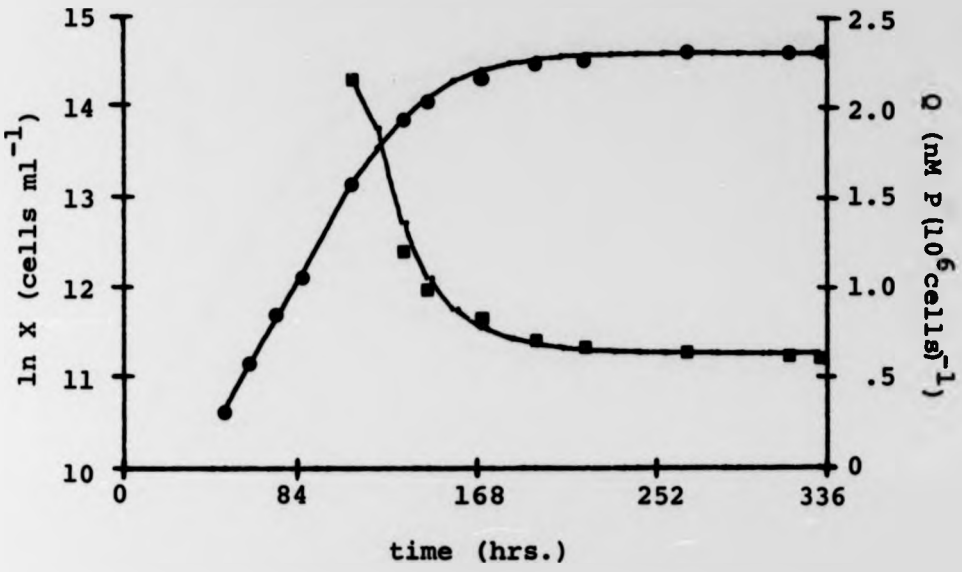



Figure 9. Ln biomass - ● from the exponential phase and phosphorus cell quota - ■ from the transitional phase to the end of experiment 4 (64.15 W $m^{-2}$). The lines are the predictions by the model (equations 1a and 5-7) after estimating the following parameters (taken from Tables 7 and 9):

| | | |
|---|---|---|
| $u_m$ | .998 ± .024 | ($day^{-1}$) |
| $u_m$ | .2 ± 5 | (nM P($10^6$ cells)$^{-1}$ $hr^{-1}$) |
| $K_S$ | 9.5 ± 300 | (nM P $ml^{-1}$) |
| $S_o$ | .018 ± .0186 | (nM P $ml^{-1}$) |
| $\mu_m'$ | 1.08 ± .024 | ($day^{-1}$) |
| $k_Q$ | .635 ± .0062 | (nM P($10^6$ cells)$^{-1}$) |

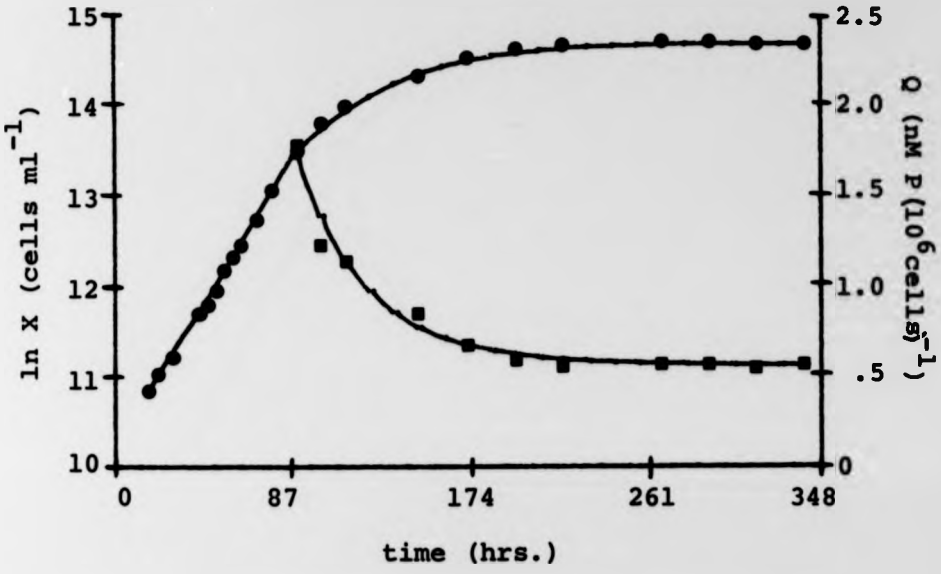



Figure 10. Ln biomass - ● from the exponential phase and phosphorus cell quota - ■ from the transitional phase to the end of experiment 5 (35.25 W $m^{-2}$). The lines are the predictions by the model (equations 1a and 5-7) after estimating the following parameters (taken from Tables 7 and 9):

| | | |
|---|---|---|
| $\mu_m$ | .893 ± .012 | ($day^{-1}$) |
| $u_m$ | .001 ± .0010 | (nM P ($10^6$ cells)$^{-1}$ $hr^{-1}$) |
| $K_S$ | .001 ± .0011 | (nM P $ml^{-1}$) |
| $S_o$ | .074 ± .0158 | (nM P $ml^{-1}$) |
| $\mu_m'$ | .720 ± .0274 | ($day^{-1}$) |
| $k_Q$ | .560 ± .0077 | (nM P ($10^6$ cells)$^{-1}$) |

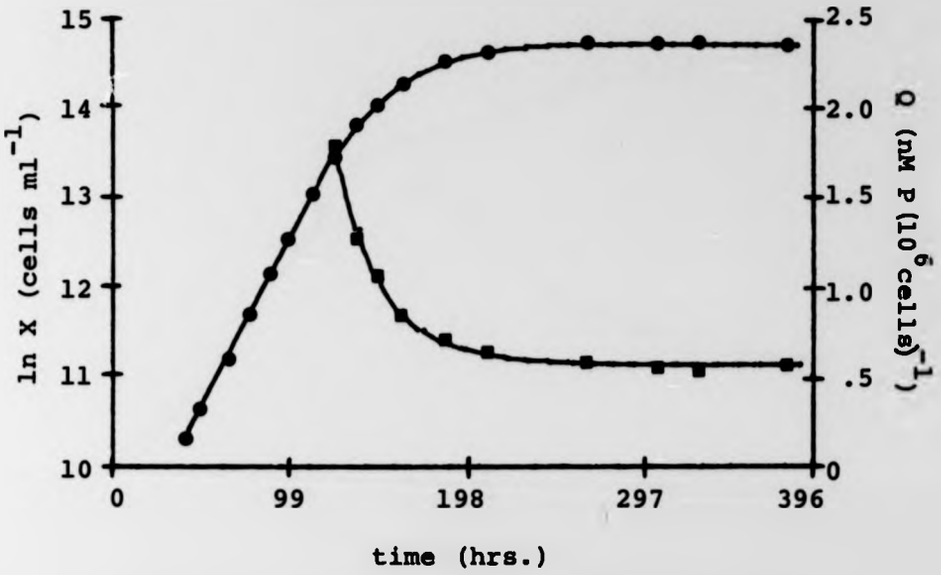



Figure 11. Ln biomass - ● from the exponential phase and phosphorus cell quota - ■ from the transitional phase to the end of experiment 6 (35.25 W $m^{-2}$). The lines are the predictions by the model (equations 1a and 5-7) after estimating the following parameters (taken from Tables 7 and 9):

| | | | |
|---|---|---|---|
| $\mu_m$ | .900 | $\pm$ .0048 | ($day^{-1}$) |
| $u_m$ | .00188 | $\pm$ .000287 | (nM P ($10^6$ cells)$^{-1}$ $hr^{-1}$) |
| $K_S$ | .00099 | $\pm$ .000176 | (nM P $ml^{-1}$) |
| $S_o$ | .034 | $\pm$ .0129 | (nM P $ml^{-1}$) |
| $\mu'_m$ | 1.003 | $\pm$ .0220 | ($day^{-1}$) |
| $k_Q$ | .567 | $\pm$ .0064 | (nM P ($10^6$ cells)$^{-1}$) |

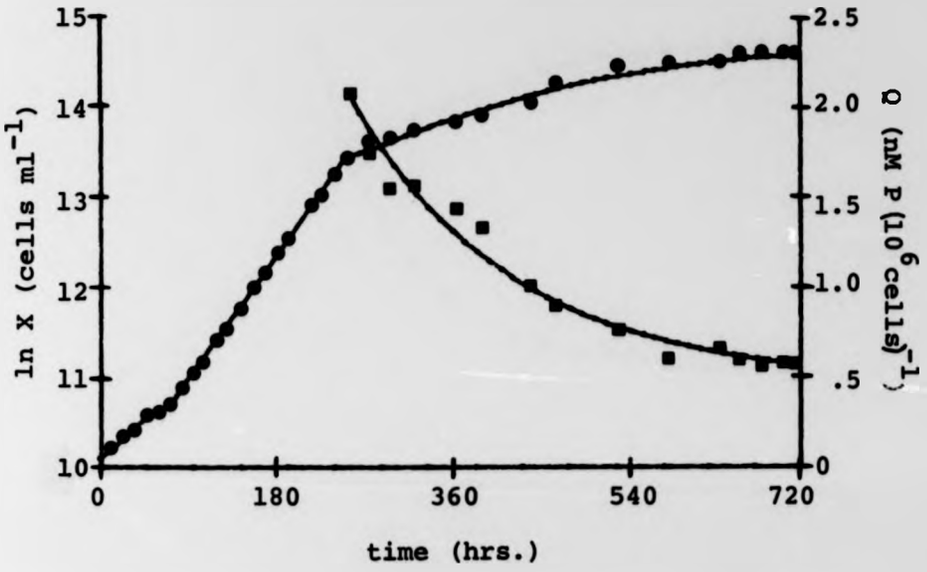



Figure 12. Ln biomass - ● from the exponential phase and phosphorus cell quota - ■ from the transitional phase to the end of experiment 7 (11.75 W m$^{-2}$). The lines are the predictions by the model (equations 1a and 5-7) after estimating the following parameters (taken from Tables 7 and 9):

| | | |
|---|---|---|
| $\mu_{m_1}$ | .235 ± .0168 | (day$^{-1}$, 8.93 W m$^{-2}$) |
| $\mu_{m_2}$ | .365 ± .0024 | (day$^{-1}$, 11.75 W m$^{-2}$) |
| $u_m$ | .002 ± .5 | (nM P (10$^6$ cells)$^{-1}$ hr$^{-1}$) |
| $K_S$ | 98.1 ± 41000 | (nM P ml$^{-1}$) |
| $S_o$ | 9.6 ± 2400 | (nM P ml$^{-1}$) |
| $\mu'_m$ | .143 ± .0093 | (day$^{-1}$) |
| $k_Q$ | .49 ± .033 | (nM P (10$^6$ cells)$^{-1}$) |

Irradiance was changed after 49 hours. $\mu m_1$ refers to the first period, $\mu m_2$ to the second (see p. 197-198).

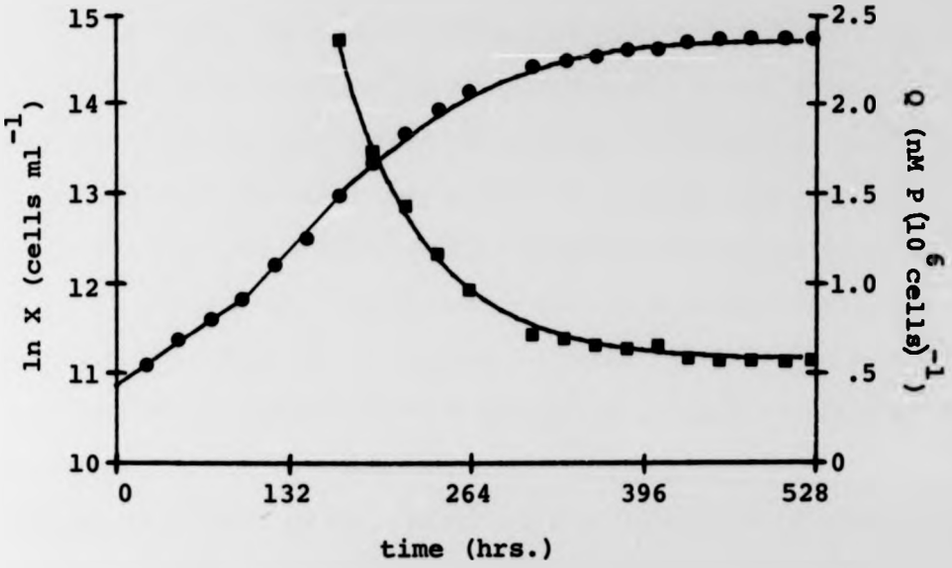



Figure 13. Ln biomass - ● from the exponential phase and phosphorus cell quota - ■ from the transitional phase to the end of experiment 8 (19.74 W $m^{-2}$). The lines are the predictions by the model (equations 1a and 5-7) after estimating the following parameters (taken from Tables 7 and 9):

| | | |
|---|---|---|
| $\mu_{m_1}$ | .247 ± .0048 | ($day^{-1}$, 14.26 W $m^{-2}$) |
| $\mu_{m_2}$ | .468 ± .046 | ($day^{-1}$, 19.74 W $m^{-2}$) |
| $u_m$ | .105 ± 3.128 | (nM P ($10^6$ cells)$^{-1}$ $hr^{-1}$) |
| $K_S$ | 10.0 ± 307 | (nM P $ml^{-1}$) |
| $S_o$ | .031 ± .0255 | (nM P $ml^{-1}$) |
| $\mu_m'$ | .461 ± .0110 | ($day^{-1}$) |
| $k_Q$ | .566 ± .0073 | (nM P ($10^6$ cells)$^{-1}$) |

Irradiance was changed after 96 hours. $\mu m_1$ refers to first period, $\mu m_2$ to second (see p.199-200).

B. Factors that Complicate the General Formulation of a Model

1. Fluctuations in S and Q

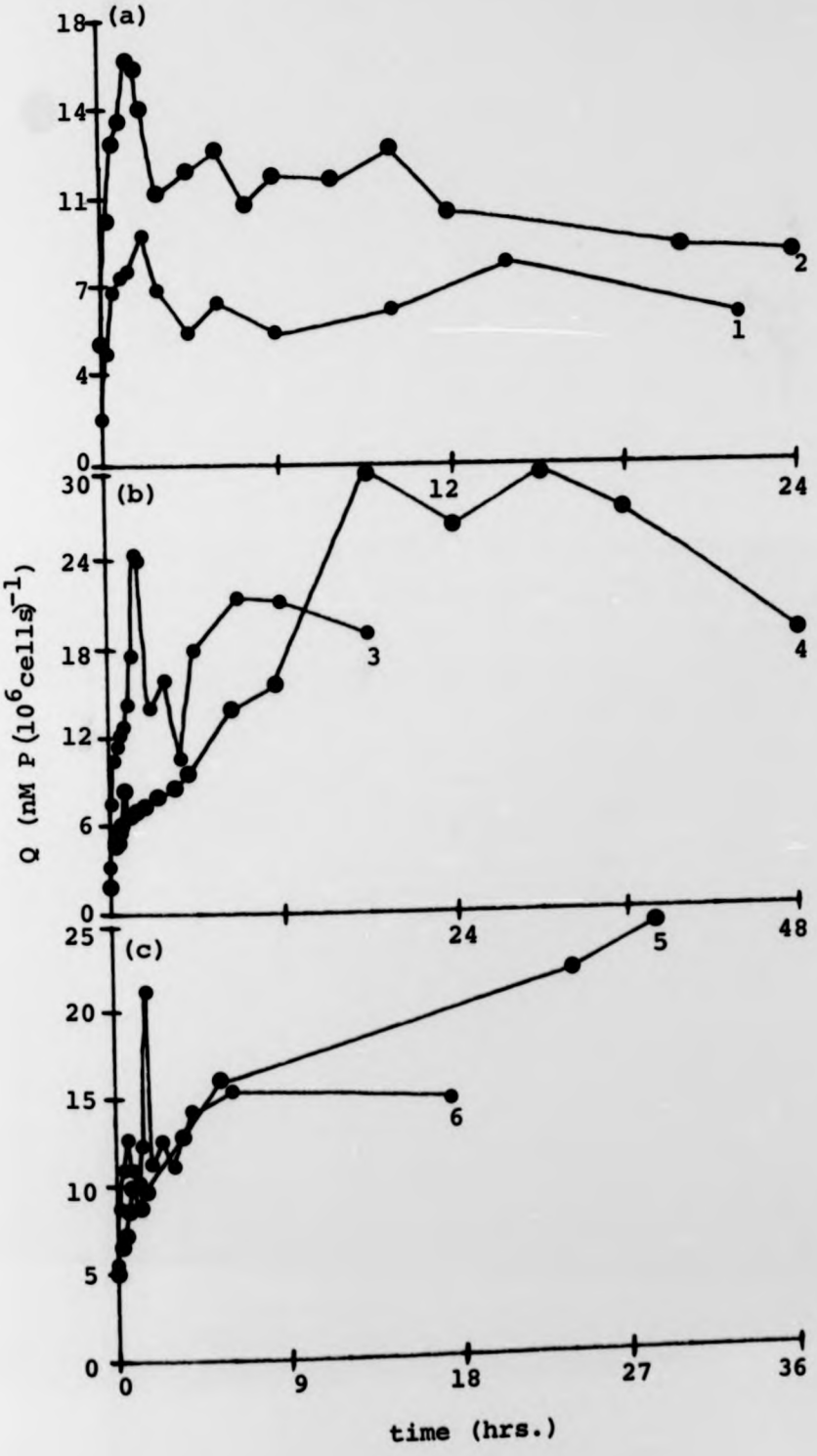
a. Initial uptake and resultant cell-quota

During the first day of an experiment, there were large fluctuations in dissolved phosphorus with associated changes in cell quota of up to 50% in fifteen minutes. Graphs of cell quota during the lag phase are given in Figure 14 for the six experiments that were inoculated with chemostat-maintained algae. Although theory (Droop, 1968) predicts a rapid initial uptake of phosphorus (which may be attributed to adsorption to the cell surface) by nutrient-deficient cells, there were at least two to three reversals of uptake in each data set (more may have occurred), a sequence of events which is not easily explained. By the end of the first day, cell counts showed that cells had begun to divide and PP measurements showed that the maximum value of phosphorus-Q had been reached. The maximum measure of Q during the lag phase will be referred to as $Q_m$. As can be seen in Figure 14, $Q_m$ was sometimes reached within two hours. In Figure 15, $Q_m$ from each experiment is plotted against the initial biomass, $X_o$ = mean biomass before cell division, and the values are in Table 3. There is a significantly high correlation between $Q_m$ and $X_o$ but the true relationship may be complicated if $Q_m$ is also related to irradiance. For all the experiments the range of dissolved phosphorus at $Q_m$ was .52-1.10 nM P $ml^{-1}$ (36-76% of the total phosphorus).

The initial, rapid uptake can be characterized by a simple rate constant (nM P $(10^6$ cells$)^{-1}$ $hr^{-1}$) as given under

$u_{ads}$ in Table 4. Also given in Table 4 is the time at which the first surge of the uptake ceased, and the measure of Q at that time. This period of rapid uptake ceased between .75 and 1.25 hours after inoculation. Table 4 also gives the time taken to reach $Q_m$, which was usually longer than the initial, rapid-uptake period. There is no correlation between $Q_m$ and the measure of Q after initial uptake ($r = -.092$, which is not significantly different from zero).

Figure 14. Cell quota vs time during the lag phases of experiments 1-6. The number of the experiment is under the last point of each curve. The levels of irradiance were: (a) 140.53 W $m^{-2}$, (b) 64.15 W $m^{-2}$, and (c) 35.25 W $m^{-2}$.


(a)
(b)
(c)
1
2
3
4
5
6
Q (nM P ($10^6$ cells)$^{-1}$)
time (hrs.)

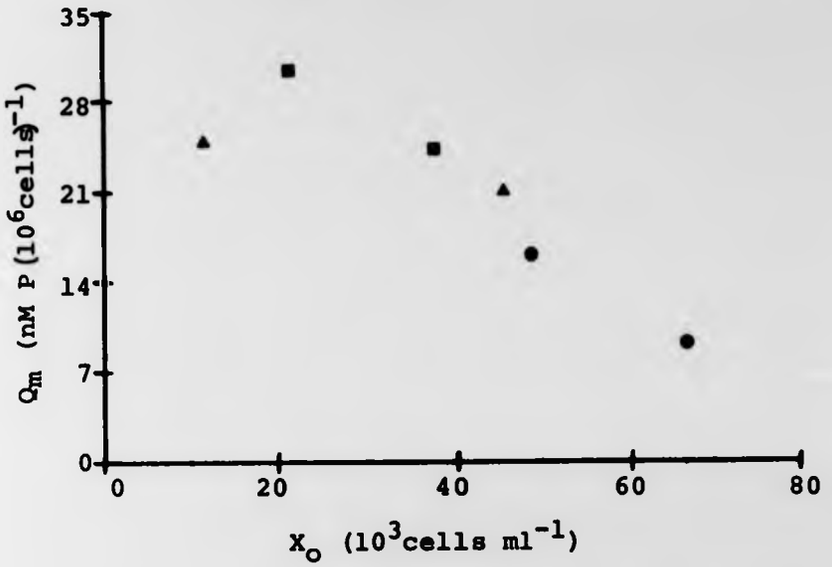



Figure 15. $Q_m$, the maximum cell quota during the lag phase, vs $X_o$, the initial or average biomass during the lag phase. Experiments 1 and 2 - ● - 140.53 W m$^{-2}$, experiments 3 and 4 - ■ - 64.15 W m$^{-2}$, and experiments 5 and 6 - ▲ - 35.25 W m$^{-2}$.

TABLE 3. $Q_m$ and $X_o$, maximum cell quota of phosphorus and the initial cell concentration. Experiments 1-6 were started with a chemostat inoculum. Values from experiments 7 and 8 (their inocula were from the starter culture) are given for comparison. The correlation coefficient (R) of $Q_m$ and $X_o$ is -.868, which is significant (p (R>o) > .975).

| | Experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| $X_o$ ($10^6$ cells $ml^{-1}$) | .0666 | .0485 | .0378 | .0214 | .0362 | .0114 | .0285 | .0533 |
| s.e. (standard error) | .0018 | .0011 | .0019 | .0010 | .0011 | .0013 | .0013 | .0016 |
| n (number of samples) | 9 | 14 | 14 | 10 | 13 | 2 | 5 | 1 |
| $Q_m$ (nM P ($10^6$ cells)$^{-1}$) | 9.29 | 16.31 | 24.49 | 32.55 | 21.22 | 24.88 | 6.30 | 12.93 |
| S (nM P $ml^{-1}$) | .82 | .65 | .51 | .64 | .67 | .99 | .27 | .35 |
| time to reach $Q_m$ (hrs) | 1.5 | 1.0 | 2.0 | 18.0 | 1.75 | 28.5 | 158.0 | 48.0 |

$X_o$ was estimated from cell counts during the lag phase. The number of samples depended on the length of this phase.

TABLE 4. Calculated values of $u_{ads}$, values of Q after initial uptake, calculated values of $u_{exp}$, and correlation coefficients of u vs S.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Experiment Number | $u_{ads}$ (nM P ($10^6$ cells)$^{-1}$ $hr^{-1}$) | Q (nM P ($10^6$ cells)$^{-1}$) | t (hr) | $u_{exp}$ (nM P ($10^6$ cells)$^{-1}$ $hr^{-1}$) | S (nM P $ml^{-1}$ | R | n |
| 1 | 5.94 | 7.91 | 1.0 | .037 | .33 | .032 | 9 |
| 2 | 11.32 | 16.31 | 1.0 | .045 | .19 | .860* | 8 |
| 3 | 9.18 | 12.52 | 1.0 | .033 | .27 | .230 | 10 |
| 4 | 5.21 | 8.55 | 1.25 | .037 | .26 | .293 | 10 |
| 5 | 9.65 | 12.87 | .75 | .036 | .20 | .071 | 15 |
| 6 | 4.825 | 10.12 | 1.0 | .071 | .20 | .731* | 12 |
| 7 | A | A | A | .038 | .07 | .463 | 17 |
| 8 | A | A | A | B | B | .351 | 36 |

Column 1 Rate of uptake (attributed to adsorption) during the initial stage of an experiment.

2 The phosphorus cell-quota at the end of the "adsorption" interval

3 The time from the beginning of an experiment, at which the "adsorption" interval ended

4 Rate of uptake during the exponential phase of an experiment. The average for all experiments is .042 (nM P $10^6$ cells$^{-1}$ $hr^{-1}$) with standard error of .005

5 Dissolved phosphorus at the end of the exponential phase

6 Correlation coefficient of uptake rate vs dissolved nutrient (data from both exponential and phosphorus-controlled phases)

7 Sample size for correlation

* The correlations were significant ($p(R>o) > .95$)

A These values are omitted because the inocula were from the starter culture

B These values are omitted because this was the light/dark experiment and thus the uptake results were not directly comparable

b. Nutrient uptake after the lag phase

It has been shown that phosphorus uptake during the initial stage of the experiments cannot be characterized as a one way movement of dissolved phosphorus into the cells. This reverse flow of phosphorus was also observed throughout the later stages of all the experiments. Therefore, it is not surprising that the correlations of specific uptake rate to dissolved nutrient are both poor and inconsistent (Table 4). Because of its discontinuous nature, it will be difficult to describe uptake with the proposed equation nor are there any other suitable equations. Although this difficulty affects parameter estimation for the models that are a series of differential equations, it does not present an insurmountable problem. Obviously, over the course of an experiment, nutrient was taken out of the medium and the nutrient concentration decreased. Moreover, the decrease in dissolved phosphorus was, in general, a process which continued until a minimum concentration was reached. Only at the lowest light level (exp. 8) - the slowest growing and the longest experiment, did the dissolved nutrient increase markedly (and erratically) after a minimum was reached.

A gross estimate of the uptake rate during the exponential phase ($u_{exp}$) can be approximated as follows:

$$u_{exp} = \frac{1}{\bar{X}} \frac{dS}{dt} \simeq \frac{1}{\bar{x}} \frac{\Delta S}{\Delta t} =$$

$$\frac{1}{e^{\frac{\ln X_{(t_o + \Delta t)} + \ln X_{t_o}}{2}}} \cdot \frac{S_{(t_o + \Delta t)} - S_{t_o}}{\Delta t}$$

where $t_o$ = beginning of the exponential phase

$\Delta t$ = length of time of the exponential phase

This approximation is made assuming that the uptake rate is constant between $t_o$ and $t_o + \Delta t$ (since this condition is not met, the rate is treated as the average rate over the period rather than an estimate of the true rate). The calculated values of $u_{exp}$ are given in Table 4 and are remarkably similar, as the standard error is 12% of the mean.

The measured concentrations of phosphorus at the end of an experiment, usually the minimum concentration during an experiment, ranged from .05-.20 $nM/ml^{-1}$. These measures of minimum dissolved phosphorus were not similar either between duplicate experiments or light levels. Estimates of the uptake constants during the phosphorus-controlled phase will be given in the parameter estimation section of the results.

2. Chlorophyll-a

In a chemostat in equilibrium, it can be assumed that the chlorophyll-a concentration remains steady. In a batch culture, the chlorophyll-a/ml (chl-a/ml) is expected to change as the numbers of cells increase, and the chl-a/cell may change throughout the course of an experiment - as the population changes from light-controlled growth to nutrient-controlled growth. Since the batch cultures in this study were grown at different light levels, differences in chlorophyll levels between experiments might also be expected. Mean chl-a/cell for the experiments are plotted in Figure 16 and, for each experiment, the data have been divided into two groups - exponential phase and phosphorus-controlled phase. The average for each group is plotted and the values are in Table 5, included are the results of an analysis of the variance between phases and light levels. The differences in the means, both between phases and amongst light levels, are significant. As expected, the chl-a/cells increased with decreasing illumination. The sign of the chl-a/cell differences between exponential and phosphorus-controlled phases was not the same at all light levels. At the two highest light levels, the chl-a/cell decreased slightly after the exponential phase. For the three lower light levels, the chl-a/cell increased after the exponential phase, and the increase was greater as the illumination decreased.

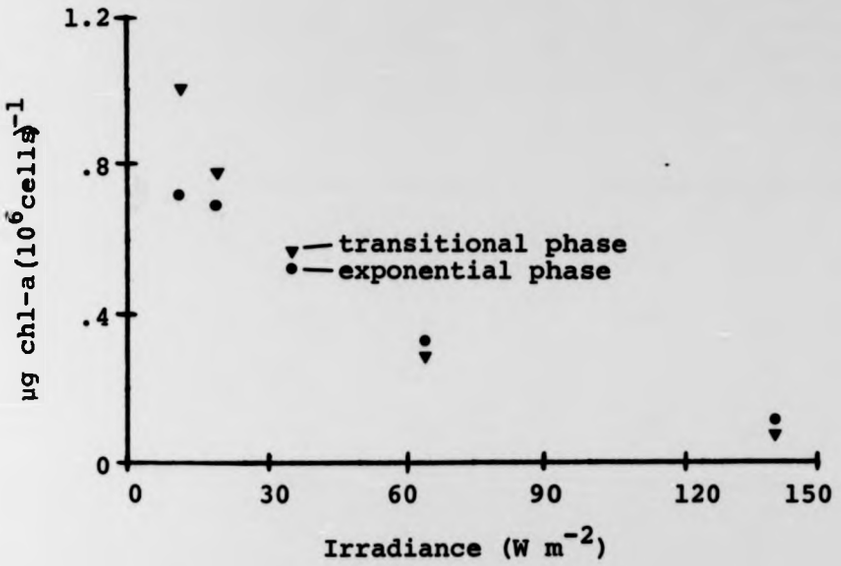



Figure 16. Mean chlorophyll-a/cell vs irradiance. Two means are shown for each experiment: exponential phase - • and transitional phase - ▲ .

Table 5. Mean chlorophyll-a/cell during the nutrient-saturated (exponential) and phosphorus-controlled phases of the batch-culture experiments. Values are given in units of µg chlorophyll-a $(10^6 \text{ cells})^{-1}$. The differences among light levels are significant at $\alpha = .001$. Within light levels, the differences between phases were significant ($\alpha = .05$) - all the chl-a/cell values for the two phases of each experiment (Appendix IV A) were analyzed with a runs test, above and below the median (Sokal and Rohlf, 1969).

| Experiment Number | Nutrient-saturated phase | | | Phosphorus-controlled phase | | |
|---|---|---|---|---|---|---|
| | chl-a | s.e. | n | chl-a | s.e. | n |
| 1 | .098 | .015 | 6 | .079 | .013 | 6 |
| 2 | .134 | .006 | 6 | .068 | .007 | 6 |
| 1 + 2 | .116 | .033 | 12 | .073 | .026 | 12 |
| 3 | .325 | .017 | 7 | .245 | .018 | 6 |
| 4 | .359 | .011 | 5 | .314 | .016 | 8 |
| 3 + 4 | .339 | .040 | 12 | .284 | .055 | 14 |
| 5 | .534 | .013 | 12 | .567 | .012 | 10 |
| 6 | .511 | .012 | 9 | .584 | .008 | 9 |
| 5 + 6 | .524 | .042 | 21 | .575 | .032 | 19 |
| 7 | .726 | .010 | 15 | 1.012 | .050 | 13 |
| 8 | .692 | .018 | 11 | .788 | .014 | 24 |

3. Dynamics During a Photoperiod

a. Growth rate and uptake

During experiment 8 (with the L/D cycle), there were two days of intensive sampling - once in the exponential phase and the other in the phosphorus-controlled phase. A sampling period was started just before the lights went out at midnight and samples were taken every two or three hours for 24 hours. Measured and derived variables from the intensive sampling periods are given in Table 6.

The highest growth rates in both days occurred within five hours on both sides of midnight (when the lights went off). Seventy-eight percent of each day's growth took place in these six to eight hours. As the experiment progressed into the phosphorus-controlled phase, the daily growth rate decreased but the cyclical trend in growth rate was still recognizable. This cycle of cell division was not evident in the continuous-light experiments (growth rates were calculated for the four-hour sampling intervals in experiment 5 and their values are given in Table 6).

Phosphorus-uptake rates were calculated for the two sampling periods and these are included in Table 6. A large proportion of the uptake during each day occurred just prior to and concurrent with the period of highest growth rate. Therefore, the uptake was not directly related to the availability of light but seemed to be associated with cell division.

TABLE 6. Measured and derived variables from the light/dark experiment. For comparison, growth rates within a day are given from a constant-light experiment.

| Sample Number | Time (24 hr clock) | Cell Size Index (small:large cells) | $\mu(hr^{-1})$ | u (nM P $(10^6$ cells$)^{-1}$ $hr^{-1}$) | µg Chl-a $(10^6$ cells$)^{-1}$ | µg Chl-a $ml^{-1}$ | Time Experiment 5 | µ Experiment 5 |
|---|---|---|---|---|---|---|---|---|
| 9 | 2320 | -- | .023 | -.074 | .761 | .270 | 0800 | .040 |
| 10 | 2350 | -- | .004 | .344 | .764 | .271 | 1200 | .019 |
| 11 | 0100 | -- | .033 | .282 | .748 | .276 | 1600 | .044 |
| 12 | 0300 | -- | .035 | .137 | .702 | .278 | 2000 | .054 |
| 13 | 0500 | 2.74 | .038 | .164 | .651 | .278 | 0000 | .035 |
| 14 | 0700 | 3.45 | .015 | -.117 | .636 | .280 | 0400 | .034 |
| 15 | 0800 | 4.00 | .015 | -.032 | .601 | .268 | 1200 | .035 |
| 16 | 1000 | 2.53 | -.007 | -.098 | .614 | .270 | | |
| 17 | 1200 | 1.94 | -.006 | .022 | .670 | .292 | | |
| 18 | 1500 | 1.45 | .021 | .069 | .710 | .329 | | |
| 19 | 1900 | .86 | .0003 | -.119 | .762 | .345 | | |
| 20 | 2300 | 1.03 | .025 | -.018 | .728 | .373 | | |
| 23 | 0000 | .88 | .008 | .010 | .883 | .854 | | |
| 24 | 0200 | 1.09 | .024 | .014 | .880 | .893 | | |
| 25 | 0400 | 1.35 | .036 | .032 | .811 | .884 | | |
| 26 | 0600 | 1.74 | .003 | -.043 | .859 | .942 | | |
| 27 | 0800 | 1.85 | .023 | .010 | .821 | .942 | | |
| 28 | 1100 | 1.65 | -.004 | -.019 | .834 | .946 | | |
| 29 | 1400 | 1.43 | .002 | .004 | .847 | .967 | | |
| 30 | 1700 | 1.07 | -.0007 | -.004 | .884 | 1.007 | | |
| 31 | 2000 | .86 | .001 | -.029 | .869 | .995 | | |
| 32 | 2300 | .76 | .020 | -.005 | .849 | 1.030 | | |

b. Cell-size and growth rate

After five hours in the first intensive sampling period, it was observed that the average size of the cells (as indicated on the Coulter-Counter oscilliscope) was slowly decreasing. By adjusting the "window" size on the counter, it was possible to obtain an index of cell size. A ratio of small cells to large cells was calculated and this index can be compared to growth rate.

Growth rate varied throughout a L/D period as did the cell-size ratio - the calculated values are given in Table 6. Smaller cells (a higher ratio) are an indication that cells have divided, and larger cells are presumably ready to divide. It can be inferred from the information in Appendix IV C that the cells became medium sized as the experiment progressed towards the stationary phase (and the daily growth rate decreased).

In Figure 17, the size ratio and growth rate are plotted throughout the day (values are in Table 6) and there seems to be a distinct cycle of changes in cell sizes and growth rates. This pattern is not so obvious during the phosphorus-controlled phase. In Figure 18, the values of size index and growth rate, calculated from cell counts taken at 1000 hours, are plotted through time. The correlation between these two variables is high ($r = .964$, $p(r > 0) > .999$) and this relationship may be important in the construction of a model to describe the within-day growth dynamics of algae in a changing- or diurnal-light environment.

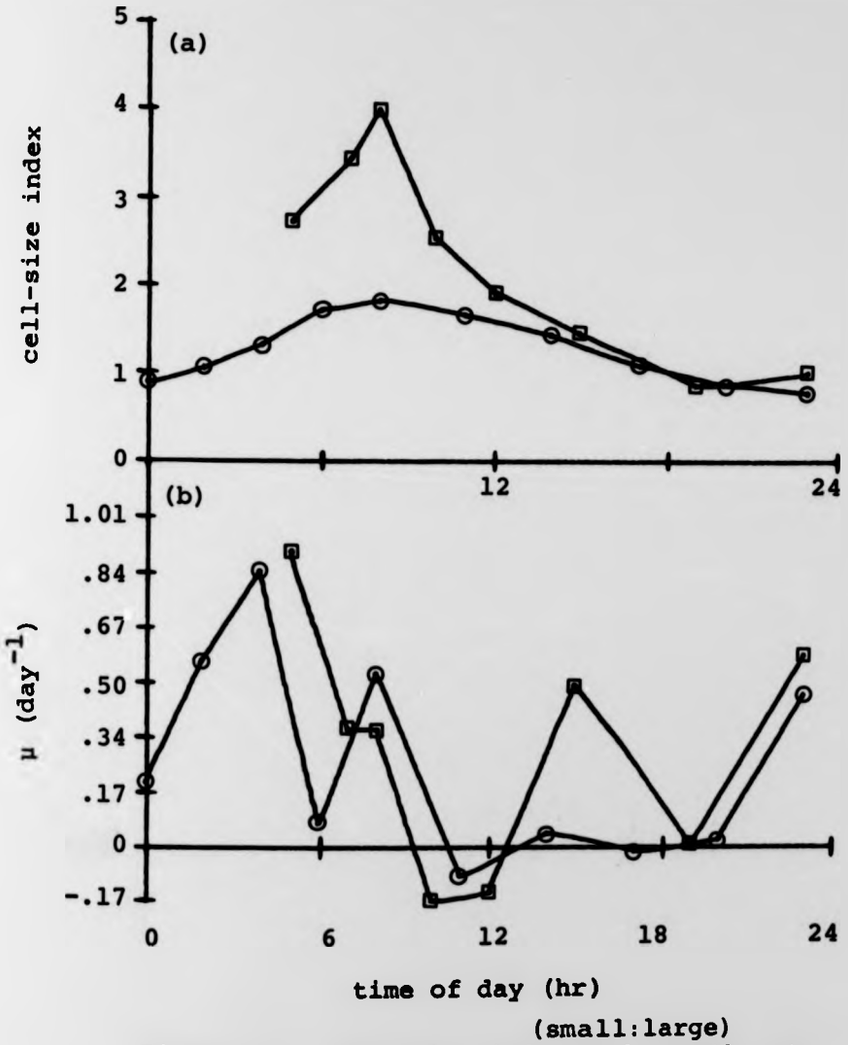



Figure 17. Cell-size index and growth rate within a 24 hour period during the exponential phase - □ and the transitional phase - O of the L/D experiment (No. 8).

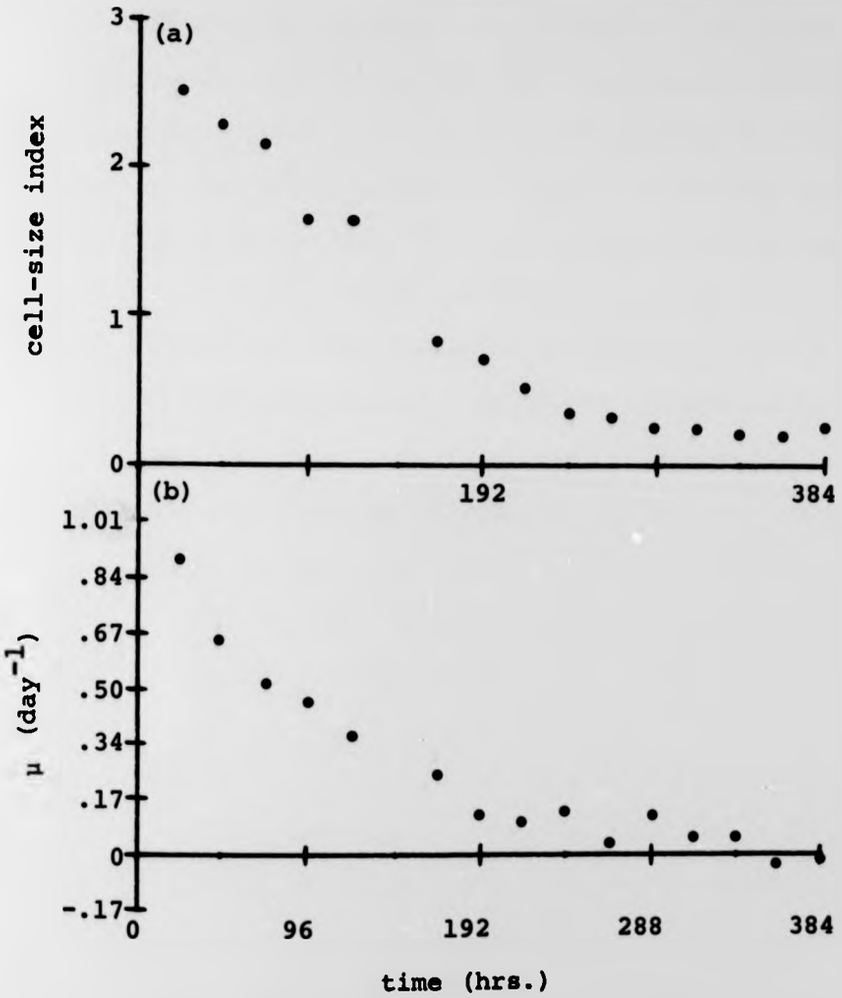



Figure 18. Cell-size index (small:large) and growth rate vs time for samples spaced at 24 hour intervals during the L/D experiment (No. 8).

c. Chlorophyll-a/cell

There were changes in chl-a/cell during the L/D experiment that can be directly related to the availability of light. Measures of chl-a/ml and chl-a/cell are given in Table 6. First it is apparent that there was a general increase in chl-a/cell as the culture progressed into the phosphorus-controlled phase (this was presented earlier). Within a daily cycle, the chl-a/cell decreased at night as the cells divided and began to increase a few hours after the lights came on. The measures of chl-a/ml attest to this, as they stayed constant at night and increased in the daytime. The synthesis of chlorophyll-a was strongly related to the availability of light.

C. The Effects of Irradiance on Maximum Growth Rate

After the lag phase, the algae started to grow at a steady, exponential rate. The rate was calculated for each experiment (by regression of ln X against time) and taken to be the maximum growth rate ($\mu_m$) for the given illumination. The maximum growth rate for each experiment is plotted against irradiance in Figure 19. The points at the three highest light levels are for replicate experiments and an analysis of variance of these six values indicates that they are significantly different (the variance amongst light levels was greater than the variance between replicates). An analysis of variance also indicates that the growth rates at the two highest light levels are different (these results and analyses are given in Table 7). The other four points in Figure 19 were obtained from the last two experiments, in which the cultures were grown at a low illumination until enough data were collected to obtain a significant regression. Then the illumination was increased to speed up the growth rate, and the subsequent points were fit by a new regression. The measures of irradiance for experiment 8 (L/D) were multiplied by 16/24 to allow comparison with the other results.

Since maximum growth rate increased with light to an optimum level and then decreased slightly with a further increase in illumination, the equation by Steele (1962) was chosen to describe the data. A respiration constant was included in the equation which was fitted to the ten data points:

$$\mu_m = \alpha I e^{-\beta I} - r$$

where:

$I$ = irradiance (watt $m^{-2}$)

$\alpha = .041 \pm .005$ ($m^2$ $watt^{-1}$ $day^{-1}$)

$\beta = .013 \pm .004$ ($m^2$ $watt^{-1}$)

$r = .110 \pm .082$ ($day^{-1}$)

$\mu_{opt} = 1.164 \pm .304$ ($day^{-1}$)

The maximum growth rate at the optimum irradiance ($\mu_{opt}$) has been derived from $\alpha$ and $\beta$ (see Jassby and Platt, 1976) and is given here to show the optimum growth rate as estimated by Steele's equation. The precision of the parameters is not very good, especially the estimate of r.

The curve predicted by Steele's equation is drawn in Figure 19. The shape of the curve does not depict two aspects of the data: the slope at the lower light levels should be steeper, and the change to light-saturated growth appears to be more abrupt than the model predicts. Other equations can be fit to the data (Appendix V), such as the linear model by Blackman (1905) which fits the data very well; but none predicts a decrease in growth rate (after an optimum has been reached) with increasing irradiance.

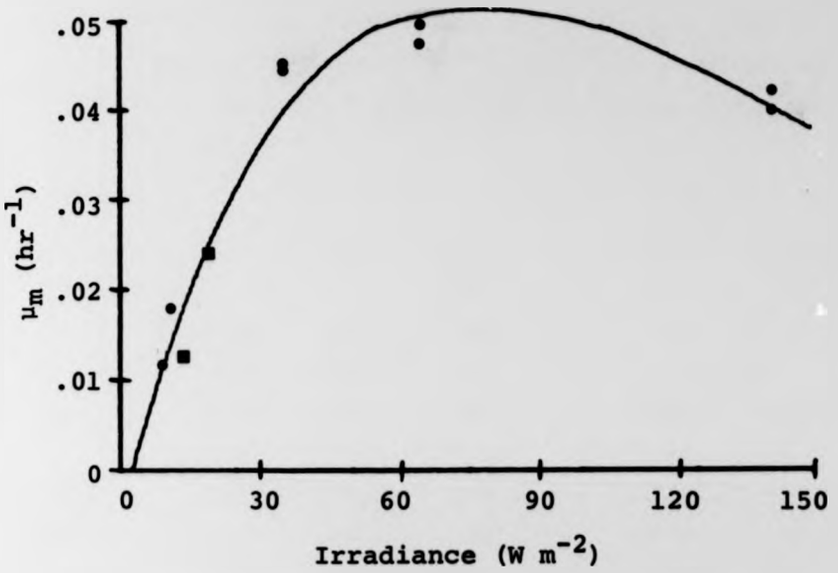



Figure 19. $\mu_m$, as calculated for the exponential phase of each experiment, vs irradiance. The square points are from the light/dark experiment (No. 8). The line is the curve predicted by the following equation:

$$\mu_m = .041\ I\ e^{-.013I} - .110$$

TABLE 7. Calculated values of $\mu_m$ at different illuminations. The exponential rate of growth during the nutrient-saturated phase of each experiment is given in units of $hr^{-1}$ ± the standard error (the standard deviation of $\mu_m$) and in units of $day^{-1}$. An analysis of variance between experiments 1 and 2, and 3 and 4 gives a difference significant at $p = .035$.

| Experiment Number | $\mu_m$ $hr^{-1}$ | S.E. | $\mu_m$ $day^{-1}$ | n | F[1] | Irradiance W $m^{-2}$ |
|---|---|---|---|---|---|---|
| 1 | .0339 | .0016 | .814 | 6 | 450 | |
| 2 | .0355 | .0004 | .852 | 6 | 8471 | |
| 1+2 | .0352 | .0009 | .845 | 12 | 1444 | 140.53 |
| 3 | .0399 | .0006 | .958 | 7 | 3855 | |
| 4 | .0416 | .0010 | .998 | 5 | 1755 | |
| 3+4 | .0408 | .0009 | .979 | 12 | 2098 | 64.16 |
| 5 | .0372 | .0005 | .893 | 12 | 6531 | |
| 6 | .0375 | .0002 | .900 | 9 | 25169 | |
| 5+6 | .0375 | .0002 | .900 | 21 | 26933 | 35.25 |
| $7_1$ | .0098 | .0007 | .235 | 5 | 180 | 8.93 |
| $7_2$ | .0152 | .0001 | .365 | 15 | 18312 | 11.75 |
| $8_1$ | .0103 | .0002 | .247 | 5 | 2019 | 14.26 |
| $8_2$ | .0203 | .0019 | .487 | 5 | 120 | 19.74 |

[1] The F represents the ratio of explained/unexplained variance for each regression of ln cell concentration on time (Sokal and Rohlf, 1969). All regressions were significant at (at least) $p. < .01$.

D. Phosphorus-Controlled Growth

1. μ vs Q (finite difference)

As mentioned in the introduction, it is possible to parameterize both Droop's and Caperon's models by transforming the cell counts into finite-difference measures of growth rates and calculating the corresponding mean cell-quotas. Droop's model can be fitted to the data by linear regression of μ on 1/Q. Both models can be fitted to the data by nonlinear regression of μ on Q, using the algebraic forms of the models (solving for $\mu = \frac{1}{X}\frac{dX}{dt}$ in equations 5 and 8). The results of both curve-fitting exercises are presented in Figures 20 to 24 and in Table 8. Combined data sets for replicate experiments were used for the results shown in Figures 20 to 22. This was necessary because, with uncombined data sets, the estimates of the parameters in Caperon's model were sensitive to the constraints for the upper and lower bounds of the parameters. This indicated that the values for the parameters in Caperon's model were not unique for the uncombined data sets (due to inadequacies in the model, or the small sizes of the data sets, or the lack of precision of the data).

For Droop's model, however, the range of the constraints for the upper and lower bounds of the parameters were wide, and widening the constraints did not alter the estimates. The standard errors of the estimates in Droop's model were usually less than ten percent of the estimate. The standard errors for the estimates in Caperon's model indicated that the estimates were unsatisfactory because the error was often larger than the estimate. For this reason, the estimates of the parameters in Droop's model were the ones

used in the analysis to determine the effect of irradiance on phosphorus-controlled growth.

The total sum of squared error for the two models (shown in Table 8) suggests that Caperon's model is the better of the two; however, an objective comparison of the two models demonstrates that the sum of squared error for both models is not significantly different. The significance of the variance ratio can be compared using a scheme as presented by Chiu *et al.* (1972):

$$F_{m,i} = \frac{A}{B} = \frac{\text{larger variance}}{\text{smaller variance}}$$

where: m = number of models being compared

i = degrees of freedom of numerator[2] (n - p)

variance = (SSE)/(n - p)

n = number of data

p = number of parameters in the given model

Therefore the variance ratio (F) of the two SSE's can be calculated:

$$F_{2,48} = \frac{.00031/(50-2)}{.00019/(50-3)} = 1.60$$

The variance ratio is not significantly different from 1.0.

---

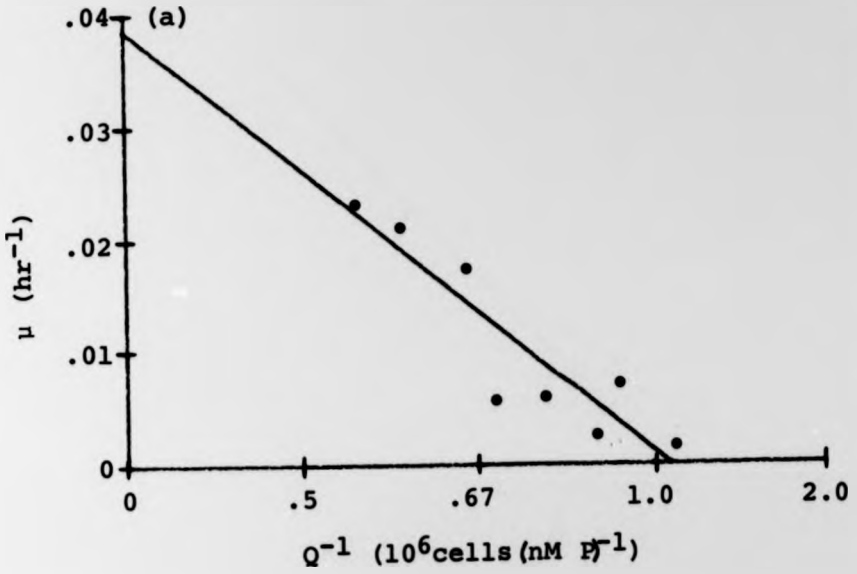
[2]There is some confusion about how to calculate the degrees of freedom when the samples are grouped by experiment and the number of data is the number of experiments. The degrees of freedom are decreased and the variance ratio becomes:

$$F_{2,3} = \frac{.00031/(5-2)}{.00031/(5-3)} = 1.08$$

The variance ratio is not significant and is predictably smaller than the first ratio. While it is irrelevant to argue which ratio is appropriate in the present study,

there may be situations in which one ratio is significant and the other is not - this matter needs to be clarified.

Figures 20-24. In these graphs are plotted finite-difference measures of $\mu$ vs 1/Q and Q. Figures 20-22 represent grouped data from 2 experiments (at the same light level). The lines are the predictions of Droop's model in (a) and Droop's model, 1, and Caperon's model, 2, in (b), after estimating the parameters given in Table 8.



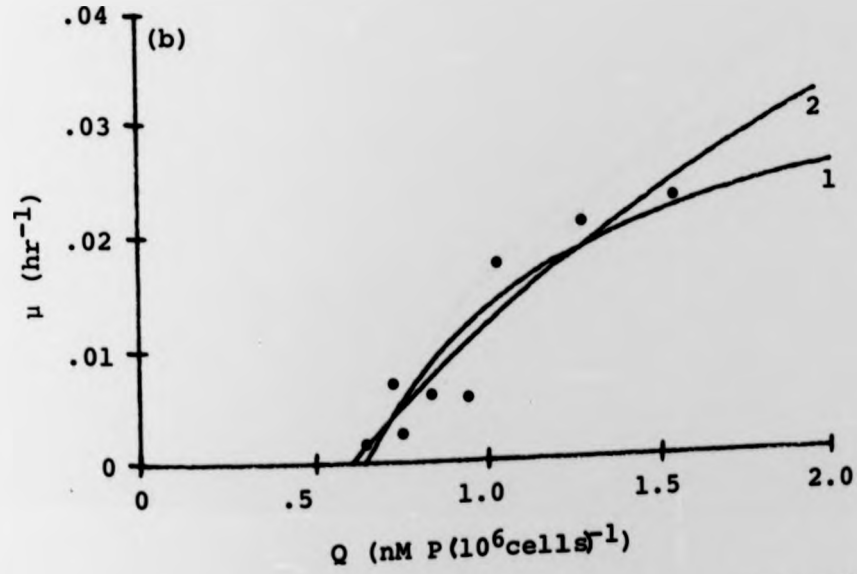




Figure 20. Experiments 1 and 2 (140.53 W $m^{-2}$).

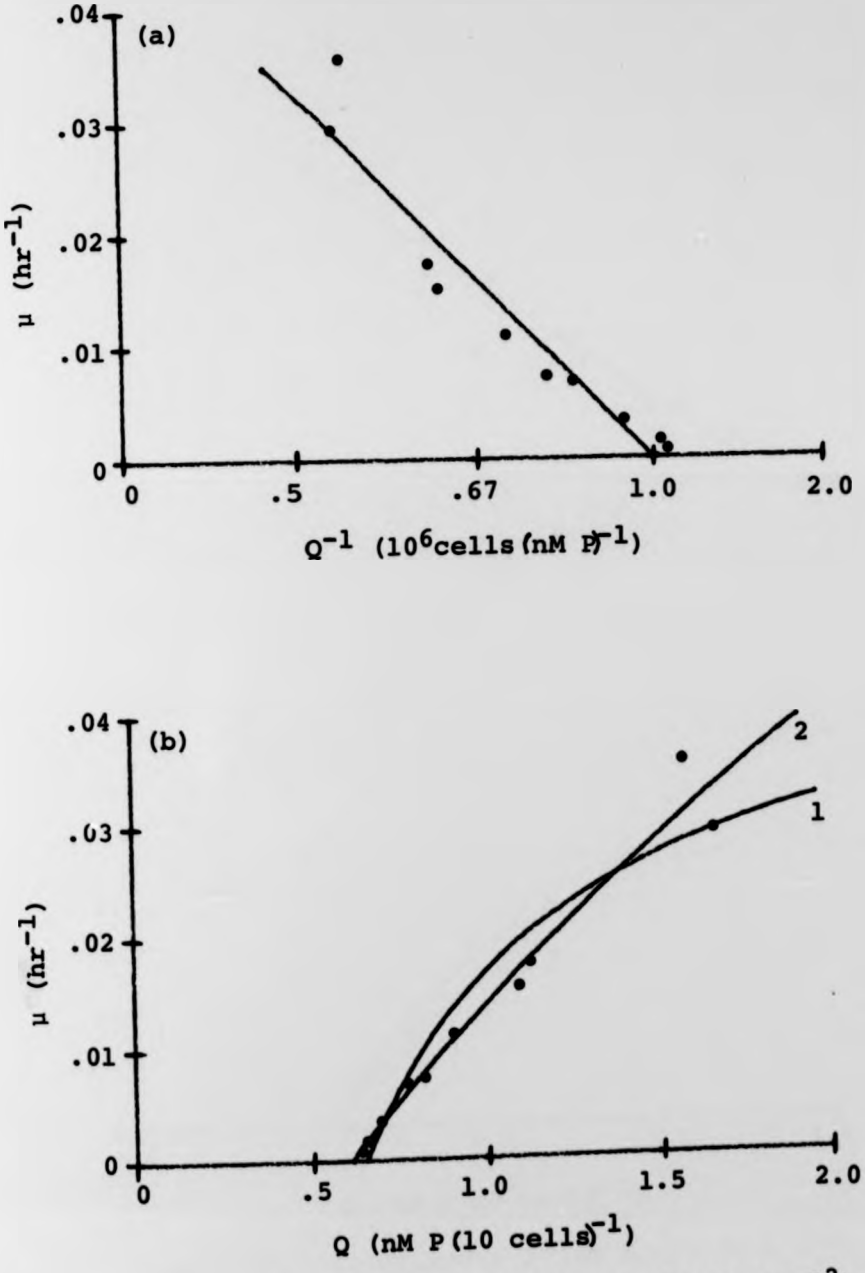



Figure 21. Experiments 3 and 4 (64.15 W $m^{-2}$).

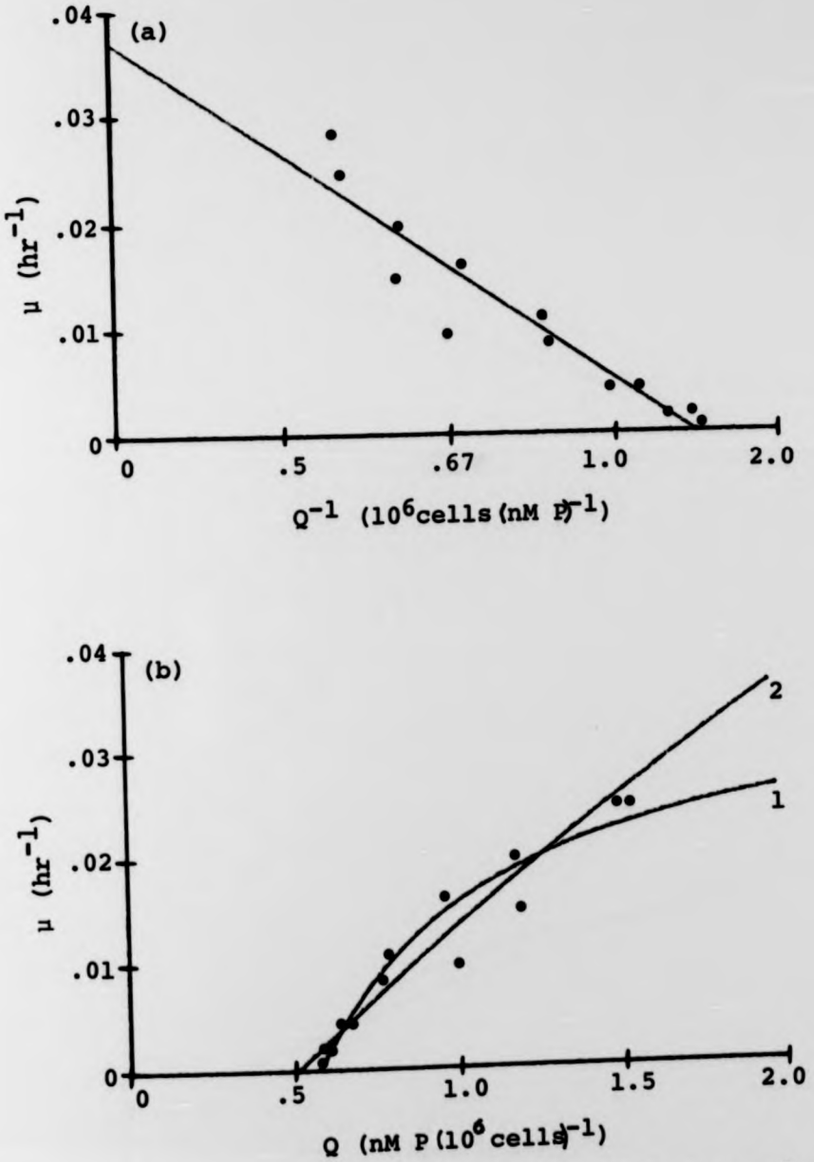
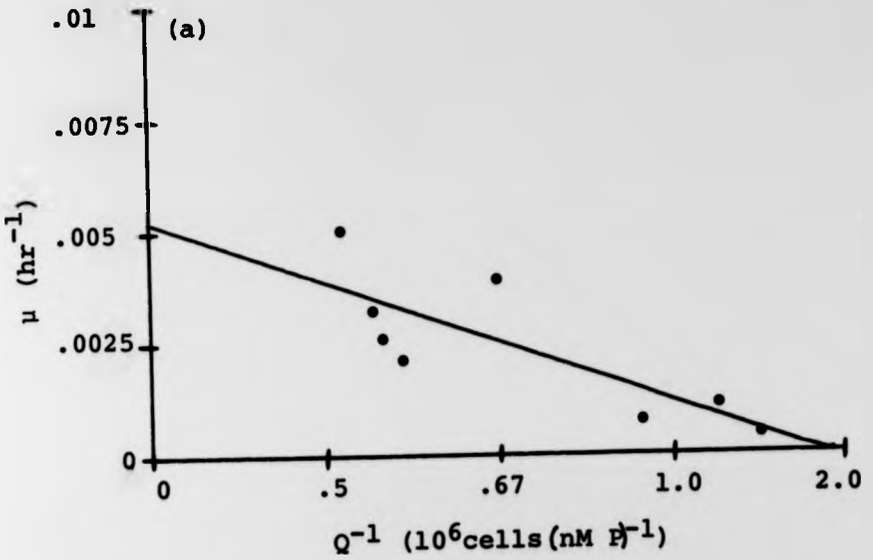



Figure 22. Experiments 5 and 6 (35.25 W m$^{-2}$).



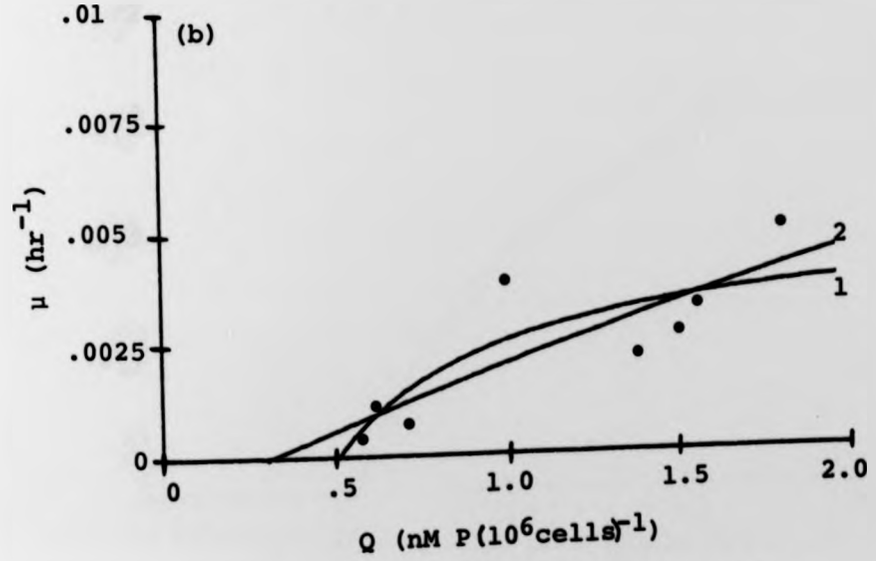
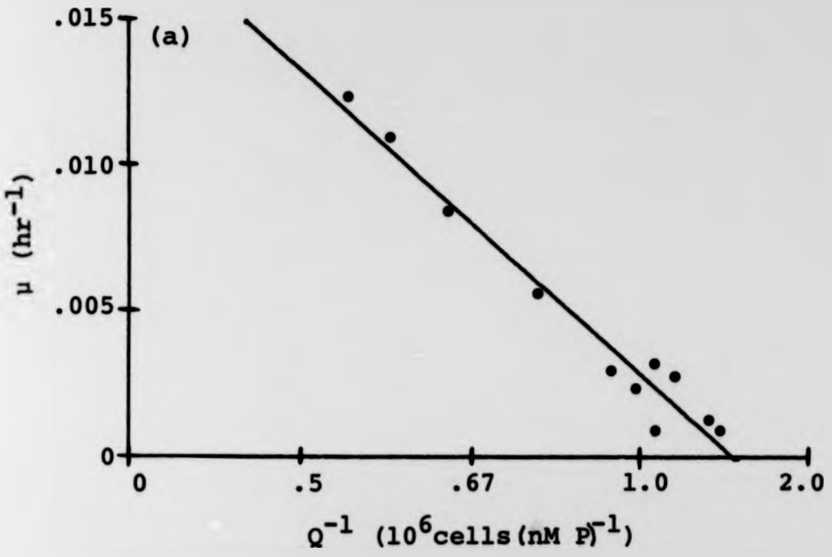




Figure 23. Experiment 7 (11.75 W m$^{-2}$).



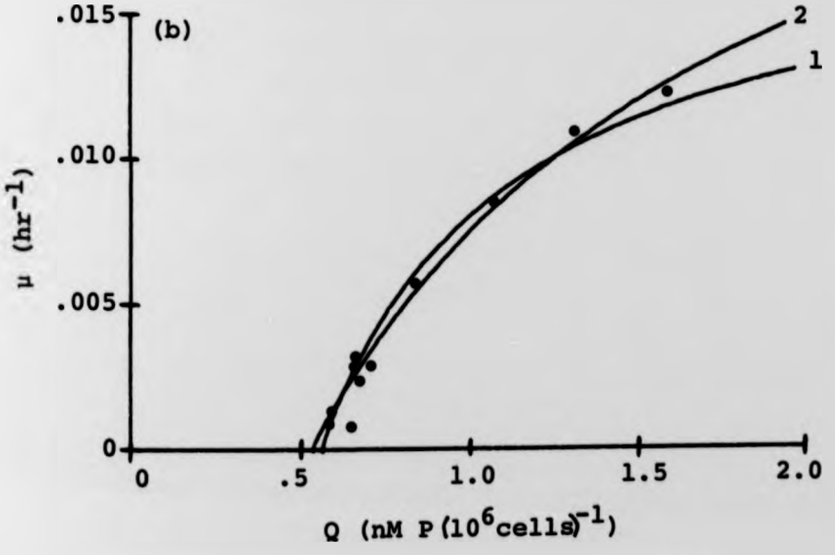




Figure 24. Experiment 8 (19.74 W m$^{-2}$).

TABLE 8. Estimated values of the parameters in Droop's and Caperon's models for $\mu$ vs Q and 1/Q.

Droop's Model - $\mu$ vs 1/Q, from: $\mu = \mu_m' - \mu_m' k_Q \frac{1}{Q}$

| Experiment Number | $\mu_m'$ ($hr^{-1}$) | S.E. | $k_Q$ (nM P/$10^6$ cells) | S.E. | n |
|---|---|---|---|---|---|
| 1+2 | .039 | .0054 | .65 | .040 | 8 |
| 3+4 | .049 | .0040 | .660 | .024 | 10 |
| 5+6 | .0371 | .0028 | .571 | .022 | 13 |
| 7 | .0053 | .0010 | .51 | .082 | 8 |
| 8 | .0186 | .0010 | .561 | .013 | 11 |

Droop's Model - $\mu$ vs Q, from: $\mu = \mu_m' (1 - k_Q/Q)$

| Experiment Number | $\mu_m'$ | S.E. | $k_Q$ | S.E. | Sum of squared error | n |
|---|---|---|---|---|---|---|
| 1+2 | .039 | .0054 | .65 | .040 | $9.2 \times 10^{-5}$ | 8 |
| 3+4 | .049 | .0040 | .661 | .024 | $1.0 \times 10^{-4}$ | 10 |
| 5+6 | .0371 | .0028 | .570 | .022 | $1.0 \times 10^{-4}$ | 13 |
| 7 | .0053 | .0010 | .51 | .082 | $6.9 \times 10^{-6}$ | 8 |
| 8 | .0186 | .0010 | .562 | .013 | $7.5 \times 10^{-6}$ | 11 |

Caperon's Model - $\mu$ vs Q, from: $\mu = \mu_m' \left(\frac{Q-Q_o}{K_Q+Q-Q_o}\right)$

| Experiment Number | $\mu_m'$ | S.E. | $Q_o$ ($k_Q$) | S.E. | $K_Q$ (nM P/$10^6$ cells) | S.E. | Sum of squared error | n |
|---|---|---|---|---|---|---|---|---|
| 1+2 | .11 | .228 | .60 | .102 | 3.0 | 8.5 | $7.2 \times 10^{-5}$ | 8 |
| 3+4 | .20 | .268 | .61 | .044 | 5.0 | 8.5 | $4.2 \times 10^{-5}$ | 10 |
| 5+6 | .25 | .64 | .50 | .059 | 8.0 | 25.0 | $6.2 \times 10^{-5}$ | 13 |
| 7 | .03 | .38 | .31 | .63 | 10.0 | 138.0 | $6.9 \times 10^{-6}$ | 8 |
| 8 | .028 | .0104 | .53 | .031 | 1.3 | .79 | $5.4 \times 10^{-6}$ | 11 |

2. The transient models fit to X, Q, and S

The preferred parameter-estimation technique for use with transient data is the one that fits the solution of the differential form of the model to the time-series of state variables, X, Q and S. Each time-series data set was taken from the last sample during the exponential phase to the end of the experiment. The two models for nutrient-controlled growth, equations 5-10, were fitted to the data set for each experiment. In theory, the estimates of the parameters are better when the data sets are larger, so the models were also fitted to the combined data from replicate experiments (there is an option for this type of treatment in Bard's estimation program).

Caperon's model could not be fitted to the data. The estimation procedure always reached an endpoint that was not a global minimum and thus was unsatisfactory for purely technical reasons (rather than because the estimated values of the parameters were unsatisfactory or the predictions of the model were unreasonable). Droop's model was fitted to the data sets and the estimated values of the parameters are given in the legends for Figures 6 to 13.

The results of the estimation with the various data sets are given in Table 9. Although the estimation procedure converged when fitting Droop's model to the separate data sets, it did not do so with two of the combined data sets. Consistent values of the growth parameters, $\mu_m'$ and $k_Q$, were obtained from the separate data sets, but the estimated values of the uptake parameters ($u_m$, $K_S$, and $S_o$) were not similar even for the replicate experiments. It was probably this reason that two of the combined data sets

did not converge - the estimation procedure could not "choose" a single set of values for the uptake parameters. Thus the effects of irradiance on uptake cannot be further studied here because the estimates of the parameters in the uptake portion of the model seem to have no general meaning. The estimates of the parameters in the growth portion of Droop's model, however, are consistent between replicate experiments and thus may be compared amongst light levels.

TABLE 9. Estimated values of the parameters in the differential form of Droop's model (the standard errors are given under Figures 6-13).

| Experiment Number | $u_m$ (nM P ($10^6$ cells)$^{-1}$ $hr^{-1}$) | $K_S$ (nM P $ml^{-1}$) | $S_o$ (nM P $ml^{-1}$) | $\mu_m'$ ($day^{-1}$) | $k_Q$ (nM P ($10^6$ cells)$^{-1}$) |
|---|---|---|---|---|---|
| 1 | .6* | 9.9* | .12 | .96 | .594 |
| 2 | .5* | 9.8* | .104 | 1.22 | .704 |
| 3 | .005* | .001* | .098 | 1.28 | .64 |
| 4 | .2* | 9.5* | .018 | 1.08 | .635 |
| 5 | .001* | .001* | .074 | .720 | .560 |
| 6 | .00188 | .00099 | .034 | 1.003 | .567 |
| 7 | .002* | 98.* | 9.6* | .143 | .49 |
| 8 | .105* | 10.0* | .031 | .461 | .566 |
| 1+2 | .4* | 9.9* | .104 | 1.05 | .648 |
| 3+4 [1] | .05 | .001 | .067 | 1.15 | .642 |
| 5+6 [1] | .002 | .001 | .057 | .893 | .569 |

* The standard errors of the estimates were greater than the value of the estimate.

[1] The estimation procedure failed to converge with these data sets.

E. The Effects of Irradiance on Phosphorus-Controlled Growth

1. $Q_m'$ vs irradiance

In this study $Q_m'$ is defined as the cell-quota of phosphorus at which the algae switch from exponential growth to phosphorus-controlled growth. It is difficult to obtain a precise value of $Q_m'$ from batch-culture data because it is impossible to determine exactly when the changeover occurs. $Q_m'$ can be estimated by taking the logarithmic average of Q at the last sampling in the exponential phase and Q at the next sampling. The values of Q and the estimates of $Q_m'$ are given in Table 10 along with the estimates of $k_Q$ and $Q_m'/k_Q$ for each experiment.

The differences between the two measures of Q used to estimate $Q_m'$ for experiments 1-6 are large because the cultures were still growing very fast. There is no significant difference between the estimates of $Q_m'$ and there is no trend in the variation between experiments. This indicates that phosphorus-controlled growth began at the same cell-quota for the different irradiances and, that there was a definite switch from light to phosphorus control. The discontinuity in growth rate between exponential and phosphorus-controlled phases is obvious in Figures 6-13.

Table 10. Estimates of $Q_m'$, $k_Q$, $Q_m'/k_Q$ at the different illuminations. $Q_1$ and $Q_2$ are the values used to obtain $Q_m'$.

| Experiment Number | Irradiance ($W\ m^{-2}$) | $Q_1$ | $Q_2$ (nM P ($10^6$ cells)$^{-1}$) | $Q_m'$ | $k_Q$ | $Q_m'/k_Q$ | Mean $Q_m'/k_Q$ |
|---|---|---|---|---|---|---|---|
| 1 | 140.53 | 1.276 | 1.790 | 1.66 | .594 | 2.79 | 2.38 |
| 2 | " | 1.008 | 1.537 | 1.38 | .704 | 1.96 | |
| 3 | 64.16 | 1.252 | 1.908 | 1.72 | .64 | 2.69 | 2.81 |
| 4 | " | 1.198 | 2.148 | 1.86 | .635 | 2.93 | |
| 5 | 35.25 | 1.227 | 1.730 | 1.59 | .560 | 2.84 | 2.87 |
| 6 | " | 1.266 | 1.783 | 1.64 | .567 | 2.89 | |
| 7 | 11.75 | 1.733 | 2.061 | 1.89 | .49 | 3.86 | |
| 8 | 19.74 | 1.719 | 2.360 | 2.01 | .566 | 3.55 | |

2. $\mu_m'$ vs irradiance

All of the estimates of the growth parameters in Droop's model are given in Table 11. A comparison of the estimates of $\mu_m'$ between the three techniques shows that they are very similar. As was stated earlier, the best estimates are made by the technique that fits the differential equations to the original data (column 3 in Table 11), so the following analysis is based on those estimates.

The estimated values of $\mu_m'$ are plotted against irradiance in Figure 25a. There is an obvious decrease in $\mu_m'$ with decreasing light, following the general shape of a rectangular hyperbola. An analysis of variance of the differences amongst the estimates of $\mu_m'$ at the three highest light levels shows that the differences amongst these light levels are insignificant (a result explained by the extent of the variability within replicates). However, the differences between the highest estimates (1.153 $day^{-1}$ for experiments 3 and 4) and the lowest value (.143 $day^{-1}$ for experiment 7) is over 700%, and a t-test shows the difference is significant. An analysis of variance based on a linear regression of all the estimates of $\mu_m'$ against light intensity also indicates the $\mu_m'$ is strongly related to light intensity.

Various equations for rectangular hyperbolas were fit to the $\mu_m'$ vs irradiance data and the results are presented in Appendix V. A two-step, linear model by Blackman (1905) gives the best fit, but this is due to the small size of the data set - resulting in two, linear regions in the data: a plateau at the high light levels (six points which are not significantly different) and a region of increasing

growth rate with increasing irradiance (only two points) at the lower light levels. It is doubtful that the linear model would fit so well with a larger data set.

The model derived from the Monod equation for substrate-limited growth, equations 14-17 provides an uncomplicated (one equation having two parameters) description of the data:

$$\mu'_m = \mu'_{opt} (1 - I_{o/I}),$$

where: $\mu'_{opt} = 1.267 (\pm .087)$ day$^{-1}$

$I_o = 10.81 (\pm 1.30)$ watts m$^{-2}$

The symbol $I_o$ could be replaced by $k_I$ for the sake of consistency. The curve predicted by the equation is drawn in Figure 25a. Steele's equation, used earlier to describe the nutrient-saturated growth vs rate irradiance data, was not suitable because phosphorus-controlled growth did not decrease at the highest irradiance.

TABLE 11. Values of $\mu_m'$ and $k_Q$, as estimated by the three parameter-estimation techniques.

| Experiment Number | Irradiance ($W\ m^{-2}$) | $\mu_m'$ ($day^{-1}$) 1 | 2 | 3 | $k_Q$ (nM P ($10^6$ cells)$^{-1}$) 1 | 2 | 3 |
|---|---|---|---|---|---|---|---|
| 1 | 140.53 | .94 | .94 | .96 | .594 | .596 | .594 |
| 2 | 140.53 | 1.09 | 1.09 | 1.22 | .74 | .74 | .704 |
| 3 | 64.16 | 1.31 | 1.31 | 1.28 | .67 | .67 | .64 |
| 4 | 64.16 | 1.05 | 1.05 | 1.08 | .651 | .650 | .635 |
| 5 | 32.25 | .76 | .76 | .720 | .56 | .56 | .560 |
| 6 | 32.25 | 1.04 | 1.04 | 1.003 | .589 | .589 | .567 |
| 7 | 11.75 | .127 | .127 | .143 | .51 | .51 | .49 |
| 8 | 19.74 | .444 | .446 | .461 | .561 | .562 | .566 |
| 1+2 | 140.53 | .93 | .93 | 1.05 | .65 | .65 | .648 |
| 3+4 | 64.16 | 1.17 | 1.17 | 1.15 | .660 | .661 | .642 |
| 5+6 | 32.25 | .89 | .89 | .893 | .571 | .570 | .569 |

| Column | Estimation Technique |
|---|---|
| 1 | $\mu$ vs $1/Q$ |
| 2 | $\mu$ vs $Q$ |
| 3 | $\frac{dX}{dt}$, $\frac{dQ}{dt}$, and $\frac{dS}{dt}$ vs observed X, Q, and S |

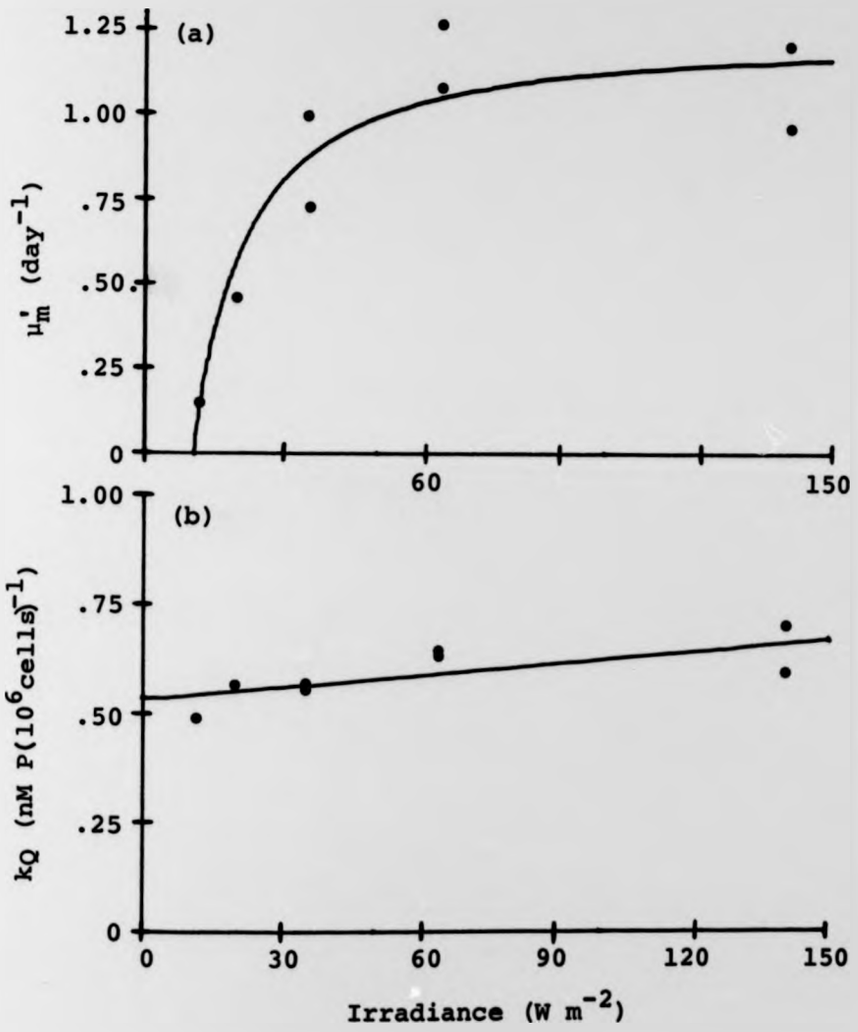



Figure 25. $\mu_m'$ and $k_Q$ vs irradiance. The values are from column 3 in Table 11. The lines are the curves predicted by the equations:

(a) $\mu_m' = 1.267 \left(1 - \frac{10.81}{I}\right)$

(b) $k_Q = .0008\ I + .553$

3. $k_Q$ vs irradiance

The estimates of $k_Q$ are in Table 11. A comparison of the estimates of $k_Q$ between the three estimation techniques shows that there are only slight differences among them. The estimates in column 3 (from the fit of the differential equations to the data) are used in the following analysis.

The values of $k_Q$ decrease slightly with decreasing irradiance (Figure 25b). Even though the difference between the highest and lowest values is only about 30%, a linear regression of $k_Q$ vs irradiance is significant ($p = .04$, the line is drawn in Figure 25b). The equation for the regression is:

$$k_Q = .0008\ (\pm\ .0003)I + .553\ (\pm\ .024). \qquad (19)$$

# DISCUSSION

## A. Results from Batch-Culture Experiments

### 1. Light-controlled (exponential) growth

The values of $\mu_m$ measured during the exponential phases of the batch cultures demonstrate a strong relationship with irradiance. During the exponential phases of the batch cultures, the algae were "nutrient-saturated" and irradiance, through the photosynthetic system, was controlling growth. The supposition that irradiance, rather than $CO_2$, was controlling the growth rate can be supported by two lines of argument:

1) The plots of ln X vs time were linear; however, if $CO_2$ was controlling the growth rate, it would do so in a continuously progressive fashion causing the plots to curve. Also, the biomass was never large enough to affect growth rate through $CO_2$ limitation - Myers and Graham (1959) found that biomass up to .13 mg/ml had no effect on growth rate. Using the conversion given by Droop (1974) for Monochrysis (Pavlova), the biomass in the batch cultures never was greater than .03 mg C/ml.

2) The culture medium was buffered, set to pH 8.0, and extra carbonate was added (80 mg/l). Thus the carbonate buffer system was close to the ideal conditions for maintaining maximum levels of free $CO_2$.

Light inhibition of photosynthesis has often been reported in natural phytoplankton (Ryther, 1956; Ryther and Menzel, 1959; Talling, 1957; Talling, 1960; Steele, 1962; and Steemann Neilsen, 1949) and in laboratory experiments (Ryther, 1956; Tamiya et al., 1955; and Steemann Neilsen, 1962). An alternative explanation for some of these observations, especially in oligotrophic waters, is that the supply of nutrient in an incubation bottle is more quickly

depleted at higher light levels, causing a decrease in photosynthesis. Indeed, this can be simulated using a simple model of algal growth (Hornberger et al., 1975). Productivity experiments are not designed either to show changes in growth rate and nutrient levels throughout the experiment, or to distinguish between growth conditions other than irradiance.

A comparison of the rates of exponential growth during the light-controlled phases for the two highest light levels demonstrates inhibition of growth rate due to the high illumination. Of course, there are explanations other than inhibition of growth rate through inhibited photosynthesis - there may be increased respiration at higher light levels, resulting in a lower net production, causing a decrease in growth rate. There is convincing evidence supporting the hypothesis that respiration (usually called photorespiration) increases with increasing light (Laws and Caperon, 1976; Talling, 1957; Caperon, 1967; and Brown and Richardson, 1968).

The equations for algal growth controlled by irradiance (e.g. during the exponential phase of a batch culture) may be written:

$$\frac{dX}{dt} = (\alpha I e^{-\beta I} - r)\, X = \mu_m X$$

$$\frac{dQ}{dt} = u - (\alpha I e^{-\beta I} - r)\, Q$$

$$\frac{dS}{dt} = -uX$$

where: $\alpha = .041 \pm .005\ m^2\ W^{-1}\ day^{-1}$

$\beta = .013 \pm .004\ m^2\ W^{-1}$

$r = .110 \pm .082\ day^{-1}$

The parameter "r" may be thought of as respiration, but I prefer to think of it as a constant needed to describe the data. Notice that $r_Q$ in the cell-quota equation has units of negative nutrient uptake - an interesting concept that cannot be supported by the data (at least as a constant rate of excretion).

These equations for nutrient-saturated growth provide a description of growth rates over a wide range of irradiances. At a given level of irradiance, growth is predicted to proceed at a constant, exponential rate. The process of uptake, u, is left undefined, although uptake might be given a value equal to $\mu_m Q_m'$ and the rate of uptake would, in this scheme, remain constant until the minimum concentration of nutrient in solution is reached (of course the rate of uptake would decrease as S decreased). Once the dissolved nutrient is depleted ($S = S_o$) or $Q = Q_m'$, growth changes from a constant rate to a changing rate controlled by the decreasing phosphorus cell quota.

2. Phosphorus-controlled growth

The purpose of this study was to determine the interactive effects of irradiance and phosphorus on algal growth. The phosphorus cell-quota controlled the rate of growth during the transitional phases of the cultures. The growth dynamics during the phosphorus-controlled phase for each experiment were characterized by $\mu_m'$ (maximum phosphorus-controlled growth rate) and $k_Q$ (subsistence quota for phosphorus). A comparison of $\mu_m'$'s for all the experiments demonstrated that $\mu_m'$ decreased markedly and $k_Q$ decreased slightly with decreasing irradiance. Thus it has been shown that irradiance and internal concentration of phosphorus (the cell-quota of the controlling nutrient) have a combined (i.e. simultaneous) effect on the growth rate of Pavlova lutheri.

I have chosen to describe the interaction between light and phosphorus by multiplying the optimum growth rate by an expression for the effect of the cell quota and an expression for the effect of irradiance.

The equations for the transitional phase of a batch culture, with both irradiance and phosphorus controlling the growth rate, may be written:

$$\frac{dX}{dt} = \mu'_{opt} \left(1 - \frac{I_o}{I}\right) \left(1 - \frac{k_Q}{Q}\right) X$$

$$\frac{dQ}{dt} = u - \mu'_{opt} \left(1 - \frac{I_o}{I}\right) \left(1 - \frac{k_Q}{Q}\right) Q$$

$$\frac{dS}{dt} = -uX$$

where: $\mu'_{opt} = 1.27 \pm .085 \text{ day}^{-1}$

$I_o = 10.83 \pm 1.28 \text{ W m}^{-2}$

$k_Q = .595 \pm .023 \text{ nM P}(10^6 \text{ cells})^{-1}$

The value of $k_Q$ is the mean for all experiments. A more precise treatment would be to substitute the equation for $k_Q$ as a function of irradiance, equation 19. In these equations and the equations given in the previous section, uptake has not been given a mathematical expression because a suitable one is not available (this will be discussed in the next section).

Figure 26 shows the type of batch-culture curves of biomass predicted by the equations in this and the previous section. Figure 26a is the multiplicative model and Figure 26b is the threshold model. The major difference between the two models is the point at which control changes from irradiance to phosphorus cell-quota (black dots on the curves). In the threshold hypothesis, phosphorus takes over control when the phosphorus-controlled growth rate equals the light controlled growth rate (in this case $\mu_m'$ is equal at all light levels). Thus, the cell-quota at crossover would be smaller at lower light levels (note the crossover points in Figure 26b).

The curves in Figure 26a match the experimental results - the transition point was nearly the same at all light levels. This shows that the interaction between irradiance and phosphorus is such that phosphorus affects growth rate when the cell-quota reaches a certain level (regardless of irradiance). $\mu_m'$ decreased with decreasing light and therefore is a function of both light and phosphorus cell-quota.

The prediction of both of the above models would be slightly different if self-shading occurred during the experiments. Self-shading would cause the growth rates

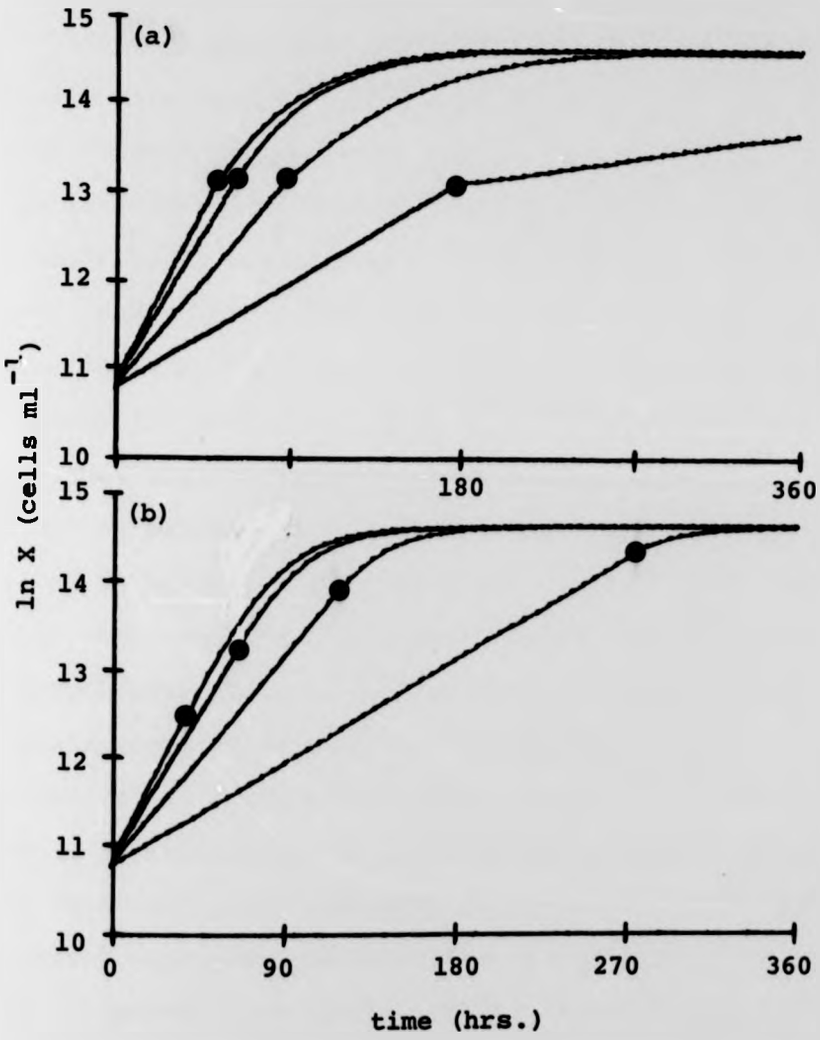



Figure 26. The theoretical batch-culture curves predicted by two hypotheses of light/phosphorus cell-quota interactions: (a) multiplicative and (b) threshold. Each line represents a different level of irradiance, and the dots represent the transition point between constant exponential growth and phosphorus-controlled growth.

to decrease due to the decreasing irradiance caused by the increasing biomass.

In previous studies (Droop, 1974, 1975; and Rhee, 1974, 1978), it was shown that there is a threshold effect on growth rate when two nutrients are limiting. The nutrients in both studies were not directly involved with the photosynthetic mechanism (vitamin $B_{12}$ and phosphorus in Droop's study and nitrogen and phosphorus in Rhee's study). Senft (1978) has shown that there is a multiplicative effect on photosynthesis when light and phosphorus cell-quota are both below their maximum levels. Senft stated that his conclusions, pertaining to photosynthesis, need not be paralleled in growth. Since the general conclusions in Senft's (1978) study and this study are similar, it would be interesting to measure both photosynthesis and growth rate under light/phosphorus control to determine relationships between the two processes.

Maddux and Jones (1964) supply data that suggest that there is an interaction of light and nutrients on growth rate. The only other work with irradiance and a nutrient (silicate) supposedly demonstrated an either/or (threshold) effect on growth rate (Davis, 1976). Davis et al. (1978) reinterpreted the 1976 data set in terms of a cell-quota model, but they did not discuss the implications on Davis' earlier threshold conclusions. When luxury uptake and phosphorus cell-quota are considered, the simplest interpretation of the effect of irradiance and silicate cell-quota on growth is that there was a combined effect. The two culture conditions that provided data for comparison

were chemostats grown at the same dilution rate (.04 $h^{-1}$) and different irradiances (normalized to 100% and 30%). Other pertinent data are given below (taken from Davis, 1976) - the units are not necessary for comparison:

| illumination | 100% | 30% |
|---|---|---|
| silicate cell-quota | .28 | .40 |
| biomass | 38.1 | 27.6 |
| dilution rate (μ) | .04 | .04 |

Davis (1976) maintained that under both irradiances silicate was controlling the growth rate - this was substantiated by the results of a perturbation experiment which resulted in uptake of silicate under both irradiances. Disregarding the perturbation experiment (which was inconclusive and subject to an alternative interpretation - see Davis et al., 1978), a comparison of the cell-quotas at different light levels shows that a higher cell-quota was present at the lower light level. This observation can be stated in terms of the cell-quota model: a higher cell-quota in the 30% light culture maintained the same growth rate as the 100% light culture. Thus, the decreased irradiance must have been affecting the silicate-controlled cultures.

Davis' results are inconclusive because his experimental design was inadequate - it did not supply enough pertinent data. Two different designs for chemostat experiments which would provide conclusive data are:

1) Maintain chemostats at the same dilution rate and same silicate input. Measure the cell-quota at a larger number and a wide range of irradiances, making sure that silicate remained limiting. This technique is analogous to that of Rhee (1978).

2) Maintain the chemostats in a few, wide ranging, irradiances and the same silicate input. Run the chemostats at a variety of dilution rates, measuring cell-quota so that $\mu$ (the dilution rate) can be related to Q. This technique is analogous to Droop's (1974).

For both designs to provide unambigious data, the silicate cell-quotas must always remain below the maximum for silicate control and the lowest irradiance must not be below $I_o$, the compensation level of irradiance.

In this study, there was no significant difference between the estimates of the $\mu_m'$ (for phosphorus) at the three highest irradiances; nonetheless, more replicate measures are necessary to determine whether the rates are equal or different. Chemostat cultures of phosphorus-controlled algae grown at different, high-light levels may provide more conclusive data concerning inhibition of growth.

3. Nutrient uptake

Uptake of orthophosphate by algae has been widely studied for a long time; however, there is no general theory of phosphorus uptake that describes the data in this study. The development of a theory of uptake has been hindered by studies that measured biomass in terms of particulate nutrient (the limiting nutrient), so that uptake rate equalled growth rate (Dugdale, 1967; Eppley et al., 1969; and many others). These investigators have made a mistaken attempt to determine a value of $K_s$ (the half-saturation constant of nutrient in solution) for growth rate. Their model, wherein uptake rate equals growth rate, can only be applied to chemostat cultures, where the steady-state nature of the system simplifies the forms of the Monod and cell-quota models so that they are algebraically equivalent. A general model for uptake must be based on the fact that uptake and growth are uncoupled processes (Caperon, 1968; Droop, 1968; and Ketchum et al., 1958). Since there is still confusion concerning a conceptual model for nutrient-uptake, a descriptive model is introduced in the following discussion and some supporting definitions are given in Table 12.

Figure 27 is an idealized diagram of the uptake process depicting cell-quota, nutrient in solution, and the number of cells. The cell-quota compartment should be conceptualized as a container that is filled by uptake and emptied by growth. The level of the cell-quota never goes lower than $k_Q$. The encircled numbers in Figure 27 are rates, processes, or affects that are discussed below:

Table 12. Definitions for discussion of nutrient uptake.

$Q$ - cell-quota: concentration of nutrient in the cell.

$k_Q$ - subsistence quota: the minimum level of nutrient in the cell. When $Q = k_Q$, growth stops.

$Q_m$ - the maximum amount of nutrient that the cell can store.

$Q_m'$ - a nutrient controls growth when its cell-quota is less than $Q_m'$. There is a value of $Q_m'$ for each nutrient (the same is true for the above parameters) and, if the cell-quotas of two or more nutrients are below their respective $Q_m'$, the nutrient with the smallest value of $Q/k_Q$ is the one controlling growth rate.

adsorption - the physical or physico-chemical process whereby nutrients from the medium attach to the cell surface.

absorption - assimilation: a biochemical process whereby the nutrient is taken into the cell (this step is considered to be much slower than adsorption, so in most cases, absorption = uptake).

uptake - adsorption plus absorption. Uptake is measured as the change in particulate nutrient. Specific uptake rate =

$$\frac{d\,(\text{particulate phosphorus})}{dt}\,\frac{1}{X}, \text{ or } -\frac{dS}{dt}\,\frac{1}{X}.$$

surge uptake - the initial, rapid uptake by nutrient deficient algae which occurs in a perturbation experiment or when algae are transferred from a nutrient-poor to a nutrient-rich medium (Caperon, 1968; Cloern, 1977; Conway *et al.*, 1976; Kuenzler and Ketchum, 1962; Sakshaug and Holm-Hansen, 1977; and Spencer, 1954).

luxury uptake - the uptake which occurs when $Q \geq Q_m'$: the continued uptake of a nutrient that is not controlling growth rate. Luxury uptake refers to uptake that is in excess of immediate needs - when an increase in the cell-quota does not increase the growth rate (Davis *et al.*, 1978; Droop, 1974 and 1975; Fitzgerald and Nelson, 1976; Nyholm, 1975; and Rodhe, in press).

limiting or controlling nutrient - (see $Q_m'$ above) the nutrient with the smallest value of $Q/k_Q$. This is analogous to Leibeg's law of the minimum (pertaining to yield, which is here applied to growth rate - as was first done by Blackman, 1905). This definition might only hold for stored nutrients. It is not clear if the effects of factors such as light, $CO_2$, salinity, temperature, and pH - should be considered. When all nutrients but one are in excess, that nutrient controls growth when its $Q$ becomes less than $Q_m'$. When $Q > Q_m'$ for all nutrients, (either light or) intrinsic biochemistry controls growth.

nonlimiting or noncontrolling nutrient - all nutrients except for the one controlling growth rate.

nutrient starved - $Q = k_Q$.

nutrient deficient - $Q < Q_m$.

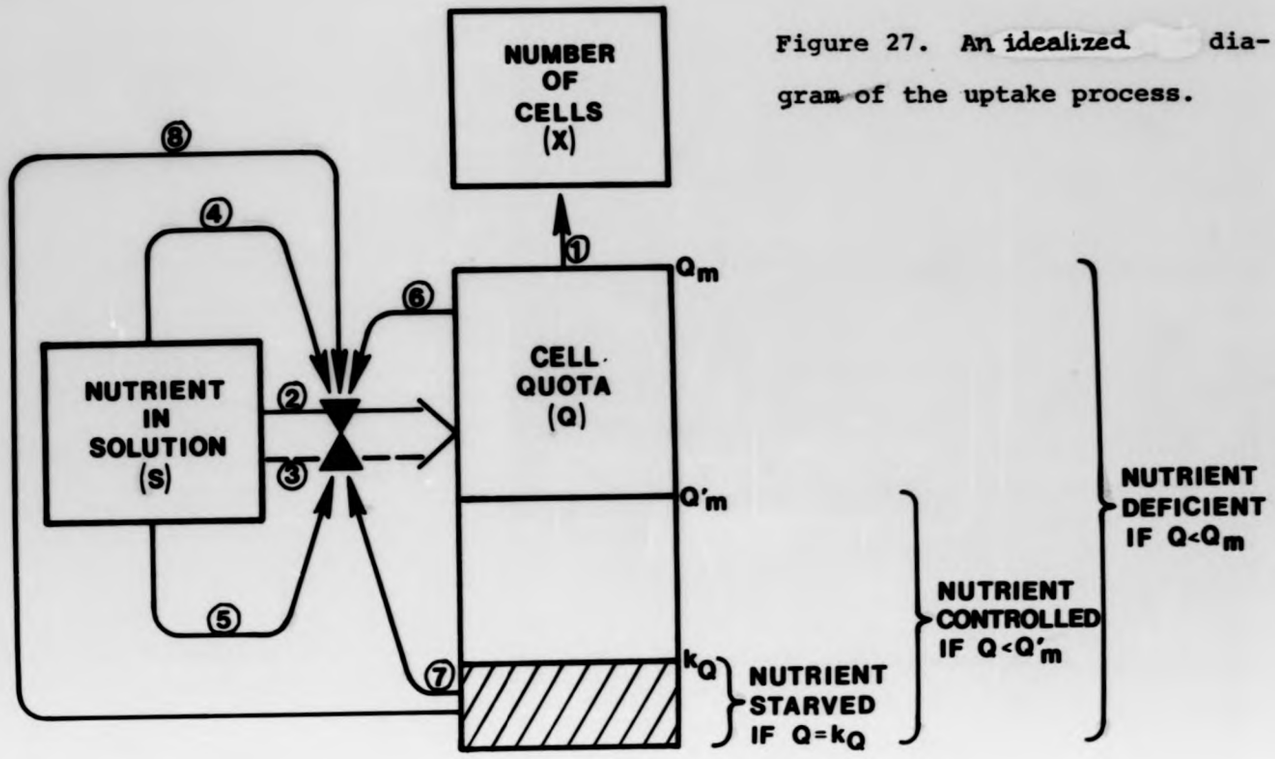
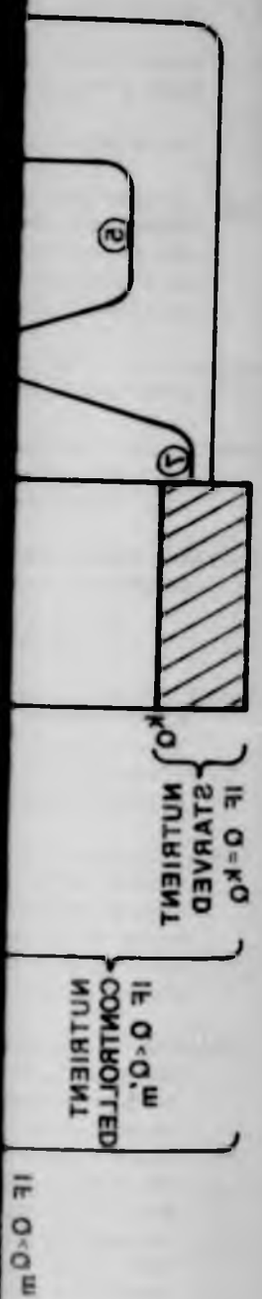



Figure 27. An idealized diagram of the uptake process.




(1) The cell-quota decreases as the cell numbers increase (the units of Q are conc/cell).

(2) Rate of absorption of nutrients.

(3) Rate of adsorption to the cell surface (the initial rapid surge, as observed in this study).

(2) and (3) Uptake rate (assimilation rate). Thus an equation can be written for the change of cell quota (process 1):

$$\frac{dQ}{dt} = u - \mu Q, \text{ or}$$

$$\frac{dQ}{dt} = \text{uptake rate} - (\text{growth rate} \times \text{cell-quota}).$$

(4) and (5) The concentration of nutrient in solution may control the uptake rate, as described by nutrient kinetics:

$$u = u_m \frac{S}{K_S + S}, \text{ for the assimilation rate.}$$

It is possible that the rapid rate of adsorption might be controlled by the nutrient in solution and another equation for uptake due to adsorption might be appropriate:

$$u = u_{ads} \frac{S}{K_{ads} + S}, \text{ for the adsorption rate.}$$

For most purposes, a separate equation for adsorption is unnecessary; however, care should be taken in perturbation experiments to ensure that the adsorption mechanism is saturated.

(6) The uptake rate might be inhibited by a feedback-control mechanism - this inhibition probably takes place only when Q is greater than $Q_m$ (Brown and Harris, 1978; Davis et al., 1978; and Rhee, 1973 and 1974). An equation describing such a mechanism has been presented by Rhee (1973) and is modelled in terms of product inhibition as Q approaches $Q_m$. An alternative description of the process of luxury uptake has been presented by Droop (1974). In Droop's model the uptake rate of the noncontrolling nutrient is a function of the nutrient in solution, a luxury coefficient, and the rate of uptake of the controlling nutrient. Both investigators stress the fact that it is necessary to distinguish between processes of uptake, depending on whether a nutrient is controlling or not controlling growth (Caperon, 1968; Droop, 1974 and 1975; Nyholm, 1975; and Rhee, 1974).

(7) Adsorption may be affected by the cells becoming disorganized if there is an abrupt change in the nutrient environment (Lean and Nalewajko, 1976; and Spencer, 1954). Also, it is possible that the cell surface is maintained for different strategies of uptake in different environments. If the environment changes, there might be subsequent changes in the cell surface. This might explain the lack of rapid, initial uptake in experiments 7 and 8 (started with stationary-phase cells).

(8) Assimilation (absorption) might be slowed down or disrupted after the cell has been in a nutrient-controlled state for a long time (Kuhl, 1974). A decreased rate of uptake by nutrient-controlled cells might be related to the increased activity in the enzyme alkaline phosphotase (Fitzgerald and Nelson, 1966; Healey, 1973; Healey and Hendzel, 1975; and Kuhl, 1974) which aids in 'stripping' the cell of any phosphorus in excess of $k_0$. Another explanation for decreased uptake by stationary-phase cells is that some metabolic activity may be necessary to maintain phosphorus uptake sites or to keep active the enzyme systems that control the process of uptake.

Three different types or patterns of uptake have been defined by various workers using silicate (Conway and Harrison, 1977; Conway et al., 1976; and Harrison et al., 1976) and phosphorus (Cloern, 1977). The three types of uptake are:

1) surge uptake by nutrient deficient cells;

2) internally controlled uptake which is a function of growth rate; and,

3) externally controlled uptake when the nutrient in solution is small enough to affect the rate of uptake.

A simpler and more general classification of the types of uptake (similar to that presented by Nyholm, 1975) is given below:

Two types or modes of uptake:

1) surge - rapid uptake resulting from adsorption and assimilation into nutrient deficient cells. The amount of nutrient taken out of the

medium is a function of the degree of deficiency. The rate of uptake is a function of the nutrient in solution and, possibly, product inhibition.

2) internally controlled - the storage pools are full or decreasing and uptake is a function of growth rate or the nutrient in solution, and, possibly, product inhibition.

Notice that the above two modes are both affected by the nutrient in solution, if the concentration is low enough to decrease the uptake rate. When all nutrients are in excess and all storage pools are filled, the uptake may be expressed as:

$$u = \mu_m Q_m$$

a relationship that was first noted by Mackereth in 1953. In this study $Q_m$ did not remain constant, nor was it the same for all experiments. It is possible, therefore, that the nutrient concentration was too low (1.44 mM $ml^{-1}$) to saturate the uptake mechanism and thus uptake, during the exponential phase, was a function of the concentration in the medium and not directly related to the growth rate. Thus it is possible that the uptake rate can be lower than maximum and the growth rate continues at a maximum (as long as Q is greater than $Q_m'$).

When a nutrient is controlling growth rate, the uptake of the controlling and noncontrolling nutrients may be expressed as:

controlling nutrient -

u = f (nutrient in solution); and,

noncontrolling nutrient -

u = f (uptake of controlling nutrient and concentration of nutrient in solution; or, the growth rate; or, nutrient in solution and inhibition products).

The exact description of the uptake mechanism may be irrelevant for growth prediction. Nyholm (1977) has noted that since uptake is rarely growth-rate controlling in transient conditions, any approximation of uptake has little effect on simulation of growth. Once $Q \geq Q_m'$ we can assume that uptake is at least equal to $\mu Q_m'$ and will continue to be so until uptake is controlled by S (nutrient in solution). When, through growth, Q becomes smaller than $Q_m'$, Q takes over control of growth. Thus, for growth prediction, the value of $Q_m'$ should be known.

For uptake prediction, the value of $Q_m$ needs to be known. $Q_m$ is especially important in growth prediction when there are two or more species competing for the available nutrient (Stewart and Levin, 1973). $Q_m$ represents excess storage to be used in the future - a species with a smaller $Q_m$ or slower uptake mechanism has a disadvantage.

Another complication in the description of the uptake mechanism is the effect light has on uptake. Whether or not the energy necessary for uptake is provided directly from photosynthesis is an unresolved question. Some workers have shown enhanced nutrient uptake in light (Nitrate, nitrite, or ammonia by Caperon and Zieman, 1976; Dugdale and Goering, 1967; Eppley and Coatsworth, 1968; Grant and Turner, 1969; and, Hattori, 1962. Silicate by Guillard *et al.*, 1973. Phosphorus by Healey, 1973; Kuenzler, 1970; Kuenzler and Ketchum, 1962; and, Kuhl, 1962) while other studies have shown uptake at night or no difference (Nitrate by Laws and Caperon, 1976; and, Caperon, 1968. Phosphorus by Schneider and Frischkneckt, 1977; and, Taft *et al.*, 1975).

It seems reasonable to assume that energy bound by photosynthesis is used to maintain the uptake mechanism; however, in a nutrient-saturated cell, the difference between light and dark uptake may be minimal. Also, under certain conditions, uptake may be related to a periodic rate of cell division and thus not directly related to presence or absence of light (as long as the necessary energy is available).

There is a belief by some workers (Davis et al., 1978) that they have described uptake/growth mechanisms in terms of cellular biochemistry. I think that they are mistaken - the biochemistry in their studies is usually based on the simple Michaelis-Menten expression for substrate-limited product formation and internal pool (or pools) of nutrients. Very little work has been done (Rhee, 1973 is one exception) on the biochemical aspects of algal growth and uptake - the pathways, reactions and products relevant to growth and uptake. Since most mathematical models are empirical, it seems pointless to argue which model is the most pure or mechanistic description of the processes. A conceptual description of the uptake process should be studied and agreed upon. Once a framework has been determined, then a mathematical description can be chosen. For a physiologist or an ecologist, a biochemical model might be too complicated and incorporate too much precision - a simpler description is more useful, especially when experimental design and standard chemical analysis can only provide "rough" observations of the physiological state of the algae.

4. Chlorophyll-a/cell

Algae adjust or adapt to low-light levels by increasing the amount of chlorophyll-a per cell (chl-a/cell) which results in an increase in photosynthesis per unit light (and sometimes per cell) but a decrease in photosynthesis per unit chlorophyll (Beardall and Morris, 1976; and Steemann Nielsen and Jørgensen, 1968). Chl-a/cell may also be adjusted in response to changes in growth rate and to changes in cell-quota of certain nutrients. The above information does not help to explain the changes in chl-a/cell that were observed in the cultures in this study.

It is generally accepted that chl-a/cell decreases with nutrient limitation (Healey, 1973); however, chl-a/cell does not decrease in the same manner for all nutrients. Chl-a/cell decreases when there is a deficiency in cellular nitrogen, phosphorus or carbon (Davis, 1976 and Sakshaug and Holm-Hansen, 1977) but phosphorus-limited cells have a higher concentration of chl-a than do nitrogen-limited cells (Rhee, 1978). Thus, chlorophyll-a/cell is dependent upon the extent of nutrient limitation and is probably affected by more than one nutrient. If, for example, both nitrogen and phosphorus were deficient to the same degree (their respective values of $Q/k_Q$ were nearly equal) then the amount of chl-a/cell might be more severely affected than if only one of the nutrients were deficient. Conversely, if the physiological state of the algae is ideal and constant, such as during the exponential phase of growth, chl-a/cell remains constant and might be used as reliable measures of biomass (Caperon and Meyer, 1972a;

and Sakshaug and Holm-Hansen, 1977).

Since chl-a/cell is a function of the light environment and the nutrient status, there is not a direct method of using chl-a/cell as a simple indicator of the physiological state of the cell or of environmental conditions. It is also impossible to predict the photosynthetic potential of an algal population or community by measuring chl-a/biomass - when a population adapts to a different light environment, the photosynthetic mechanism saturates at different light levels (Beardall and Morris, 1976; Coombs et al., 1967; and Yentsh and Lee, 1966).

Algal physiologists need to determine the relationships between irradiance and chl-a/cell and between the cell-quotas of different controlling nutrients and chl-a/cell. My view is that continuous-culture experiments would be more useful than batch cultures in determining these interrelationships. Once the general relationships are known, batch-culture experiments could be designed to determine the dynamics of changes of chl-a/cell.

The manner in which irradiance is measured might be an important factor when interpreting light/chl-a relationships - light can be measured as incident or as transmitted. Eppley and Dyer (1965) measured transmitted light and found that chl-a/cell remained constant over a range of irradiances. The incident light was constant in their experiments but they assumed that by allowing cell density to change they would change the light environment (a common assumption when designing turbidostat experiments). Photosynthesis/cell might decrease as transmitted light de-

creases, but algae seem to adjust chl-a/cell in response to the level of incident light (obviously it is desirable to keep cell density low, as in this study, and provide adequate stirring) and the use of filters to change irradiance might be a better alternative to varying cell density.

Chl-a/cell remained fairly constant during the exponential (light-controlled) phases of all experiments. It seems reasonable to assume that during the exponential phases the cells maintained a chlorophyll level that allowed for optimum functioning of the photosynthesis and growth mechanisms.

The chl-a/cell from the phosphorus-controlled phases of the experiments might be interpreted in terms of the two controlling factors - light and phosphorus cell-quota, depending on whether or not photosynthesis was in excess of needs. At the higher light levels of experiments 1 through 4, photosynthesis was presumably in excess of needs since carbon fixation had maintained a growth rate of $\mu_m$ (during the exponential phase) - a rate which was greater than any $\mu$ during the phosphorus-controlled phase. A decrease in the amount of chl-a could thus occur without affecting the growth rate. A certain amount of energy and resources are necessary to maintain and produce chl-a. Once phosphorus controls the growth rate, a decrease in chl-a (and, presumably, carbon fixation) would not affect the growth rate - there is no "need" for the optimal rate of carbon fixation. Thus, during the phosphorus-controlled phases at the higher light levels, only phosphorus cell-

quota controlled the rate of growth.

During the phosphorus-controlled phases of experiments 7 and 8, both irradiance and phosphorus cell-quota were affecting the growth rate. It is possible that the cell responded to the decreasing rate of growth by increasing the level of chl-a/cell to increase (or simply to maintain, or to slow down the decrease of the) growth rate by making more efficient use of the photosynthetic mechanism. The "strategy" at lower light levels, at the onset of P-control, might be to increase chl-a/cell - carbon fixation is below saturation levels. The efficiency of the photosynthetic mechanism might decrease (carbon fixation/chl-a might decrease relative to the "optimal" efficiency of the exponential phase) but the efficiency of growth might benefit (growth rate relative to light and phosphorus).

Experiments 5 and 6 were in the middle region, bordering the two sets of conditions, and chl-a/cell did not appreciably change between the exponential and nutrient-controlled phases.

5. Effects of light/dark cycle

The model presented in this study does not satisfactorily describe the dynamics of algae within a daily time scale when there is a diurnal cycle of light. This problem would be further complicated if irradiance was to change constantly - such as under natural conditions. The complications which arise from a diurnal and transient-light environment can only be studied in terms of transient or cyclical behavior and is especially important to phytoplankton ecologists.

It has been observed in this study that phosphorus uptake was associated with cell division - probably after cell division as the cells increased in size. Another observation was that chlorophyll was synthesized only in the presence of light. Other light/dark experiments with Pavlova (Monochrysis) have shown that cell-quota changed systematically throughout the cycle (Laws and Caperon, 1976) and that cell division took place during specific periods (Caperon and Ziemann, 1976 observed cell division taking place just after the light went out - similar to results in this study).

General experiments have been carried out to study the effects of daylength and irradiance on growth rate (Jitts et al., 1964; Tamiya et al., 1955; and Eppley and Renger, 1974). Holt and Smayda (1974) have studied how algae adapt to optimum combinations of daylength and irradiance. Still very little is known about the cellular mechanisms that adjust to changes in daylength - there is no information that is useful in terms of a model.

The relationship between cell-size and growth rate may also be important in a diurnally varying photic environment. As with chlorophyll, the cell size/growth rate relationship is not obvious and is probably interrelated with other variables, such as irradiance, cell-quota, and nutrient uptake (Sorokin and Krauss, 1962; and Stross et al., 1973). Durbin (1977) reports (but does not interpret) observations on the cell size/growth rate relationship for a diatom. The clarification of this relationship will most likely result from batch-culture experiments rather than chemostats, wherein the cell sizes are fairly constant. A few batch-culture experiments and a series of periodic chemostats (chemostats maintained in diurnal-light, as in Fritsch and Gotham, 1979) can provide a large amount of data which could be analyzed by time-series analysis to determine if changes in cell size are periodic and if they are correlated to nutrient uptake and cell division. The relationship between cell size (cell volume or possibly carbon/cell) and growth rate (rate of change in cell numbers) probably has a form similar to that of the cell-quota model. Cell size is likely to increase as a function of uptake (and possibly photosynthesis) and decrease as a direct function of growth. Growth rate (of cell numbers) is thus likely to be a composite function of cell size and nutrient status.

6. Comparison with previous work

Some of the results from the batch-culture experiments can be compared to the results of other experiments with Pavlova (Monochrysis), especially to Droop's (1974) chemostat results. Droop (1975) later used the values of the parameters in his growth model, derived from the chemostat experiments, to show that the model could describe growth in batch cultures. Thus, a comparison with Droop's work can only be made with his chemostat results from which his parameter values were derived, and not with his batch culture results. Differences between the results in this study and those of Droop's (1974), however, might not be due to differences between chemostat and batch culture. Our algal strains, media, glassware, and tubing were the same; however, I used different lights, and our radiochemical techniques were different.

The two, maximum growth rates, $\mu_m$ and $\mu'_m$, taken from the experiments at optimum irradiance (experiments 3 and 4) are very similar to Droop's estimates and are given in Table 13. This indicates that the light levels for Droop's chemostat experiment were near the optimum level. The estimate of $\mu_m$ is similar to estimates by Caperon and Ziemann (1976 - .94 $day^{-1}$) and Laws and Caperon (1976 - .94 $day^{-1}$), but is lower than the estimate obtained by Sakshaug and Holm-Hansen (1977 - 1.3 $day^{-1}$), whose cultures were grown in full strength sea water.

In Table 13, there are large differences (between columns) in the estimates for $k_Q$ and $S_o$, which may be due to systematic differences in our methods of radiochemical

Table 13. Values of various growth parameters estimated from chemostat and batch-culture data.

| | Chemostat (Droop, 1974) | Batch (this study) |
|---|---|---|
| $\mu_m$ $(day^{-1})$ | .9 | .978 ± .029 |
| $\mu_m'$ $(day^{-1})$ | 1.18 | 1.18 ± .136 |
| $S_Q$ $(nM\ P\ ml^{-1})$ | .004 | .069 ± .016 (range = .018 - .122) |
| $K_Q$ $(nM\ P(10^6\ cells)^{-1})$ | .37 | .595 ± .023 |
| $Q_m'$ $(nM\ P(10^6\ cells)^{-1})$ | 1.56 | 1.72 ± .070 (3.46 ± .214) |
| $Q_m'/k_Q$ (dimensionless) | 4.21 | 2.89 ± .074 (5.82 ± .213) |

measurement, culture conditions, methods of estimation, or a combination of factors. Droop's lower estimate of $k_Q$ is associated with higher cell counts (up to $3.4 \times 10^6$ cells/ml in Droop's chemostats at low growth rate and up to $2.3 \times 10^6$ cells/ml at the end of my experiments) - as would be expected if neither of our radiochemical measurements were biased (a lower subsistence quota should result in more cells). The difference between our estimates of $k_Q$ are significant as the estimates are over two standard errors apart (the standard error is approximately .05 nM/ml). Droop (1975) also observed in his batch-culture experiments a lower subsistence quota than mine - indicating that the difference between our results are due to differences in our techniques of radiochemical measurement or culture conditions (medium, glassware, mixing, etc.).

The extremely low estimate of $S_o$ from the chemostat cultures is only 5% of the average value obtained during the stationary phase in my batch cultures (Table 10). It should be noted that the chemostat estimate (.004 nM/ml) was extrapolated from data with a range of .3 - .03 nM/ml. A comparison of the mean value of $S_o$ in this study and the lowest value of S measured in Droop's (1975) phosphorus-limited batch cultures, .125 nM/ml, are reasonably similar. Droop (1975) explained the high levels of dissolved nutrients in the stationary phase (high apparent $S_o$) of his batch cultures by postulating that the remaining dissolved phosphorus was not available for uptake - it was possibly a bound, organic form of phosphorus, or a product of excretion or sequestration.

In Droop's batch cultures, the dissolved phosphorus started increasing after 20 days, which he explained in terms of cell death and lysis. The explanation is reasonable as the cultures were in the stationary phase for up to three weeks and cell numbers were decreasing. Only in experiment 7 of my work did the dissolved nutrient increase after growth had ceased. This experiment lasted over one month, and death and lysis may thus be the explanation for the increase in dissolved phosphorus. In this experiment, as opposed to Droop's, there was no decrease in cell counts - possibly because the culture was well-mixed and dead cells were kept in suspension. The death and lysis explanation is, for another reason, not completely satisfactory because if an increasing proportion of the cells were dead or dying, a decrease in the acid ratio of the chlorophyll measurements should have been, but was not observed.

The complete form of Droop's (1974 and 1975) model has a pool of bound nutrient which increases over time as a function of biomass. If the bound nutrient increases as a function of biomass and it remains bound throughout the experiment so that the pool can only increase, then why are there differences between the observed $S_o$ for my replicate experiments, and what is the mechanism which allows different experiments to have a high or low $S_o$? The existence of a bound form of phosphorus has been postulated to explain certain observations, but there is no direct evidence of such a pool (Droop, personal communication). It should be remembered that a phosphorus binding factor was a tentative suggestion (Droop, 1974) and its existence

was postulated by analogy with the vitamin $B_{12}$ binding factor, whose existence is well documented.

The estimate of $Q_m'$ given in column two of Table 13 was calculated by taking the average of the estimates of $Q_m'$ for each experiment (the individual estimates were given in an earlier section). This gives an estimate of $Q_m'$ = 1.72 nM/$10^6$ cells, and thus $Q_m'/k_Q$ = 2.89. $Q_m'$ can also be calculated by substituting the definition that $\mu = \mu_m$ when $Q = Q_m'$ into equation 5 to obtain equation 13a, thus:

$$\mu_m = \mu_m' \quad (1 - k_Q/Q_m'),$$

which can be rewritten:

$$Q_m'/k_Q = \frac{1.18}{1.18 - .978} = 5.82$$

and therefore, $Q_m'$ = 3.46. This estimate of $Q_m'$ is given in brackets in Table 13, next to the value calculated from the mean of the observed values of Q.

The two estimates of $Q_m'$ which were estimated from the batch culture are significantly different. I interpret this as an indication that the mathematical derivation of $Q_m'/k_Q$ from growth rates ($\mu_m'$ and $\mu_m$) may be a use that exceeds the rigor allowable with what is, after all, an empirical model (Droop, 1977). The discrepancy between observed and estimated values of $Q_m'$ indicates that the model provides poor predictions at high cell quotas. A comparison between the chemostat and batch-culture estimates of $Q_m'$ and $Q_m'/k_Q$ may not be meaningful since the chemostat estimates were obtained by extrapolating to theoretical values (based on the model), while the batch-culture estimates were obtained from direct observations.

It is difficult to determine the meaning of the differences and similarities between the chemostat and batch culture cell-quota parameters. The results are inconclusive - they may be coincidental, there may be a systematic measurement bias, or there may be differences in the way the algae behave in chemostat and batch culture.

B. Techniques for Culturing Algae and Analysis of Data

1. Precautions to be taken with batch cultures

Batch cultures are not commonly used in experimental studies of algal physiology, because of the difficulties in controlling both the experimental conditions and the prehistory of the algae to be cultured. This study has shown that if adequate precautions are taken in the design of the experiments the results are reproducible. Also, that if the physical and chemical environment are closely controlled, the behavior of the algae may be rigorously analyzed. Some aspects of the design of the experiments in this study that might have been helpful in obtaining useful and reproducible results are restated in the following list:

1) The cultures were mechanically stirred (rather than relying on the turbulence from the air supply), keeping the cultures well-mixed and preventing settling.

2) The concentration of the limiting nutrient (phosphorus) was chosen to produce a final biomass that would not be affected by self-shading, nor by availability of $CO_2$.

3) The amount of the initial inoculum was chosen to allow at least five doublings of cell numbers, ensuring that the growth phases of each experiment lasted long enough to provide an adequate amount of data for analysis of results.

4) The small amount of culture taken at each sampling minimized the effects of decreasing culture volume. Larger sampling volumes would have necessitated using a multiple-flask experiment, wherein a whole vessel is harvested for each sampling (Daley and Brown, 1973).

5) Small sample volumes were easily and quickly filtered under a low vacuum, minimizing damage to cells.

6) The batch-culture medium was the same as the supply medium to the chemostats, thus minimizing the shock of transfer.

7) The first six of the eight experiments were inoculated with algae that had been grown in nearly the same physical and chemical, steady-state environment (the chemostats), assuring that the 'prehistory' of each inoculum was similar for these experiments.

8) Pavlova is almost an ideal alga for experimentation. It is robust and, being single celled, it can be precisely counted.

In general the experiments were designed to meet the control-criterion described by Myers (1962) and Spencer (1954): in batch cultures with adequate supply of $CO_2$ and nutrients, illumination governs growth rate until a nutrient is depleted. Experiments by Spencer (1954) show that $CO_2$ did not become limiting until the cell concentrations of Nitzschia (an alga much larger than Pavlova) was almost an order of magnitude larger than the cell concentration in this study ($15 \times 10^6$ cells vs $2 \times 10^6$ cells).

Rodhe (in press) made the general statement that pretreatment of a culture is important when results from different experiments are to be compared. Other studies (Schneider and Frischnecht, 1977; and Tilman and Kilham, 1976) have shown that well-defined physiological conditions are important when measuring phosphorus-uptake rates. Experiments by Daley and Brown (1973) also indicate that reproducible results are possible when culture conditions are closely controlled and pretreatment is standardized, and their study also lends support to the findings of this study that, when the initial conditions of the physiological state of the algae (e.g. cell quota) can be measured, rates of growth from various experiments can be compared. The last two experiments in this study were inoculated with a culture that had an undefined prehistory and the

results were satisfactory once the algae began exponential growth. Prehistory of a culture may be a significant factor only when studying initial uptake, uptake in general, or length of the lag phase. The important point is that the experimental design was such that all nutrients but one were in excess and remained so. Thus the selected nutrient eventually came into control of growth, so the parameters of nutrient-controlled growth could be estimated.

2. Batch vs continuous culture

There is a prevailing opinion among algal physiologists that continuous-culture techniques are the only means for determining growth/nutrient relationships of algae (Ahlgren, 1977; and Soeder et al., 1971). Part of this opinion is based on the belief that only continuous cultures are able to provide reproducible results (Myers, 1962). Jannasch (1974) stated that batch cultures have an advantage in that the culture system is simplistic as compared to an apparatus for continuous culture, but he also pointed out that the ever changing growth environment in a batch culture is a disadvantage. In his article discussing the terminology of steady-state vs continuous culture, Jannasch (1974) argued that a chemostat has a disadvantage as an experimental technique in that it does not reproduce a natural habitat. He further pointed out that batch and chemostat cultures represent two extremes of artificial culture and that a natural habitat has some characteristics of both culture systems.

This study has shown that results from batch-culture experiments are reproducible and the "ever changing growth environment" can be measured and analyzed in terms of a general model of nutrient-controlled algal growth. Continuous culture has become the standard experimental tool of algal physiologists and what has been learned from chemostat experiments is often claimed to be applicable to transient situations. This is especially true in ecosystem modelling, where growth relationships and parameter estimates from chemostats are applied to simulate transient

systems (Grenney et al., 1973a) Lehman et al., 1975; O'Brien, 1974; and Hornberger et al., 1975).

The results from the experiments in this study indicate the model for nutrient uptake, which is satisfactory for chemostats, is inadequate as a description of the uptake dynamics observed in a batch culture. This discrepancy may be due to the nature of chemostat experiments in which rates are not directly measured but are calculated by deduction - assuming a steady-state: in a chemostat, the cell quota is constant, so

$$\frac{dQ}{dt} = 0 = u - \mu Q,$$

and therefore,

$$u = \mu Q = DQ.$$

The information gained from the above analysis may not be appropriate as a description of transient behavior. Only in the long run might uptake rates average out to the values predicted by equation 10 - as Droop (1975) demonstrated. In a batch culture, as demonstrated by this study, uptake rates are likely to change continuously and simple, steady-state kinetics (as derived from a chemostat) are not appropriate.

There are possibly other examples of interpretations of chemostat results that are not applicable to dynamic systems. Batch-culture experiments should be carried out to determine whether a relationship which describes chemostat data is a general one which may be applied to batch-culture data. The important point is that batch cultures are useful and, in some situations, should be considered as an alternative to continuous culture.

3. Perturbation experiments

Caperon and Meyer (1972b) were among the first investigators to make use of an experimental technique called a perturbation experiment. Such an experiment usually takes the form of interrupting a chemostat or batch culture in one of several ways:

1) Stop the dilution of a chemostat, thus initiating a batch culture,

2) Perturb a chemostat through the spike addition of added nutrient and continue dilution,

3) Same as 2, except the dilution is stopped and a batch culture is initiated,

4) Perturb a batch culture through the spike addition of nutrient,

5) Change the dilution rate or the rate (amount) of supply of nutrient.

These types of experiments have become a common addition to studies with algal cultures (Harrison and Davis, 1977; Conway et al., 1976; Nyholm, 1977; Conway and Harrison, 1977; and Cloern, 1977). The advantage of the perturbation experiment is that a culture with a controlled prehistory is forced into a transient mode, providing data otherwise unobtainable from an experiment under its original conditions.

Caperon and Meyer (1972b) discussed this type of experiment especially in relation to the study of uptake kinetics and there is some disagreement as to the degree of perturbation necessary to provide the most useful data set. Koya and Humphrey (1967) discussed some of the theoretical aspects of the behavior of a perturbed chemostat system. Although this technique has many advantages, some results from perturbation experiments demonstrate

that care must be taken when interpreting data through the use of mathematical models.

When a culture is perturbed, the first observable response by the algae is a rapid uptake of nutrient. As a direct consequence of the addition of nutrient to the supply already in the cell, the growth rate increases (there may be a lag period depending on the physiological state of the cells and the degree of perturbation) in a manner that can be predicted by a cell-quota model. Perturbation experiments are therefore very useful for studying the dynamics of uptake and some investigators (Conway and Harrison, 1977; Nyholm, 1977; and Conway et al., 1976) have performed such experiments demonstrating the response which has been described and modelled by Droop (1977, for a summary). The uptake response (this has been discussed in section V. A. 3) has been divided into three types: (1) an initial surge if a nutrient pool is partially or totally empty; (2) uptake internally controlled by growth rate or by the uptake of a controlling nutrient - in the latter case, one nutrient is in control and the other is taken up as "luxury"; and, (3) externally controlled by the concentration of the nutrient in solution (Conway et al., 1976; and Cloern, 1977). This qualitative approach might be a necessary step back from attempting to describe all aspects of uptake in terms of a rigorous model.

There are several problems that may affect the interpretation of the uptake response in perturbation experiments. Prehistory of the culture might be important

especially if the algae have been starved and their "cellular machinery" has adjusted to a nutrient-poor environment. Thus the surge of initial uptake might be a combination of adsorption and readjustment of the cells' uptake system to a new external environment - in this case uptake or absorption is not being observed. Also, if the degree of perturbation is not large enough to saturate all adsorption/absorption sites (these sites are just a useful concept, but one which has not been fully described or quantified), the initial surge is affected by the external concentration of nutrient and the rate of surge uptake is less than the maximum.

4. Parameter estimation

Few difficulties were encountered in the analysis of the batch-culture data. It was possible not only to fit models to data, but also to use the values of the parameters in one model to test a hypothesis. Thus, contrary to some earlier suggestions (Fogg, 1965; and Harrison et al. , 1977), batch-culture data can be analyzed in a quantitative manner with no loss in mathematical rigor, such as would result from finite-difference methods.

Bard's technique of nonlinear parameter estimation (1967b) was previously shown to work with similar equations analogous to those in the present study and simulated data (Lederman et al., 1976), but the technique was never applied to real data. One basis for choosing an estimation procedure is that, when it is known that the model is correct, the estimated values of the parameters should be consistent between simulated replicate experiments (Lederman, 1974). Conversely, if the estimation technique is capable of fitting a given model to simulated data, then consistent results with real data add credibility to the model. Although it can never be proven that a theoretical model is the "correct" model, it has here been demonstrated that consistent estimates can be obtained - thus these results show that batch cultures are reproducible and the data can be analyzed with a stable estimation procedure using nonlinear differential equations.

It is impossible to determine whether a model is an exact representation of a set of data because there are "errors" in the data - some of the errors are experimental

or due to measurement, but others are due to natural fluctuations in all data sets. An equation (or model) can be selected to give any desired shape of a rectangular hyperbola, and a model can thus be chosen so that its predictions will describe the general properties of a data set.

Jassby and Platt (1976) examined a set of models with similar properties. A similar study was carried out by Nyholm (1977). In both of their studies, the models were fit to real data sets. These two studies are good examples of the problems that are associated with the interactions between estimation procedures, models, and choosing the best model. It can be shown for each study that the models are so similar that it would be impossible to choose one model as the best (Lederman and Tett, unpublished manuscript presented in Appendix VI).

Another problem that is often ignored in many studies which attempt to formulate models of the uptake and growth processes of algae is that a data set is interpreted in terms of one explanation only (only one concept or model is used to describe an observed response). There are two basic growth models: one which describes uptake as growth - this model relates growth to external nutrient concentration and attempts to determine a value of $K_s$ (concentration of nutrient in solution) for growth; and, the other which separates uptake and growth into two processes which are interrelated - the cell-quota model. The results in Davis (1976) were first interpreted in terms of an uptake model, but his conclusions would have been different if he had

based his interpretation on a cell-quota model. Davis did reinterpret his results in a later paper (Davis et al., 1978), however, he did not reevaluate his conclusions.

An unfortunate aspect of not considering various models is that the discussion of the relative worth of various models is then based on subjective evaluations or separate investigations. When a relatively complicated model (Davis et al., 1978) was proposed as the best description of a data set, it was difficult for other investigators to determine whether an alternative model was indeed a poorer description of the data. Droop (1978) argued that the model proposed by Davis et al. (1978) was unnecessarily complex and that a two-pool, cell-quota model would be difficult to parameterize. Davis and Harrison (1978) replied with an argument based on their conceptualization and said that since their model described the data, it was both correct and better. This disagreement could have been solved easily and objectively by fitting both models to the data and determining which model provided the better fit to the data.

It has been demonstrated in this study that models can be objectively compared when using the same data set and the same estimation technique. For example, Droop's model was chosen over Caperon's because the estimated values of the parameters in Caperon's model were not consistent for the different experiments. The goodness of fit of the two models was not significantly different, even though Caperon's model has the advantage of an extra parameter. In another example of model-comparison, the fit of several models to the maximum growth rate vs irra-

diance data was studied in order to choose a model that was both useful and simple (Appendix V).

In all attempts to discriminate between models that describe the same phenomenon, it has been shown that different models can give predictions that are so similar that it is not possible to choose the best model only on the basis of goodness of fit (smallest sum of squared error). The choice of the best model amongst similar models must often be based on other criteria, such as tradition or simplicity.

## C. Models of Nutrient-Controlled Growth

### 1. The cell-quota model

This study demonstrates the versatility of the cell-quota model, formalized by Droop (1968), as a description of transient growth of nutrient-controlled algae. The portion of the model that predicts growth rate as a function of the cell quota was shown to be reliable (compare the curves predicted by the model with data in Figures 6-13) even though the uptake dynamics could not be precisely described. Positive and negative uptake rates were observed, making it impossible to describe uptake as a simple, continuous function of external concentration of nutrient. The erratic uptake caused the values of Q to fluctuate, but the model still worked because the fluctuations were in effect averaged in the interpretation of the data and treated merely as part of the statistical error distribution of Q's. In justification of this treatment it can be assumed that the cell responds to either the average cell-quota or a separate phosphorus pool that is a function of the average total particulate phosphorus.

The observation that growth rate is independent of medium nutrient concentration was first made by Ketchum (1939). The concepts of a cell reservoir of nutrient, uptake and growth being separate processes, and a subsistence level of nutrient/cell were presented by Mackereth (1953) and later by Gerloff and Skoog (1954), who also discussed the concept of luxury consumption. Other workers noted the importance of cellular stores of nutrient in relation to growth rate (Fitzgerald and Nelson, 1966; Kuenzler

and Ketchum, 1962; and Price and Carell, 1964). Droop (1968) formalized the cell-quota model with a mathematical expression, and later developed a comprehensive model of nutrient-controlled growth (see Droop 1977 for a review). The model has been shown to be correct for several nutrients and a variety of species of algae (Caperon, 1968 and 1969; Cunningham, 1976; Fuhs, 1969; Nyholm, 1975, 1976 and 1977; Paasche, 1973a and b; and Rhee, 1973, 1974 and 1978). Nonetheless, many recent studies, influenced by the original and important work of Dugdale (1967), still interpret nutrient-controlled culture experiments in terms of an uptake model, where growth rate is a function of nutrient in solution (e.g. Kilham, 1975; Davis, 1976; Tilman and Kilham, 1976; and Conway and Harrison, 1977).

Once the cell-quota model is accepted as a better description of nutrient-controlled algal growth, the more complicated process of nutrient uptake can be studied by designing experiments that can be interpreted in a manner that does not confuse uptake with growth rate. A simple and general model of the growth and uptake dynamics of algae is especially important to ecologists who rely on information obtained from physiological studies.

2. Application of growth models

A very common application of algal growth models is in the field of ecological modelling. Most ecological models are constructed in terms of nutrient or energy flows, and a basic aspect of all of the models is that nutrients control the rates of most processes (Chen, 1970; Dugdale, 1967; Grenney et al., 1973b; and Prober et al., 1972). Lehman et al. (1975) presented a comprehensive model of algal growth and production by synthesizing most of the knowledge obtained from laboratory experiments. Ecological modellers often need more information than has been made available through laboratory experiments.

Since the natural environment is complicated and several nutrients or conditions can be near the levels at which they control growth rate, many models for primary production of algal growth are formulated with terms that predict a multiplicative interaction (Chen, 1970; Lehman et al., 1975; Male, 1973; and Middlebrooks and Porcella, 1971). Ahlgren (1977) supports the use of multiplicative terms for several limiting factors by referring to work by Baule (1917). The multiplicative description of nutrient interaction seems to be the predominant concept (Beinfang and Gunderson, 1977; and Rodhe, in press), as opposed to a threshold explanation (only one nutrient at a time controls growth rate).

Lehman et al. (1975), who proposed a multiplicative model, pointed out that a multiplicative interaction results in an extremely low prediction of growth rate when the supply or two or more nutrients are very low. Rhee (1978) demonstrates the difference between a multiplicative

and a threshold prediction, and his work (Rhee, 1974 and 1978) and Droop's (1974) are the only experiments studying "multiple" limitation. Both Droop and Rhee have shown that there is a threshold interaction between nutrients - only one nutrient at a time controls growth rate.

The results from the batch-culture experiments in this study demonstrate that the interaction between light and a nutrient, phosphorus, is multiplicative. It can be postulated that a multiplicative interaction may be expected when substrate availability separately affects the photosynthetic and growth mechanisms. In this case, when one substrate such as light, $CO_2$, temperature, or pH affects the photosynthetic light reaction processes and another substrate such as nitrogen, phosphorus, or silicate affects the dark reaction or other metabolic processes, the result is a reduction of growth rate that is more severe than the reduction due to limitation of either one on its own. This explanation of colimitation should be useful in ecological models and growth prediction. There is a need, however, to perform more laboratory experiments with multiple limitation to determine the combinations of nutrients and conditions that produce a threshold or a multiplicative interaction. The general question concerning multiple limitation of growth is still to be answered: what happens when various combinations of nutrients are controlling growth rate, and under these conditions, what is happening inside the cell?

The cell-quota growth model may be used to describe the growth dynamics of phytoplankton under natural condi-

tions (Tett et al., 1975) and in laboratory microcosms (Jones et al., 1978). Tett et al. (1975) have used the phosphorus/carbon ratio to explain differences in phytoplankton growth rates and chlorophyll status of cells, yet not enough is known to be sure of what factors are causes and what are the affects.

The parameter estimation technique used in this study could be applied to transient ecological data. A problem with ecological experiments is the lack of control over environmental conditions. This problem has been partially solved through the use of in situ enclosures (Blinn et al., 1977; Antia et al., 1963; Gamble et al., 1977; Schelske and Stoermer, 1971; Powers et al., 1972; and Menzel and Case, 1977). Experiments in enclosure could provide transient data - similar to batch-culture data - that could be quantitatively interpreted using Bard's parameter estimation technique.

3. Primary production and growth

There are two areas of algal biology between which no direct link has been made - photosynthesis and algal growth. These areas are of fundamental interest to both algal physiologists and production ecologists. In order to model the processes of photosynthesis and growth, it is necessary to determine the relationship between nutrient availability, photosynthesis and growth rate.

Platt et al. (1975) present, in a clear and logical manner, a framework within which photosynthesis, primary production, and growth can be discussed. Their practical approach is concerned with those aspects of photosynthesis and growth that are most important in nature, namely, photosynthesis relative to light and chlorophyll and the effects of nutrients on photosynthesis (by limiting chlorophyll-a, controlling growth rate, or directly effecting photosynthesis).

The simplest definition of primary production is: the increase of biomass measured as particulate carbon. A general equation can be written:

$$\text{Primary production} = \text{net photosynthesis} \times \text{biomass} - \text{excretion} \times \text{biomass}$$

$$\frac{d\,(\text{particulate carbon})}{dt} = \left(\frac{1}{\text{hr}}\right) \times (\text{particulate carbon}) - \left(\frac{1}{\text{hr}}\right) \times (\text{particulate carbon})$$

Net photosynthesis is the difference between gross photosynthesis and respiration - processes related to light, temperature, and nutrient storage. Photosynthesis can also be expressed relative to chlorophyll, but, as mentioned in the discussion of the chlorophyll results, light effects

the level of chl-a/cell and photosynthesis per unit chlorophyll decreases with decreasing light.

Another conceptualization of the relationship between photosynthesis and growth is that photosynthesis provides energy and carbon, and the rate of photosynthesis is a function of light, temperature, and the availability of certain cellular components. Growth rate is a function of nutrients, temperature, and the rate at which carbon (and/or) energy is made available. Thus growth might be controlled either by the net rate of carbon fixation or by the quota of a nutrient; and, a nutrient (other than carbon) might effect cell division without effecting the photosynthetic mechanism. Some nutrients, such as phosphorus, could effect both photosynthesis and growth; however, there is no evidence to suggest that a nutrient which is controlling growth rate is consequently controlling the rate of photosynthesis (Senft, 1978).

The relationship between chl-a/cell and irradiance observed in the batch cultures demonstrates the cells' adaptability to different light levels. The changes in chl-a/cell within an experiment, after the phosphorus quota controlled the growth rate, also demonstrates the effects that the changes in cell-phosphorus have on chl-a/cell; and, the effects of phosphorus control were different at different light levels.

The relationship among chlorophyll/nutrient cell-quota/light intensity/primary production/growth rate are equally important to ecologists not only because of the processes' fundamental importance to production ecology but also because it is easier to deduce production rates from the

easily measured variables, chlorophyll and light than to measure the rates directly. Such information might clarify the differences in primary production that are observed over a period of time at the same or similar sampling locations (such as presented by Jassby and Platt, 1976).

The parameter estimation techniques used in this study would also be useful with models of primary production (the applicability to photosynthesis vs light is discussed in Appendix VI). Experiments could be designed to study the effects of temperature, irradiance, and nutrients, each variable alone and in combinations. These experiments should be carried out with axenic, single-species cultures as the first step in developing models for natural populations.

There are problems associated with modelling primary production of natural phytoplankton because phytoplankton are multispecies assemblages. Williams (1973) questioned the use of a single expression to describe nutrient uptake by an assemblage of several species. He demonstrated that such treatment could result in biased estimates of kinetic parameters. The conclusions of Williams' study apply to modelling of processes other than uptake - such as multispecies growth vs cell quota (measured as particulate phosphorus/total cell count, or the ratio of phosphorus to carbon) or primary production relative to chlorophyll, nutrients, or temperature.

One method of modelling multispecies growth, uptake, or primary production is to model the dynamics of each species in the phytoplankton. The values of the model parameters are necessary for each species. It is impos-

sible, with present chemical techniques, to separate the response of an assemblage into the responses of the individual species (except for cell counts to determine cellular growth rate). Therefore the values of the parameters must be obtained from single-species cultures. If, however, the response of each species in a multispecies experiment could be measured, it is possible, using Bard's (1967b) estimation technique, to fit a set of multispecies equations to batch-culture data (Lederman et al., 1976).

Another method of modelling the dynamics of the phytoplankton is to treat it as an entity and accept the resultant errors in estimation and prediction (the error discussed by Williams, 1973). Such a model is less rigorous than a multispecies model but the phytoplankton model, in many situations, might be the only practical alternative. MacIssac and Dugdale (1969) have used a single expression to describe nutrient uptake by phytoplankton and they obtained a reasonable fit of the model to the data. Lederman et al. (1976) showed that it is possible to fit a single-species model (the phytoplankton is treated as an entity) to simulated, multispecies data.

In a study of phytoplankton photosynthesis vs concentration of inorganic carbon, Caperon and Smith (1978) demonstrate the applicability of a single expression describing the multispecies response. They argue that the parameter that is most likely to be biased due to the single expression treatment, the half-saturation constant for the reaction, is also the parameter that has the largest standard error under the best conditions. Thus, the variation in

the estimate of the half-rate constant due to the single expression treatment is likely to be small relative to biological variation.

There are other problems associated with modelling multispecies dynamics. A set of parameters that characterize the phytoplankton might not provide useful predictions into the future since the species composition is continuously changing. The parameter values would most likely change as different species become dominant. Also, it is possible, that the response of different species might be controlled by separate environmental variables, such as diatoms controlled by silicon, bluegreen algae controlled by phosphorus, and green algae controlled by temperature. These conditions would definitely have to be accounted for and might limit the predictive usefulness of a phytoplankton model.

The relationship between photosynthesis and growth might be studied by monitoring photosynthesis in a batch-culture experiment as carried out in this study. Photosynthesis could be continuously measured in a batch-culture by monitoring oxygen concentration using equipment and techniques as developed by Kelly et al. (1974) for use in rivers. The main difficulty with monitoring $0_2$ concentrations in a batch culture is that the culture is vigorously mixed and air is bubbled through it - there would be a rapid rate of reaeration. Since the stirring and bubbling is relatively constant, it would be possible to calculate the reaeration coefficient and thus solve the equation for $0_2$ production (Hornberger and Kelly, 1972). Net primary

production (measured as net particulate production) could be measured as the change in particulate carbon during the batch experiment. It would then be possible to compare net $O_2$ production (photosynthesis-respiration) to net carbon production. The rates of net production might then be studied relative to environmental or cellular control (irradiance, temperature, or nutrients).

# CONCLUSIONS

The continuous culture of algae, such as the chemostat, has been and will continue to be a very useful method for the study of algal physiology. Some of the strengths of continuous culture may also be weaknesses: the algae might adapt to the experimental conditions, and the steady-state relationships might be too simple. From steady-state observations it is sometimes difficult to determine cause and effect, or to predict the responses of natural, non-steady-state phytoplankton to changing environmental and physiological variables. An alternative to steady-state culture is to study algae in experiments in which certain variables are continuously changing, such as in batch cultures.

There are strong justifications for using batch-culture experiments to develop both conceptual and mathematical models. The dynamics of algal growth cannot be verified with steady-state experiments. The most common application of relationships derived from steady-state cultures is to predict the transient behavior of algae. Thus, if models can be validly used to describe transient behaviour, it should also be possible to formulate and parameterize the models through the use of transient experiments. Batch-culture experiments should not be avoided because little is known about them. This argument is self defeating - batch cultures will not be understood until they are studied.

This study has shown that is is possible to overcome the main difficulties of batch-culture experiments - re-

producibility and the analysis of results. The data from batch cultures were used to parameterize a model of algal growth and the estimated values of the growth parameters were both consistent and reasonable. Thus, with proper experimental design and control, reproducible results were obtained and parameter estimation techniques were used to fit models to batch-culture data. Moreover, it was possible to use the batch-culture results to test a hypothesis and to extend the cell-quota model for algal growth to include irradiance/phosphorus interactions.

The results of the study provide new information on the effects of both light and phosphorus on algal growth. Similar information might have been obtained from chemostats and turbidostats; however, steady-state cultures would have necessitated the use of more equipment and would, most likely, have taken more time. The batch-culture results are more pertinent for use in simulation modelling than would be the results from steady-state cultures, which would still have to be verified under transient conditions.

The batch-culture results point out a weakness in the continuous-culture derived model of phosphorus uptake. The transient-uptake data from the batch-culture experiments demonstrated that the steady-state uptake equation, used in Droop's model (and others) and which describes, a single, one-way process, is not satisfactory - although the equation may work as a long term, "average" description of uptake. The uptake process seems to be complicated and it is not likely that the physiological processes will be described with a simple model. Thus, the ecologist may

have to employ simplified models which "work" because the uptake process is rapid relative to growth, yet, are not accurate as descriptions of physiological processes.

The model of algal growth in equations 1-7 was extended to include the effects of irradiance on exponential (nutrient-saturated) growth and phosphorus-controlled growth. The general growth model successfully described transient growth, even though the uptake portion was not adequately described - this attests to the robustness of the cell-quota model.

By using current models, ecologists attempt to explain natural events, such as algal blooms and species succession, in terms of information obtained from steady-state studies. There is no reason to expect that transient phenomena can be explained by steady-state relationships. It is therefore necessary to study and explain a controlled, dynamic, laboratory experiment in order to better understand and explain natural events. Information on light/phosphorus interactions on growth rate would be useful to ecologists whatever the source, and the fact that transient data were analyzed might prove to be especially pertinent.

A purpose of experimentatation is to provide data for hypothesis testing and the most effective experimentation uses careful design and control to provide answers to simple questions. These answers are the basis for mathematical models that tell us little more than we already know - critical experimentation provides new information and the basis for models.

## SUMMARY

The purpose of this study was to determine the effects of illumination and the cellular concentration (cell-quota) of phosphorus on the growth rate of the alga, Pavlova (= Monochrysis) lutheri Green (Droop). The hypothesis that was tested was that the dynamics of phosphorus-controlled growth are the same at all illuminations. The alternative hypothesis was that there is a combined effect of illumination and phosphorus cell-quota on growth rate. Data were obtained from batch cultures grown at five illuminations, and the values for the parameters in a cell-quota model (Droop 1973) were calculated for each illumination. The values of the parameters, obtained at different illuminations, were compared to determine the effects of illumination on the growth of the algae during the exponential and phosphorus-controlled phases of the cultures.

This study demonstrates that it is possible to use algal batch-culture techniques as part of the experimental design to test a hypothesis. It was possible to quantitatively analyze the transient data and the results were reproducible. Illumination and phosphorus limitation had a combined effect on the growth rate of Pavlova lutheri during the time when phosphorus cell-quota was controlling growth.

In more detail the results were as follows:

1) Reproducible batch-culture experiments were carried out at various illuminations. All the cultures went from a light-controlled, exponential phase to a phosphorus-controlled, transitional phase.

2) The smaller the number of cells in the inoculum taken from a phosphorus-limited chemostat, the larger the value of $Q_m$ (the maximum cell-quota) after the initial uptake (uptake and adsorption). The prehistory of the inoculum is probably important in this relationship.

3) Chlorophyll/cell was higher at lower illuminations. Within an experiment, during the phosphorus-controlled phase, the chlorophyll/cell decreased with time at the two highest light levels and increased with time at the lower light levels.

4) The exponential growth rate ($\mu_m$) increased as a function of illumination and was inhibited at the highest light level.

5) Three different parameter-estimation techniques were used to fit Droop's growth model to the batch-culture data. The preferred technique, which fits the differential form of the equations, has never before been used with algal batch-culture data.

6) The estimates from the batch culture were compared with chemostat estimates from Droop (1974). Maximum growth rates ($\mu_m$ for exponential growth and $\mu_m'$ for phosphorus-controlled growth) were similar, but the batch-culture estimates of subsistence quota and maximum cell-quota ($k_Q$ and $Q_m$) were 50% greater than the chemostat estimates.

7) The differential form of the steady-state uptake equation did not satisfactorily describe the transient uptake data.

8) The hypothesis that nutrient-controlled growth rate is not affected by illumination was rejected. There was also a significant but slight decrease in $k_Q$ with decreasing light.

9) A set of equations can be written to describe the batch-culture, growth dynamics. These equations are based on Droop's cell-quota model, and take into account both illumination and phosphorus quota.

10) One experiment was carried out in a diurnal light cycle of 16 hours on/8 hours off. The growth model was successfully fitted to data spaced at 24 hour intervals. The dynamics within 24 hours were not modelled.

11) In the diurnal-light experiment, the population cell-size changed throughout the daily cycle during both the exponential and phosphorus-controlled phases. During the phosphorus-controlled phase, the growth rate decreased with time and the population approached a constant average cell-size.

12) Light inhibition of growth rate was observed during the light-controlled, exponential phases of the cultures grown at 140 W $m^{-2}$ (measured as photosynthetically active radiation). This illumination was greater than natural irradiances, as the energy from the light source was not equally distributed in the visible region. Instead the light source radiated a spectrum of energy closely matching the absorption spectrum of photosynthetic pigments.

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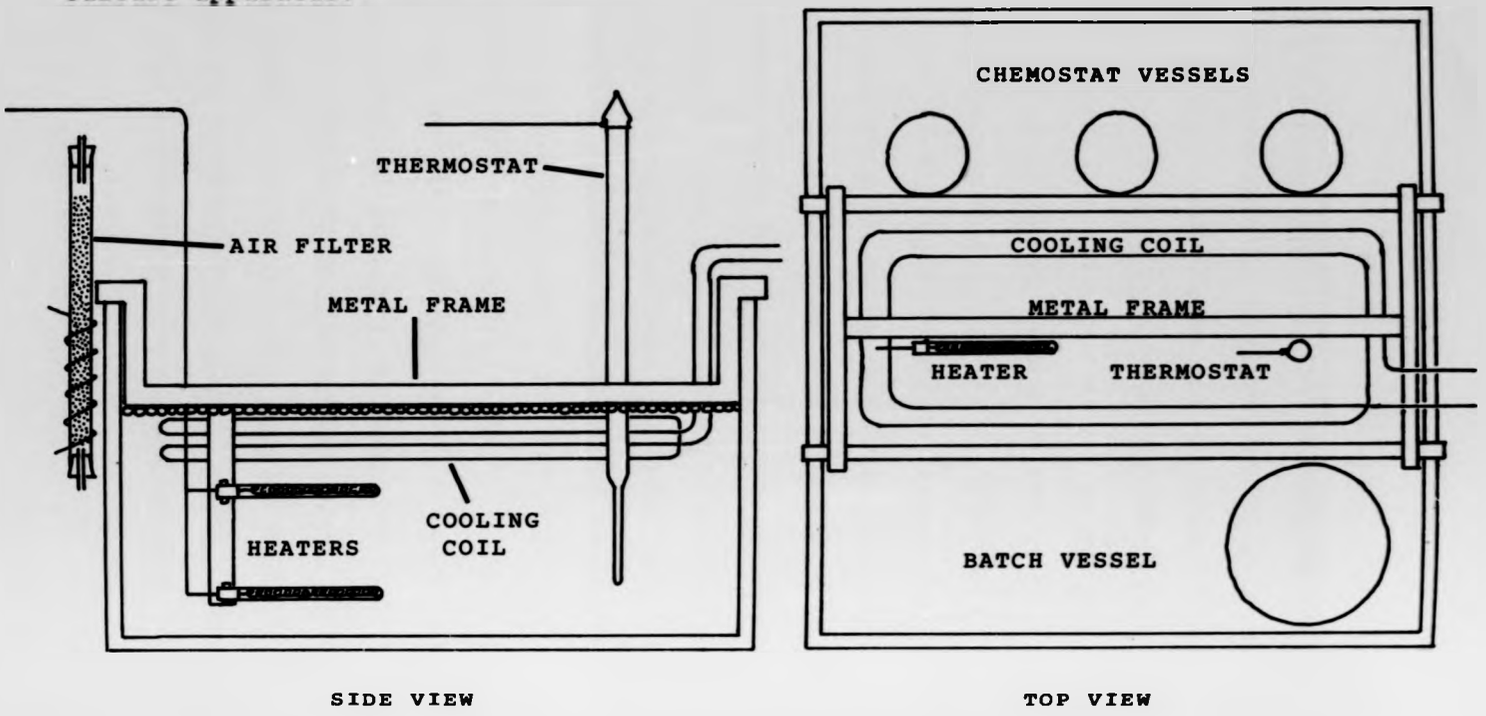
# APPENDICES

# APPENDIX IA

## Water Tank for Culture Apparatus

The cultures were grown in 250 ml or 2 l flasks which were held to a metal frame in a perspex water tank (Figure 28). The tank was constructed of 1 cm thick, clear-perspex sheet with outside dimensions of 62 cm by 62 cm base and walls 36.5 cm high. The tank was filled with distilled water to a level slightly above the shoulders of the culture flasks. It was cooled with tap water continuously running through a submerged copper coil and thermostatically controlled by two 150W submersible heating elements, maintaining a constant temperature of 20.5°C. The two side walls and the bottom were covered on the outside with two layers of reflecting, metalized PVC sheet. Small plastic balls covered the surface of the water, acting as a reflective surface and decreasing evaporation. The top of the water bath was covered with black plastic sheeting to keep out dust and light. For the low light and light-dark cycle experiments the whole apparatus was surrounded in blackout plastic.

Figure 28. The water tank for the culture apparatus.



# APPENDIX IB

## Lights

The cultures were illuminated with two banks of fluorescent lamps placed at opposite sides of the water bath. Two types of 30W 900mm lamps were used, Gro-Lux and Northlight/Colour Matching (Thorn House, Glasgow). Gro-Lux lamps have a spectral distribution of energy that is characterized by a peak between 610 and 675nm and the Northlight lamps have a relatively flat spectral distribution in the visible region (Figure 29). Both types of lamps radiate very little energy in the ultraviolet or infrared regions. Three lamps of each type were mounted alternately at two inch intervals in a concave array (Figure 30). Behind the lamps was a curved mirror made of .41mm thick metalized PVC (Lexel Lamb Ltd., Milton). The lamps were used in pairs and the spectral distributions of the various combinations of lamps were measured. Photosynthetically active radiation (PAR) was measured with a LI-COR Quantum Sensor (LI-190S) (Table 14). It was necessary to decrease the light intensity below that of a pair of lamps per side. This was achieved by placing approximately neutral optical filters on the clear sides of the water tank. 3, 6, and 13 layers of tracing paper resulted in approximately 25, 50, and 95 per cent decreases in irradiance (Figure 31).

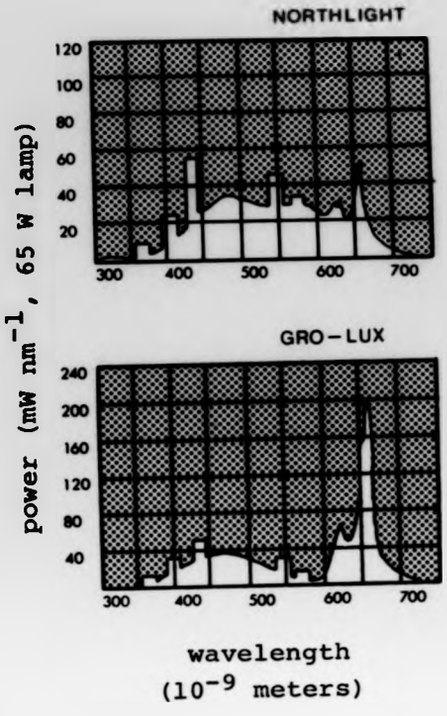



Figure 29. The spectral distribution of energy for the fluorescent lamps. Redrawn from Publ. # TL0070 /9/71 by Thorn Lighting Ltd.

Figure 30. A bank of fluorescent lights.

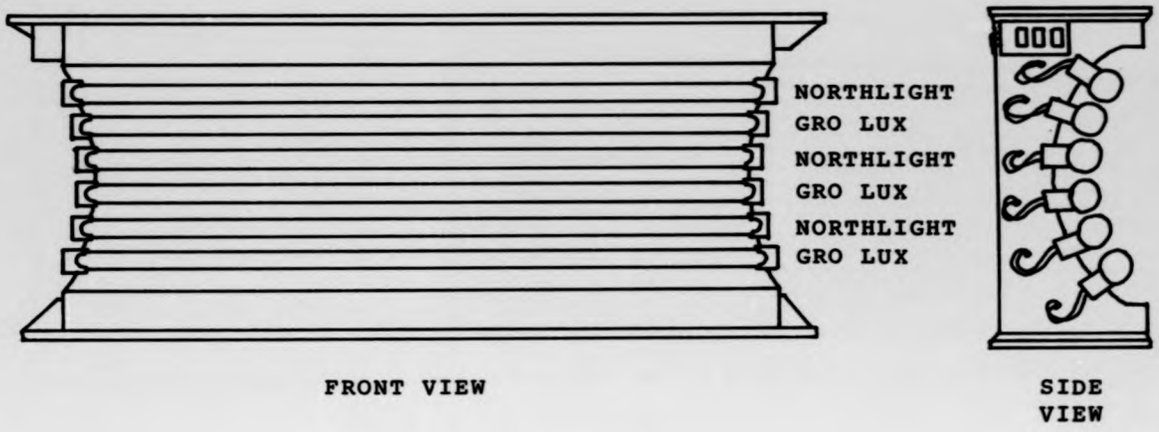


Figure 31. Transmission spectrum of tracing-paper filters.

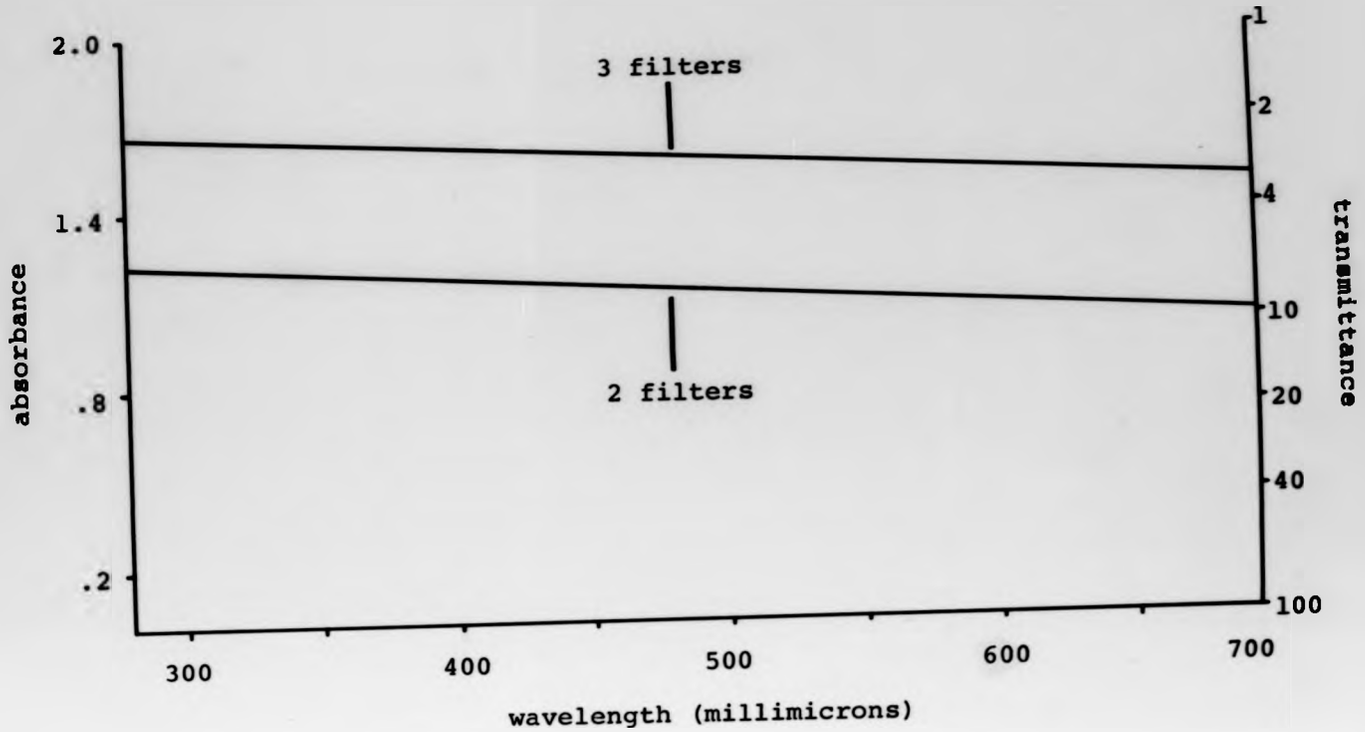


TABLE 14. Measures of photosynthetically active radiation.

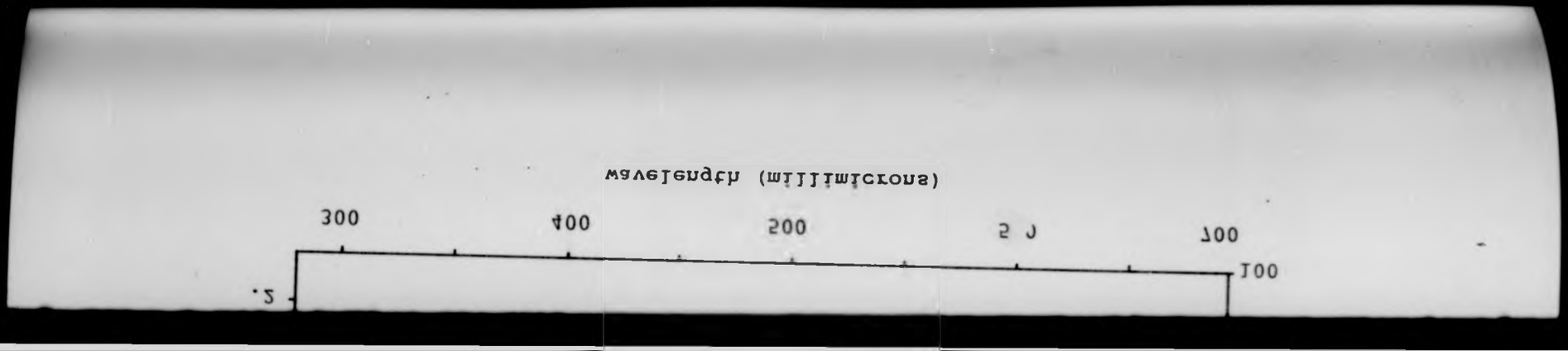
| Experiment Number | Number of filters[1] | Lamps on culture side[2] | Lamps on opposite side[2] | Irradiance culture side ($W\ m^{-2}$) | Irradiance opposite side ($W\ m^{-2}$) | Total Irradiance ($W\ m^{-2}$) |
|---|---|---|---|---|---|---|
| 1 | 0 | 3,4 | 3,4 | 94.00 | 46.53 | 140.53 |
| 2 | 0 | 3,4 | 3,4 | 94.00 | 46.53 | 140.53 |
| 3 | 3 | 1,6 | 1,6 | 45.59 | 18.57 | 64.16 |
| 4 | 3 | 1,6 | 1,6 | 45.59 | 18.57 | 64.16 |
| 5 | 6 | 2,5 | 2,5 | 24.44 | 10.81 | 35.25 |
| 6 | 6 | 2,5 | 2,5 | 24.44 | 10.81 | 35.25 |
| $7_1$ | 13 | 2,5 | 2,5 | 6.35 | 2.58 | 8.93 |
| $7_2$ | 13 | 2,5 | 2,3,4,5 | 7.29 | 4.46 | 11.75 |
| $8_1$ | 13 | 2,3,4,5 | ALL | 14.59 | 6.82 | $21.39 \times \frac{16}{24} = 14.26$[3] |
| $8_2$ | 13 | ALL | ALL | 21.86 | 7.75 | $29.61 \times \frac{16}{24} = 19.74$[3] |

[1] Number of layers of tracing paper on each side of the water bath.

[2] The numbers represent lamps from the top (1) to the bottom (6) of the light array. Even numbered lamps were "Gro-Lux" and odd numbered lamps were "Northlight".

[3] These two values are adjusted to represent a 24 hour day.





# APPENDIX IC

## Chemostat Assembly

The chemostat was made to the design by Droop (1966). The assembly consisted of a one liter, medium supply reservoir, a 250 ml spherical, reactor (culture) vessel, and a one liter, overflow receiver (Figure 32). Air was continuously pumped through the system to provide stirring, nutrient delivery, and aeration. The air was water-saturated by initially pumping it through bottles of distilled water, minimizing evaporation of the culture medium. Then the air passed through a glass tube filled with a cotton wool filter. The glass tube was heated to prevent the cotton wool from clogging. The outlet from the overflow receiver was also filled with cotton wool and was heated. All of the bungs and flexible tubing (3.2mm bore, 1mm wall) were autoclavable silicone rubber (ESCO Rubber Ltd.). The "T" joints were nylon and all the flasks and rigid tubes were glass. One chemostat assembly was held to a frame that stood on top of two walls of the water tank, and the other two assemblies stood on top of one of the light banks. Each of the three flasks in an assembly had an inlet-outlet tube to facilitate inoculation, addition of medium, sampling, and emptying the reservoir. The open ends of the tubes were clamped and submerged in 95% ethanol. An assembly could be easily removed from its stand to be autoclaved as a unit.

The dilution rate of the chemostat resulted from an addition of fresh medium at discrete intervals and could be adjusted in two ways: the amount of medium added at

each interval could be changed by moving the delivery tube up or down, altering "h" in Figure 32; or, the length of the interval between additions of medium could be adjusted. At the end of an interval a timer triggered two solenoid valves (SV) to close the normal passage of air. This increased pressure in the supply reservoir and raised medium into the delivery tube. After five seconds, SV1 opened, pressure equalized, and the medium in the metering tube lowered to the top of "h". Fifteen seconds later, SV2 opened and the delivery tube emptied the dose of medium into the reactor flask. The addition of medium raised the level of medium in the reactor flask, causing it to overflow into the receiver flask. This chemostat was not a continuous-culture apparatus in a strict sense; however, the dosage interval, being 15 minutes, was short enough to make the apparatus an acceptable approximation.

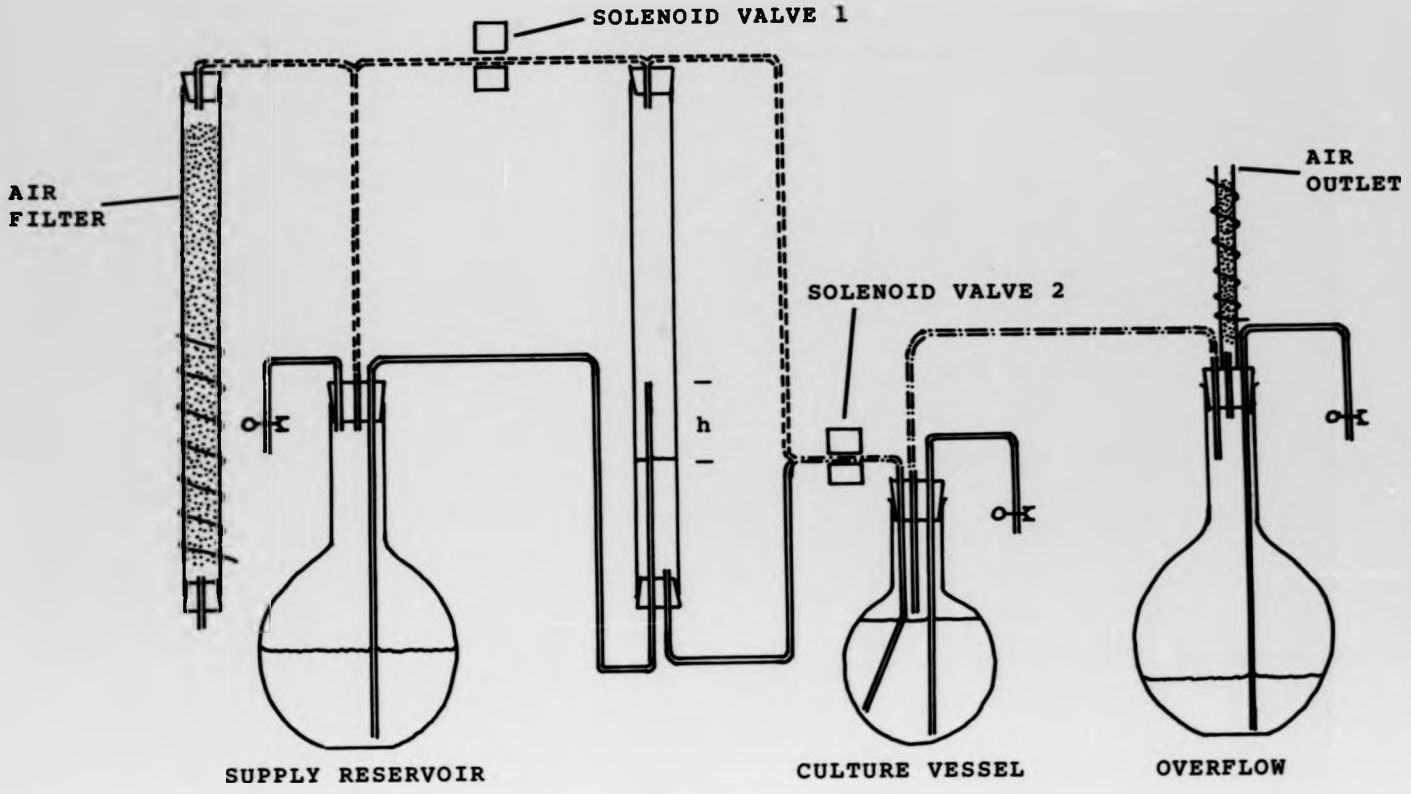



Figure 32. A chemostat assembly. Solid lines carry medium, dashed lines carry air, and dot-dash lines carry either medium or air.

# APPENDIX ID

## Medium

1. Recipe for half-strength seawater medium (S-104)

Chemicals in S-104 dry mix prepared in bulk for 100 liters. All chemicals were Analar grade from BHD Chemicals supplied from MacFarland Robson Ltd., unless noted (a "*" indicates the supplier was Sigma Chemical Company Ltd.).

| | | |
|---|---|---|
| NaCl | 1.6 | kg |
| $CaSO_4 \cdot 2HOH$ | 50.0 | g |
| $MgSO_4 \cdot 7HOH$ | 250.0 | g |
| KCl | 40.0 | g |
| Glyclglycine* | 50.0 | g |
| Glycine* | 25.0 | g |
| $KNO_3$ | 10.0 | g |
| $Na_2EDTA$ | 5.0 | g |
| KBr | 3.25 | g |
| $SrCl_2 \cdot 6HOH$ | 650.0 | mg |
| $AlCl_3 \cdot 6HOH$ | 25.0 | mg |
| RbCl | 10.0 | mg |
| $LiCl \cdot HOH$ | 5.0 | mg |
| KI | 2.5 | mg |
| $MnSO_4 \cdot 4HOH$ | 20.3 | mg |
| $CuSO_4 \cdot 5HOH$ | 1.9 | mg |
| $CoSO_4 \cdot 7HOH$ | 240.0 | µg |
| $ZnSO_4 \cdot 7HOH$ | 2.2 | mg |
| $Na_2MoO_4 \cdot 2HOH$ | 120.0 | µg |
| $FeSO_4 \cdot 7HOH$ | 250.0 | mg |
| vitamin $B_1$ | 5.0 | mg |
| vitamin $B_{12}$ | 10.0 | µg |

The ionic concentration in S-104, 20g dry mix per liter.

| | liter$^{-1}$ | | mg $l^{-1}$ | molar(M) strength | |
|---|---|---|---|---|---|
| Na | 6.3 | g | 6300. | .274 | M |
| K | 263.6 | mg | 263.6 | 6.742 | mM |
| Mg | 247.5 | mg | 247.5 | 10.18 | mM |
| Ca | 116.4 | mg | 116.4 | 2.904 | mM |
| Sr | 2.14 | mg | 2.14 | 24.42 | µM |
| Fe | 500. | µg | .5 | 8.95 | µM |
| Rb | 70.68 | µg | .07068 | .827 | µM |
| Mn | 50. | µg | .05 | .910 | µM |
| Al | 27.9 | µg | .0279 | 1.034 | µM |
| Cu | 6. | µg | .006 | 94.43 | nM |
| Li | 5.75 | µg | .00575 | .829 | µM |
| Zn | 5.1 | µg | .0051 | 78.02 | nM |
| Co | .5 | µg | .0005 | 8.485 | nM |
| Mo | .48 | µg | .00048 | 5.00 | nM |
| $SO_4$ | 1.25 | g | 1250. | 13.01 | mM |
| Cl | 9.9 | g | 9900. | .279 | M |
| $NO_3$ | 61.33 | mg | 61.33 | .989 | mM |
| Br | 21.8 | mg | 21.8 | .273 | mM |
| I | 19.1 | µg | .0191 | .151 | µM |
| $PO_4$ | 1.36 | µg | .00136 | 1.44 | µM |
| $B_{12}$ | .1 | µg | .0001 | - | |
| $B_1$ | .05 | mg | .05 | - | |
| Glycine | 250. | mg | 250. | - | |
| Glyclglycine | 500. | mg | 500. | - | |
| NaEDTA | 50. | mg | 50. | - | |

2. Procedure for preparing S-104 dry mix for 100 liters

1. $Na_2EDTA$ - 5 g weighed
2. $Na_2EDTA$ added to 50 ml glass distilled water (GDW) and 2 ml of 10% NaOH
3. 50 ml GDW added to no. 2
4. $FeSO_4 \cdot 7HOH$ - 250 mg weighed
5. $FeSO_4 \cdot 7HOH$ - dissolved in 10 ml GDW
6. pH of no. 3 tested (add HCl to bring it below pH 7), all $Na_2$-EDTA should be dissolved
7. $FeSO_4 \cdot 7HOH$ solution added to $Na_2EDTA$ solution. There should be no precipitation in the combined solution.
8. $MnSO_4 \cdot 4HOH$ added to no. 7: 20.3 mg (20.3 ml of 1 ml = 1 mg)
9. $CuSO_4 \cdot 5HOH$ added to no. 8: 1.9 mg (1.9 ml of 1 ml = 1 mg)
10. $ZnSO_4 \cdot 7HOH$ added to no. 9: 2.2 mg (2.2 ml of 1 ml = 1 mg)
11. $Na_2MoO_4 \cdot 2HOH$ added to no. 10: 120.0 µg (to 1 ml of 1 ml = 1 mg, add GDW to 10 ml to give 1 ml = 100 µg, use 1.2 ml)
12. vitamin $B_{12}$ added to no. 11: 10.0 µg (5.0 ml of 1 ml = 2 µg)
13. Vitamin $B_1$ added to no. 12: 5.0 mg (5.0 ml of 1 ml = 1 mg)
14. $CoSO_4 \cdot 7HOH$ added to no. 13: 240.0 µg (to 1 ml = 1 mg, add GDW to 10 ml to give 1 ml = 100 µg, use 2.4 ml)
15. The above solution (no. 14) set aside in refrigeration
16. $SrCl_2 \cdot 6HOH$ - 650 mg weighed

17. $AlCl_3 \cdot 6HOH$ - 25 mg (2.5 ml of 1 ml - 10 mg) added to no. 16
18. RbCl - 10 mg (10.0 ml of 1 ml = 1 mg) added to no. 17
19. LiCl·HOH - 5.0 mg (10 ml of 1 ml = 1 mg) added to no. 18
20. KI - 2.5 mg (2.5 ml of 1 ml = 1 mg) added to no. 19
21. KBr - 3.25 g weighed and added to no. 20
22. The two solutions (no. 15 and no. 21) are mixed together.
23. The solution is now put into a large desiccator bowl.
24. Glycine - 25 g weighed and mixed into no. 22
25. Glyclglycine - 50 g weighed and mixed into no. 23
26. KCl - 40 g weighed and mixed into no. 24
27. $CaSO_4 \cdot 2HOH$ - 50 g weighed and mixed into no. 25
28. $MgSO_4 \cdot 7HOH$ - 250 g weighed and mixed into no. 26
29. NaCl - 800 g weighed and mixed into no. 27
30. NaCl - 800 g weighed and mixed into no. 28
31. Mix thoroughly and grind with pestle
32. Vacuum desiccate for one week, stirring and grinding twice daily
33. The dry mix is stored with a beaker of $CaCl_2$, in a dark, air-tight, 3 kg chemical supply jar.

3. Preparation of 2 or 4 liters of culture medium

1. Rinse all glassware with glass distilled water (GDW)
2. Fill 2 l polypropylene beaker to 1950 ml with GDW
3. Weigh 40 g S-104 dry mix, add to the water in the beaker and stir
4. Add 1 ml $K_2HPO_4$ from 1 ml = .5 mg stock solution
5. When dissolved, adjust pH to 8.0 with 10% NaOH
6. Add 160 mg $NaHCO_3$
7. Transfer medium to a 2 l volumetric flask and fill to level with GDW used as a rinse of the mixing beaker
8. If 4 l of medium is being prepared the medium is transferred to a 5 l vessel, the above procedure is repeated, and the second 2 l medium put into the 5 l vessel
9. Add $^{32}P$ - usually 200-500 µl, maximum activity = 20 µCi
10. Stir or shake the medium
11. When 4 l medium are prepared, two are for a batch-culture experiment. The 2 l volumetric flask is filled to level and transferred to a 5 l, autoclavable, vented vessel
12. Fill 1 l supply flasks 1/2 to 2/3 full
13. Autoclave for 10-12 minutes at 15 psi (120°C, 1.06 kg $cm^{-2}$)

## APPENDIX IE

### Names and Addresses of Suppliers

| | |
|---|---|
| Anderman and Company Ltd.<br>Central Avenue<br>East Molesey, Surrey KT8 OQ2 | Eppendorf autopipettes<br>1000, 500 and 200 µl |
| Barr and Stroud Ltd.<br>Caxton Street, Anniesland<br>Glasgow G13 1HZ | Graded Spectrum Filter<br>Type MS2 |
| Beckman - R.I.IC., Ltd.<br>Eastfield Industrial Estate<br>Glenrothes, Fife Ky7 4NG | Liquid Scintillation Vials<br>Poly Q II with screw caps |
| Buckley Membranes Ltd.<br>Chequers Parade<br>Prestwood, Gt. Missenden<br>Buckinghamshire HP16 OPN | Finnpipette<br>1000-5000 µl |
| ESCO (Rubber) Ltd.<br>14-16, Great Portland Street<br>London WIN 5AB | Silicone tubing and bungs |
| Gordon-Keeble<br>8a Chapel Street<br>Duxford, Cambridge CB2 4RJ | Syringe valve<br>Polypropylene and ethylene propylene rubber: GKSVP |
| Kodak Ltd.<br>Pegasus House<br>375 West George Street<br>Glasgow G2 4NT | Wratten filters in "T" glass Types 88A and 2B |
| Lexel Lamb Ltd.<br>107-113 Cambridge Road<br>Milton CB4 4AX | Metalized PVC, .016 inch thick |
| Londex Ltd.<br>P.O. Box 79<br>207 Anerly Road<br>London SE 20 | Solenoid switches<br>Type LF/VA |
| MacFarland Robson Ltd.<br>Burnfield Avenue<br>Thornliebank<br>Glasgow G46 7TP | BDH (Analar) chemicals and Quickfit glassware |
| M & E Seals Ltd.<br>649 North Circular Road<br>Cricklewood, London NW2 | Stirring drive belt<br>No. 471 Nitrol o-ring |
| Millipire (U.K.) Ltd.<br>Millipore House<br>Abbey Road<br>Park Royal, London NW10 7SP | Acetate membrane filter<br>HAWP02500 |

| | |
|---|---|
| The Radiochemical Centre<br>P.O. Box 16<br>Amersham, Buckinghamshire HP7 9LL | $^{32}P$ as orthophosphate in dilute HCl. Code PBS.1<br>1 mCi/ml |
| Service Trading Company<br>57 Bridgeman Road<br>Chiswick, London W4 5BB | Geared stirring motor |
| Sigma (London) Chemical Co. Ltd.<br>Norbiton Station Yard<br>Kingston-Upon-Thames<br>Surrey KT2 7BH | Glyclglycine (free base)<br>Glycine |
| Thorn House<br>Lawmoor Street<br>Glasgow G5 OTT | Fluorescent lamps and fittings |
| K.R. Whiston<br>New Mills<br>Stockport SK12 4PT | Pulleys for stirring drive |

## APPENDIX IIA

### Procedure for Starting a Batch-Culture Experiment

1. Clean all glassware: flask and top, stirring rod and holder, air and sampling lines, in a solution of RBS detergent in hot water. Soak overnight and rinse at least three times with distilled water.
2. Clean a 50 ml polypropylene syringe fitted with a polypropylene side-arm fill valve. Both the input side arm and the output front end of the valve are fitted with 25 cm of silicone tubing, clamped at the ends. The syringe is then wrapped in aluminum foil.
3. Prepare 2 l of medium using GDW and put this in a five liter, flat-bottom vessel with an air lock and clamped siphon line.
4. Clamp all loose input and output ends, wrap in cotton wool, (inserted in each end is 8 cm of glass tube to act as connector or to give rigidity) and fit into glass vials.
5. All of the above are autoclaved at 15 psi (120°C, 1.06 kg $cm^{-2}$) for 12 minutes.
6. The batch-culture vessel is clamped into its collar and screwed into the water tank. The air line is connected to give a positive pressure to the system.
7. When cooled, the medium is aseptically transferred through the appropriate lines into the batch-culture vessel (the glass tube in one line acts as a connector) - all clamped ends are rinsed, before and after opening, with 95% ethanol.
8. The chemostat which is to supply the inoculent is sam-

pled; or, for experiments 7 and 8, the starter culture is sampled. Chlorophyll-a, total and particulate phosphorus, and number of cells are measured.

9. 50 ml of medium is removed from the batch vessel through the sampling line into the 50 ml syringe (10 ml removed for experiments 7 and 8). The medium provides a sample of total phosphorus and assures that all experiments begin with a 2 l initial volume. The ends of the lines are rinsed as in step no. 7.
10. 50 ml is removed from a chemostat reactor vessel using the syringe assembly and this sample is injected into the batch-culture vessel through its sampling line (10 ml removed from the starter culture and injected into the batch vessel for experiments 7 and 8).
11. Wait five minutes to allow for mixing and then take initial sample.

# APPENDIX IIB

## Analytical Procedures

1. Cell count

A 2.5 or 5.0 ml subsample was taken with a 1000-5000 µl Finnpipette and dispensed into a 50 ml volumetric flask which was filled to volume with .45 µ - filtered, 50% seawater. Each time a count was made a 50 ml sample of the filtered seawater was counted as a blank - this corrected for particles in the seawater and noise in the power supply. Four to twelve, ten second counts were made with a Model B Coulter Particle Counter using a 50 µ aperture tube and the following settings:

amplification$^{-1}$ = 1

aperture current$^{-1}$ = .354

lower threshold = 8%

upper threshold = 100%

A coincidence correction was made on all samples with a count of over 6800 counts per second (Figure 33 and Table 15). The counter had been modified to allow continuous flow of sample, and counting was started and stopped by an electronic timer. The following equation was used to calculate cell per ml:

$$(\text{mean sample count} - \text{mean blank count}) \times \frac{\text{sample dilution factor}}{\text{seconds of counting}} \times \text{calibration} = 10^6 \text{ cells ml}^{-1},$$

where:

dilution factor = volume of dilution/sample volume (usually 50 ml/5ml = 10)

seconds counted = 10 seconds

flow calibration = $86.73 \times 10^{-6} \frac{10^6 \text{ cells seconds}}{\text{ml counts}}$

for

*Pavlova lutheri* through a 50 μ tube at the above settings.

$$\text{counts} \times \frac{1}{\text{sec}} \times \frac{10^6 \text{ cells sec}}{\text{ml counts}} = 10^6 \text{ cells ml}^{-1}$$

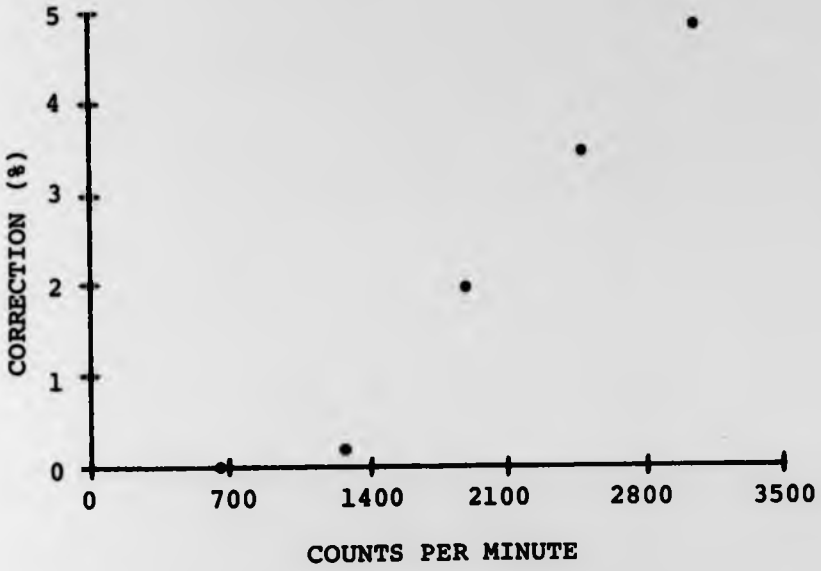



Figure 33. Coincidence correction (% coincidence) vs raw counts per minute.

TABLE 15. Data for coincidence correction of all counts. Each sample was counted eight times for ten seconds. The blank was 247 counts per ten seconds.

| Sample Number | Dilution ($\frac{\text{ml sample}}{\text{ml final volume}}$) | Mean Count (blank-corrected) | Standard Deviation (n=8) | % Standard Error | Standardized Mean Count | % Difference from sample # 1 |
|---|---|---|---|---|---|---|
| 1 | 1/50 | 6444 | 80 | .4 | 32220 | 0.0 |
| 2 | 2/50 | 12860 | 227 | .6 | 32150 | 0.2 |
| 3 | 3/50 | 18989 | 275 | .5 | 31648 | 2.0 |
| 4 | 4/50 | 24868 | 353 | .5 | 31085 | 3.5 |
| 5 | 5/50 | 30651 | 358 | .4 | 30651 | 4.9 |

2. Fluorometric determination of chlorophyll-a

1 ml of medium was subsampled using a 1000 µl Eppendorf pipette into a 12 ml polypropylene centrifuge tube. The tubes were immediately filled to a premarked 10 ml level with 100% Analar acetone stored over $NaHCO_3$. The tubes were either refrigerated in the dark overnight and then brought to room temperature in the dark, or left at room temperature in the dark for at least two hours. It was found with samples of Pavlova lutheri that all the chlorophyll-a was extracted within one hour. Cells in the extracts were then spun down in a centrifuge at 3000 rpm for twelve minutes.

The fluorescence of the extracts was measured before and after acidification using a Turner fluorometer, Model 111 equipped for chlorophyll measurement with standard door, red sensitive photomultiplier, T-5 envelope lamp, and Corning 5-60 excitation and 2-64 emission filters. The fluorometer was calibrated using 90% acetone solutions of pure chlorophyll-a which was standardized by measurement with a spectrophotometer. The calibration of the fluorometer took into account different sensitivity ranges and temperature variation. A correctly calibrated fluorometer is as accurate as the spectrophotometer against which it is calibrated but a fluorometer's main advantage is an increased precision and quicker and easier measurement.

A reading from a 90% acetone blank was subtracted from each sample reading. The intial samples from a batch-culture experiment (the first two days) contained quantities of chlorophyll-a near the lower measurement limit of the fluorometer. At the end of the lower-light experiments it

was necessary to dilute the extracts (1 ml of the 10 ml extract in the centrifuge tube was diluted to 10 ml) as the concentration of chlorophyll-a was so great as to go above the upper limit of measurement.

3. Phosphorus determinations

Total and particulate phosphorus was measured indirectly by counting the high-energy, beta-particle decay of $^{32}P$ that had been added to the medium as orthophosphate from the Radiochemical Centre Amersham. The $^{32}P$ was virtually carrier free: less than $2 \times 10^{-12}$ moles/ml of phosphorus was added with the $^{32}P$. Each time a new supply of $^{32}P$ was obtained it took 2-3 weeks for the activity in the chemostat reactor to equal the activity of the supply, and during this time there was also an equalization of activity in the algal cells. The initial level of activity for each experiment was 20 μCi/liter of medium. After the activity in the chemostats had reached steady-state it was possible to carry out two successive batch-culture experiments (each experiment lasting from two to five weeks). Since $^{32}P$ has a relatively short half life, 14 days, it was necessary to increase the amount of $^{32}P$ so that the level of activity in the cultures was high enough to allow a sample to be counted in a reasonable length of time. Therefore, a new supply of $^{32}P$ was obtained to coincide with a change in the light levels. This allowed the algae to make adjustment to new conditions while the $^{32}P$ was coming into steady-state.

Total-phosphorus samples were prepared by pipetting a 1 or 2 ml subsample using a 1000 μl Eppendorf pipette into a Beckman Poly Q II liquid scintillation vial with screw cap. Particulate phosphorus samples were prepared by filtering a 2.5, 5.0, or 10.0 ml subsample (using a 1000-5000 μl Finn-pipette) through a 25 mm Millepore HA (.45 μ) membrane filter, with a vacuum of between .14-.22 kg $cm^{-2}$. While the

sample was being filtered, the pipette was rinsed by dispensing 2.5 or 5.0 ml of filtered 50% seawater from a beaker. Another measure of the seawater was then taken with the pipette from a different beaker and was filtered, rinsing the cells, as soon as the particulate sample was dry. As soon as the sample was again dry the filter was removed, while vacuum was still being applied and put into a liquid scintillation vial.

10 ml of liquid-scintillation fluid in dioxan (BDH Chemicals supplied by MacFarland Robson - no. 19228/GF) was injected into each vial using a 0-10 ml Oxford autodispenser. The liquid-scintillation solution can absorb up to 20% of its volume of water and dissolves the 25 mm membrane filter within a minute. The screw caps were carefully tightened onto the vials and each sample was vigorously shaken. The vials also had to be shaken between each count because, if they were not, the counts decreased - probably due to settling of particulate material. This problem could in future be overcome by adding a gelling agent to the vials before shaking.

The radioactivity was counted in a Beckman LS - 150 Liquid Scintillation System using a full energy spectrum isoset ($^{3}H$, $^{14}C$, and $^{32}P$ plug-in, window setting). Each sample was counted 2 to 4 times to a level of significance between 2-5% and the counts averaged to obtain a measure for counts per minute per sample (CPM). The CPM was then corrected by subtracting an appropriate blank (scintillant or scintillant and filter) and then the particulate sample was standardized to represent the same volume as the total phosphorus sample. It was found that there was little need

to make a quench correction, especially when the total phosphorus sample was 1 ml of medium. Nonetheless, an external standard, channels ratio for each sample was measured and compared to a series of quenched standards (Table 16) - in no case was a quench correction of greater than 2% necessary.

To obtain the amount of particulate phosphorus, the corrected CPM for particulate phosphorus was divided by the corrected CPM for total phosphorus and this percentage was multiplied times the known amount of total phosphorus in the medium (1.44 nM $ml^{-1}$). Dissolved phosphorus was calculated by subtraction. Since the two samples were counted at the same time and the fractions were calculated by percentage, there was no need to account for the $^{32}P$ decay.

Another method of calculating particulate and dissolved phosphorus is to filter for the particulate phosphorus as described above and sample the filtrate for dissolved phosphorus. In this case glassware must be completely dry and clean since the filtrate could be as little as 2 ml and all of it might be needed, and even with this technique there is still a need for a measure of total radioactivity - unless one assumes that total radioactivity equals the sum of the particulate and dissolved fractions (an assumption similar to the one I made). Another way of calculating total radioactivity would be to measure it at the beginning of the experiment and then, allowing for decay, predict the total radioactivity each time a new pair of samples are counted. This technique still has drawbacks: there is an error associated with the first measure and the error is carried through to later decay predictions.

The method used in this study was chosen because, for

the largest period of each experiment, particulate and total radioactivity could be more quickly counted and there is certainly less sampling error for total and particulate radioactivity. A further advantage was that it was possible to store the samples and count several, or all, of them at the same time (though each pair of samples could be counted at any time because particulate radioactivity was expressed as a percentage of total radioactivity), giving a better estimate of total radioactivity. Thus this technique provides a measure of particulate radioactivity that is equal in precision to the other techniques mentioned, and a value for total radioactivity that should be more precise than any other single measure for total or dissolved phosphorus.

The adsorption of phosphorus to glassware is often a problem when phosphorus is used in low concentrations. I tried to overcome this problem through special treatment of the glassware. The glassware was initially acid washed and then exposed to the experimental medium. Before each experiment, the glassware was soaked for a brief period in RBS cleaning solution and then rinsed three times with distilled water. This treatment should not have "stripped" any phosphorus that was adsorbed during the initial exposure to the medium and therefore further adsorption should have been minimized. Usually adsorption can be determined by measuring total phosphorus through time. In this study, total radioactivity was measured through time and the decay in radioactivity was close to the theoretical decay curve (Figure 34), indicating that no phosphorus was lost from the system (or redissolved later in the experiment when the dis-dissolved fraction of total-P became small).

TABLE 16. Quench correction for $^{32}P$ in dioxan-based liquid scintillation fluid, nitromethane used as the quencher. The data presented represent mean values from four counts (1% s.e. for each count).

| External Standard Channels Ratio | CPM (counts per minute) | % Efficiency[1] |
|---|---|---|
| .515 | $171.0 \times 10^3$ | 100 |
| .280 | $170.3 \times 10^3$ | 99.6 |
| .172 | $168.7 \times 10^3$ | 98.6 |
| .060 | $168.0 \times 10^3$ | 98.2 |
| .008 | $166.7 \times 10^3$ | 97.5 |

[1] This efficiency represents a value relative to the lowest quenching (.515). Quench relative to absolute activity (DPM or disintegrations per minute) was not calculated because absolute measures of radioactivity were not needed to calculate the various measures of phosphorus.

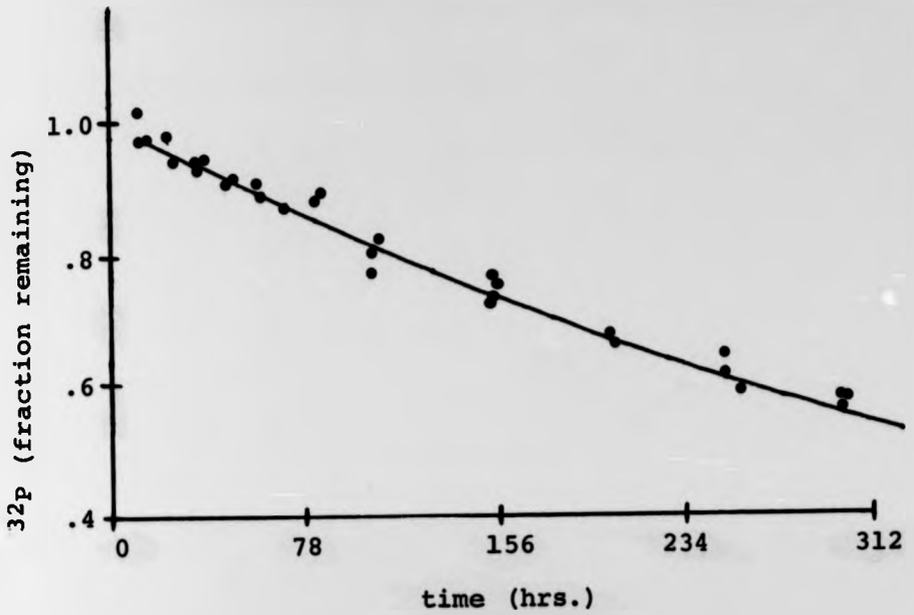



Figure 34. Observed (points) and theoretical (line) decay of $^{32}$P in experiments 1-3.

# APPENDIX III

## A. Subroutines Written for Bard's Program

### 1. Algebraic equations : μ vs Q

```
      SUBROUTINE MESQ(II,I)
      COMMON ZZ(1360),NPH,MD,LS,C1(20),X,XTH(20),A(200,10)
      IF(II.EQ.2) GO TO 200
      GO TO (1,2,3,4,5,6,7,8),MD
    1 E=C1(1)*A(I,1)-A(I,2)
      F=C1(2)-A(I,2)
      X=AMIN1(E,F)
      GO TO 10
    2 E=C1(1)*A(I,1)-C1(2)-A(I,2)
      F=C1(3)-A(I,2)
      X=AMIN1(E,F)
      GO TO 10
    3 E=C1(1)*(A(I,1)-C1(2))-A(I,2)
      F=C1(3)-A(I,2)
      X=AMIN1(E,F)
      GO TO 10
    4 X=C1(1)*A(I,1)/(C1(2)+A(I,1))-A(I,2)
      GO TO 10
    5 X=C1(1)*(1.-2.**(1.-A(I,1)/C1(2)))-A(I,2)
      GO TO 10
    6 X=C1(1)*(1.-C1(2)/A(I,1))-A(I,2)
      GO TO 10
    7 X=C1(1)*(A(I,1)-C1(2))/(C1(3)+A(I,1)-C1(2))-A(I,2)
      GO TO 10
    8 X=C1(1)*(A(I,1)-C1(2))/(A(I,1)+C1(3)-C1(2))*
     1     (C1(4)+C1(3)-C1(2))/(C1(4)-C1(2))
   10 GO TO (100,20),II
   20 GO TO (21,22,23,24,25,26,27,28),MD
   21 IF(F.LE.E) GO TO 211
      XTH(1)=A(I,1)
      XTH(2)=0.
      GO TO 100
  211 XTH(1)=0.
      XTH(2)=1.
      GO TO 100
   22 IF(F.LE.E) GO TO 222
      XTH(1)=A(I,1)
      XTH(2)=-1.
      XTH(3)=0.
      GO TO 100
  222 XTH(1)=0.
      XTH(2)=0.
      XTH(3)=1.
      GO TO 100
   23 IF(F.LE.E) GO TO 233
      XTH(1)=A(I,1)-C1(2)
      XTH(2)=-C1(1)
      XTH(3)=0.
      GO TO 100
  233 XTH(1)=0.
      XTH(2)=0.
      XTH(3)=1.
      GO TO 100
   24 XTH(1)=A(I,1)/(C1(2)+A(I,1))
      XTH(2)=-A(I,1)/((C1(2)+A(I,1))**2)*C1(1)
      GO TO 100
   25 XTH(1)=(1.-2.**(1.-A(I,1)/C1(2)))
      XTH(2)=C1(1)*(-2.**(1.-A(I,1)/C1(2)))*ALOG(2.)*(A(I,1)/(C1(2)**2)

      GO TO 100
   26 XTH(1)=(1.-C1(2)/A(I,1))
      XTH(2)=-C1(1)/A(I,1)
      GO TO 100
   27 XTH(1)= (A(I,1)-C1(2))/(C1(3)+A(I,1)-C1(2))
      XTH(2)=((A(I,1)-C1(2))/((C1(3)+A(I,1)-C1(2))**2)-
     1     1./(C1(3)+A(I,1)-C1(2)))*C1(1)
      XTH(3)=-C1(1)*(A(I,1)-C1(2))/((C1(3)+A(I,1)-C1(2))**2)
      GO TO 100
   28 XTH(1)=(A(I,1)-C1(2))/(A(I,1)+C1(3)-C1(2))*(C1(4)+C1(3)-C1(2))/
     1     (C1(4)-C1(2))
      XTH(2)=(A(I,1)-C1(2))/(A(I,1)+C1(3)-C1(4))*C1(3)*C1(1)/
     1     ((C1(4)-C1(2))**2)-C1(3)*C1(1)/((A(I,1)+C1(3)-C1(2))**2)*
     2     (C1(4)+C1(3)-C1(2))/(C1(4)-C1(2))
      XTH(3)=-C1(1)*(A(I,1)-C1(2))/(C1(4)-C1(2))*(A(I,1)-C1(4))/
     1     ((A(I,1)+C1(3)-C1(2))**2)
      XTH(4)=-C1(1)/((C1(4)-C1(2))**2)*(A(I,1)-C1(2))/(A(I,1)+
     1     C1(3)-C1(2))
      GO TO 100
  200 CONTINUE
  100 CONTINUE
      RETURN
      END
```

## 2. Algebraic equations : photosynthesis (or $\mu_m$) vs I

```
      SUBROUTINE PLSQ(II,I)
      COMMON ZZ(1868),NPN,MD,LS,DI(20),X,XTH(20),A(200,18)
      IF(II.EQ.3) GO TO 200
      GO TO (1,2,3,4,4,6,7,8),MD
    1 B=C1(2)/C1(1)
      IF(A(I,1).GT.B) GO TO 11
      X=C1(1)*A(I,1)-A(I,2)
      GO TO 9
   11 X=C1(2)-A(I,2)
      GO TO 9
    2 X=(C1(2)*C1(1)*A(I,1))/(C1(2)+C1(1)*A(I,1))-A(I,2)
      GO TO 9
    3 X=(C1(2)*C1(1)*A(I,1))/((C1(2)**2+(C1(1)**2*A(I,1)**2))**.5)
     1    -A(I,2)
      GO TO 9
    4 X=C1(1)*A(I,1)*EXP(-(C1(1)*A(I,1))/(EXP(1.)*C1(2)))-A(I,2)
      IF(MD.EQ.4) GO TO 9
      D=C1(2)*EXP(1.)/C1(1)
      IF(A(I,1).GT.D) X=C1(2)-A(I,2)
      GO TO 9
    6 X=C1(2)*(1.-EXP(-(C1(1)*A(I,1))/C1(2)))-A(I,2)
      GO TO 9
    7 Z=2.*C1(2)/C1(1)
      IF(A(I,1).GT.Z) GO TO 77
      X=C1(1)*A(I,1)*(1.-(C1(1)*A(I,1))/(4.*C1(2)))-A(I,2)
      GO TO 9
   77 X=C1(2)-A(I,2)
      GO TO 9
    8 X=C1(2)*TANH(C1(1)*A(I,1)/C1(2))-A(I,2)
    9 GO TO (100,20),II
   20 GO TO (21,22,23,24,24,26,27,28),MD
   21 IF(A(I,1).GT.B) GO TO 211
      XTH(1)=A(I,1)
      XTH(2)=0.0
      GO TO 100
  211 XTH(1)=0.0
      XTH(2)=1.0
      GO TO 100
   22 XTH(1)=C1(2)**2*A(I,1)/((C1(2)+C1(1)*A(I,1))**2)
      XTH(2)=(C1(1)*A(I,1))**2/((C1(2)+C1(1)*A(I,1))**2)
      GO TO 100
   23 XTH(1)=(C1(2)*A(I,1)*((C1(2)**2+(C1(1)*A(I,1))**2)**.5-(C1(1)*
     1     A(I,1))**2/(C1(2)**2+(C1(1)*A(I,1))**2)**.5))/(C1(2)**2+
     2     (C1(1)*A(I,1))**2)
      XTH(2)=(C1(1)*A(I,1)*((C1(2)**2+(C1(1)*A(I,1))**2)**.5
     1     -C1(2)**2/(C1(2)**2+(C1(1)*A(I,1))**2)**.5))/
     2     (C1(2)+(C1(1)*A(I,1))**2)
      GO TO 100
   24 XTH(1)=EXP(-C1(1)*A(I,1)/(EXP(1.0)*C1(2)))*A(I,1)*(1.0-(C1(1)
     1    *A(I,1)/(EXP(1.0)*C1(2))))
      XTH(2)=EXP(-C1(1)*A(I,1)/(EXP(1.0)*C1(2)))*((C1(1)*A(I,1))**2
     1    )/((EXP(1.0)*C1(2))**2)
      IF(MD.EQ.4) GO TO 100
      IF(A(I,1).LE.D) GO TO 100
      XTH(1)=0.0
      XTH(2)=1.0
      GO TO 100
   26 XTH(1)=A(I,1)*EXP(-C1(1)*A(I,1)/C1(2))
      XTH(2)=1.0-EXP(-C1(1)*A(I,1)/C1(2))*(1.0+C1(1)*A(I,1)/C1(2))
      GO TO 100
   27 Z=2.0*C1(2)/C1(1)
      IF(A(I,1).GT.Z) GO TO 277
      XTH(1)=A(I,1)-C1(1)*A(I,1)**2/(2.0*C1(2))
      XTH(2)=(C1(1)*A(I,1))**2/(4.0*C1(2)**2)
      GO TO 100
  277 XTH(1)=0.0
      XTH(2)=1.0
      GO TO 100
   28 XTH(2)=TANH(C1(1)*A(I,1)/C1(2))-(C1(1)*A(I,1)/C1(2))*(1.0-
     1     (TANH(C1(1)*A(I,1)/C1(2)))**2)
      XTH(1)=A(I,1)*(1.0-TANH(C1(1)*A(I,1)/C1(2))**2)
      GO TO 100
  200 CONTINUE
  100 RETURN
      END
```

3. Differential equations : $\frac{dX}{dt}$, $\frac{dQ}{dt}$ and $\frac{dx}{dt}$ vs X,Q, and S

```
      SUBROUTINE FUN(II,JJ,IB,T)
      COMMON C(20,20),G1(20,20),PSCA,G(20,20),F(20),Y(20),
     1       EGV(20),FF(20),TITLE(20),CUB(20),CLB(20),
     1       PNL(20),NCON,LOUT,F3,NTH,F6,F7,METH,NPH,MD,
     3       LS,C1(20)
      COMMON V(5,5),QY(5),YTH(5,20),A(100,10),ICOV,DET,
     1       IDER,M,NY,NA
      COMMON NX,NB,NTH2,NTH1,Q(10),P(10),FX(10,10),FTH(10,20),
     1       BV(20,10),TIME(100),IA(100),FN(10),XTH(10,20),
     2       XTTH(10,20)
      GO TO (1,1,2),II
    2 FX(1,3)=0.
      FX(2,1)=0.
      FX(3,2)=0.
      FTH(1,1)=0.
      FTH(1,2)=0.
      FTH(1,4)=0.
      FTH(3,3)=0.
      FTH(3,5)=0.
      IF(MD.EQ.1) GO TO 20
      FTH(3,6)=0.0
   20 RETURN
    1 P(3)=-(C1(1)*((Q(3)-C1(4)))/(C1(2)+Q(3)-C1(4)))*Q(1)
      IF(MD.EQ.2) GO TO 102
      P(1)=C1(3)*(1.-(C1(5)/Q(2)))*Q(1)
      P(2)=C1(1)*((Q(3)-C1(4)))/(C1(2)+Q(3)-C1(4))-
     1     C1(3)*(1.-(C1(5)/Q(2)))*Q(2)
      GO TO 109
  102 P(1)=C1(3)*(Q(2)-C1(5))/(Q(2)-C1(5)+C1(6))*Q(1)
      P(2)=C1(1)*(Q(3)-C1(4))/(Q(3)-C1(4)+C1(2))-Q(2)*P(1)/Q(1)
  109 GO TO (3,4),II
    4 FX(1,1)=P(1)/Q(1)
      FX(2,3)=C1(1)*(C1(2)/((C1(2)+Q(3)-C1(4))**2))
      FX(3,1)=P(3)/Q(1)
      FX(3,3)=-FX(2,3)*Q(1)
      FTH(2,1)=((Q(3)-C1(4))/(C1(2)+Q(3)-C1(4)))
      FTH(2,2)=-C1(1)*((Q(3)-C1(4))/((C1(2)+Q(3)-C1(4))**2))
      FTH(2,4)=-FX(2,3)
      FTH(3,1)=-FTH(2,1)*Q(1)
      FTH(3,2)=-Q(1)*FTH(2,2)
      FTH(3,4)=-FX(3,3)
      IF(MD.EQ.2) GO TO 402
      FX(1,2)=C1(3)*C1(5)*Q(1)/(Q(2)*Q(2))
      FX(2,2)=-C1(3)
      FTH(1,3)1T1jm.J
      FTH(1,5)=-(C1(3)*Q(1)/Q(2))
      FTH(2,3)=-Q(2)+C1(5)
      FTH(2,5)=C1(3)
      GO TO 3
  402 FX(1,2)=C1(3)+Q(1)*C1(6)/((C1(6)+Q(2)-C1(5))**2)
      FX(2,2)=(-C1(3)*(C1(5)**2-2.*Q(2)*C1(5)-Q(2)**2-C1(5)*C1(6)+
     1     2.*Q(2)*C1(6)))/((Q(2)+C1(6)-C1(5))**2)
      FTH(1,3)=P(1)/C1(3)
      FTH(1,5)=-FX(1,2)
      FTH(1,6)=-C1(3)*Q(1)*(Q(2)-C1(5))/((C1(6)+Q(2)-C1(5))**2)
      FTH(2,3)=-(FTH(1,3)/Q(1))*Q(2)
      FTH(2,5)=(FX(1,2)/Q(1))*Q(2)
      FTH(2,6)=-(FTH(1,6)/Q(1))*Q(2)
    3 RETURN
      END
```

## Appendix IIIB

Program Configuration from Bard's (1967b) Program Package

Following are two configurations of subroutines from Bard's computer program. The parameter estimation problems in this study were of two types:

1. single, algebraic equations ($\mu_m$ and $\mu_m'$ vs I, $\mu$ vs Q, and photosynthesis vs light); and,
2. differential equations (dX/dt, dQ/dt, and dS/dt vs X, Q, and S).

1. Program configuration for models in the form of a single, algebraic equation.

| Bard's Deck No. | Name | Purpose |
|---|---|---|
| 1 | MAIN | Calls first subroutine (NLMAX) |
| 2 | NLMAX | Reads input, and finds and prints maximum of the objective function (Gauss-Newton method) |
| 3 | ACCUM | Computes value of objective function and its derivatives (single equation lease squares) |
| 4 | EIG | scales a symmetric matrix (to accommodate various parameters or various state variables that have different scales of magnitude) and performs various matrix calculations |
| 5 | OUT | Detailed output (single equation least squares) |
| 6 | BOUND | Computes constraint functions and their derivatives (upper and lower bounds of parameters) |
| | DLSQ | Uses written subroutine to compute the model equation and its first derivative |

2. Program configuration for models in the form of differential equations.

| Bard's Deck No. | Name | Purpose |
|---|---|---|
| 1 | MAIN | Same as above |
| 2 | NLMAX | Same as above |
| 4 | EIG | Same as above |
| 6 | BOUND | Same as above |
| 10 | ACCUM | Computes value of objective function and its derivatives (weighted least squares with unknown weights) |
| 12 | OUT | Detailed output (weighted least squares) |
| 14 | DER | Computes model equations (models in the form of differential equations) and their first derivatives |
| 15 | PRIOR | No (known) prior distribution of parameters |
| 17 | RUN | Computes initial conditions (all are known in this instance) of the state variables, and their first derivatives |
| 20 | XTOY | The observed variables equal the state variables |
| 22 | XIJ | Performs some matrix operations (for DER) |
| | FUN | Uses written subroutine to compute the differential equations and their derivatives (with respect to the state variables and to the parameters) |

## APPENDIX IIIC

### Initial Guesses of Parameter Values

1. Droop's single equation ($\mu$ vs Q)

| $\mu_m'$ ($hr^{-1}$) | $k_Q$ (nM P($10^6$ cells)$^{-1}$) |
|---|---|
| .05 | .6 |

2. Caperon's single equation ($\mu$ vs Q)

| $\mu_m'$ ($hr^{-1}$) | $K_Q$ (nM P($10^6$ cells)$^{-1}$) | $Q_o$ (nM P($10^6$ cells)$^{-1}$) |
|---|---|---|
| .05 | 2.5 | .6 |

3. Differential equations (dX/dt, dQ/dt, dS/dt vs X, Q, and S)

Droop's Model

| $\mu_m'$ ($hr^{-1}$) | $k_Q$ (nM P($10^6$ cells)$^{-1}$) | $u_m$ (nM P($10^6$ cells)$^{-1}$) |
|---|---|---|
| .063 | .6 | .083 |

| $K_S$ (nM P $ml^{-1}$) | $S_o$ (nM P $ml^{-1}$) |
|---|---|
| .5 | .1 |

Caperon's Model

| $\mu_m'$ ($hr^{-1}$) | $K_Q$ (nM P($10^6$ cells)$^{-1}$) | $Q_o$ (nM P($10^6$ cells)$^{-1}$) |
|---|---|---|
| .05 | .5-5. | .6 |

| $u_m$ (nM P($10^6$ cells)$^{-1}$ $hr^{-1}$) | $K_S$ (nM P $ml^{-1}$) | $S_o$ (nM P $ml^{-1}$) |
|---|---|---|
| .09 | .5-5. | .05-.1 |

4. $\mu_m$ and $\mu_m'$ vs I (ten different equations)

| $\bar{\alpha}$ ($W^{-1}$ $m^2$ $day^{-1}$) | $\mu_{opt}$ ($day^{-1}$) | (r) ($day^{-1}$) | $I_o$ ($W m^{-2}$) | $K_I$ ($W m^{-2}$) |
|---|---|---|---|---|
| .05 | 1.0 | .005 | 10. | 10. |

Appendix III D. Sample Input for Bard's Program

## 1. Single, algebraic equations

```
     MUMAX VS LIGHT  USING PHVLT - EXPS 1-8
       3     2     4
0.05          1.0          -.005
      10     2
140.53        .8136
140.53        .8520
64.16         .9576
64.16         .9984
35.25         .8928
35.25         .9000
19.74         .4872
14.26         .2472
11.75         .3648
8.93          .2352
0.0           0.0          -1.0
1.0           2.5          1.0
```

```
     BATCH-CULTURE  MU'MAX VS LIGHT USING SSGRVQ
       2     2     6
.05           .6           2.5
       8     2
140.53        .9648
140.53        1.2192
64.16         1.2768
64.16         1.0848
35.25         .720
35.25         1.003
19.74         .4604
11.75         .143
0.0           0.0          1.0
200.0         200.0        200.0
```

## 2. Differential equations

```
     ALGAE BATCH CULTURE DATA ANALYSIS : EXPERIMENT 4
       5     2     1
.0833         2.5          .05          .1           .6           2.5
       3     0     3     3
1.0482        1.198        .184
1.2568        .992         .193
1.6335        .835         .076
1.9204        .715         .065
2.0838        .674         .035
2.2315        .642         .007
2.2292        .627         .043
2.2961        .606         .053
       3     1     3     0
       8
.5162         2.148        .331
24.           36.          60.          84.          108.         156.         204.0
52.
5.0           50.0         5.0
0.0           .5           0.0          0.0          0.0          .5
10.0          100.0        10.0         10.0         10.0         100.0
```

# Appendix IV

## Results

### A. Data from the Batch-culture Experiments

Included in this appendix are lists of data from the eight batch-culture experiments. Experiment 8 was the light/dark experiment. There are some missing chlorophyll-a values at the beginning of experiments - these were omitted because of unrealistic acid ratios. Some cell counts are missing because the signal to noise ratio was low due to electrical interference. Cell counts were often precise to 4 decimal places (100 cells $ml^{-1}$), depending on the background. Only three digits are given here ($10^{-3}$ x $10^{6}$ cells = $10^{3}$ cells). The values of chlorophyll-a and Q during the lag phases were calculated using the mean of X during this period. The letters "a" and "b" in the last columns of the lists mark the beginning and end of the exponential phase. The last sample in the exponential phase was treated at the first sample in the nutrient-controlled phase. The columns in each list represent:

1. Time from the beginning of the experiment (hr)
2. Time from the beginning of the P-controlled phase (hr)
3. X = cell counts ($10^{6}$ cells $ml^{-1}$) and ln X (cells $ml^{-1}$)
4. Q = phosphorus cell-quota (nM P$(10^{6}$ cells$)^{-1}$)
5. S = dissolved phosphorus (nM P $ml^{-1}$)
6. Chl-a = chlorophyll-a (µg chlorophyll-a $(10^{6}$ cells$)^{-1}$)

EXPERIMENT 1

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0 | | .057 | 10.947 | 1.946 | 1.310 | .070 | |
| 2 | .25 | | .067 | 11.114 | 4.597 | 1.134 | .100 | |
| 3 | .50 | | -- | 11.106 | 7.040 | .971 | -- | |
| 4 | .75 | | .065 | 11.074 | 7.654 | .930 | .070 | |
| 5 | 1.0 | | .068 | 11.133 | 7.909 | .913 | .067 | |
| 6 | 1.5 | | .064 | 11.065 | 9.291 | .821 | .073 | |
| 7 | 2.0 | | .065 | 11.079 | 7.150 | .964 | .079 | |
| 8 | 3.0 | | .066 | 11.103 | 5.429 | 1.078 | .089 | |
| 9 | 4.0 | | .075 | 11.220 | 6.595 | 1.001 | .046 | |
| 10 | 6.0 | | .073 | 11.200 | 5.338 | 1.085 | .082 | |
| 11 | 10.0 | | .072 | 11.179 | 6.317 | .988 | .105 | |
| 12 | 14.0 | | .082 | 11.318 | 8.157 | .769 | .091 | -a |
| 13 | 22.0 | | .095 | 11.463 | 5.977 | .872 | .053 | |
| 14 | 24.0 | | .102 | 11.529 | 5.445 | .887 | .066 | |
| 15 | 36.0 | | .148 | 11.903 | 4.263 | .810 | .104 | |
| 16 | 48.0 | | .208 | 12.244 | 3.374 | .739 | .148 | |
| 17 | 60.0 | | .311 | 12.648 | 2.564 | .642 | .133 | |
| 18 | 72.0 | 0.0 | .551 | 13.219 | 1.790 | .454 | .083 | -b |
| 19 | 96.0 | 24.0 | .964 | 13.779 | 1.276 | .210 | .117 | |
| 20 | 120.0 | 48.0 | 1.463 | 14.196 | .776 | .305 | .109 | |
| 21 | 168.0 | 96.0 | 2.050 | 14.533 | .649 | .109 | .094 | |
| 22 | 216.0 | 144.0 | 2.261 | 14.631 | .589 | .108 | .066 | |
| 23 | 268.0 | 196.0 | 2.186 | 14.598 | .645 | .031 | .054 | |
| 24 | 312.0 | 240.0 | 2.200 | 14.604 | .619 | .124 | .036 | |

EXPERIMENT 2

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0 | | .062 | 11.032 | 4.991 | 1.198 | .112 | |
| 2 | .25 | | .049 | 10.806 | 9.938 | .958 | .077 | |
| 3 | .50 | | .051 | 10.834 | 13.034 | .808 | .093 | |
| 4 | .75 | | .045 | 10.710 | 13.934 | .764 | .085 | |
| 5 | 1.0 | | .047 | 10.751 | 16.309 | .649 | .106 | |
| 6 | 1.25 | | .045 | 10.708 | 16.039 | .662 | .085 | |
| 7 | 1.5 | | .047 | 10.751 | 14.409 | .741 | .097 | |
| 8 | 2.0 | | .050 | 10.812 | 10.983 | .907 | .131 | |
| 9 | 3.0 | | .044 | 10.681 | 11.882 | .864 | .072 | |
| 10 | 4.0 | | .048 | 10.781 | 12.720 | .823 | .079 | |
| 11 | 5.0 | | .048 | 10.777 | 10.564 | .928 | .067 | |
| 12 | 6.0 | | .049 | 10.795 | 11.671 | .874 | .066 | |
| 13 | 8.0 | | .046 | 10.734 | 11.407 | .867 | .075 | |
| 14 | 10.0 | | .050 | 10.820 | 12.722 | .823 | .127 | |
| 15 | 12.0 | | .054 | 10.889 | 10.169 | .895 | .127 | |
| 16 | 20.0 | | .059 | 10.977 | 8.761 | .928 | .130 | -a |
| 17 | 24.0 | | .060 | 10.999 | 8.438 | .935 | .134 | |
| 18 | 36.0 | | .093 | 11.441 | 6.666 | .819 | .130 | |
| 19 | 48.0 | | .146 | 11.892 | 4.879 | .727 | .161 | |
| 20 | 60.0 | | .225 | 12.323 | 4.502 | .428 | .132 | |
| 21 | 72.0 | | .329 | 12.703 | 3.456 | .304 | .133 | |
| 22 | 96.0 | 0.0 | .780 | 13.567 | 1.537 | .241 | .115 | |
| 23 | 120.0 | 24.0 | 1.297 | 14.076 | 1.008 | .133 | .096 | -b |
| 24 | 144.0 | 48.0 | 1.481 | 14.208 | .877 | .142 | .068 | |
| 25 | 168.0 | 72.0 | 1.711 | 14.353 | .793 | .084 | .074 | |
| 26 | 216.0 | 120.0 | 1.920 | 14.468 | .701 | .094 | .069 | |
| 27 | 264.0 | 168.0 | 1.900 | 14.457 | .695 | .120 | .052 | |
| 28 | 312.0 | 216.0 | 1.929 | 14.472 | .697 | .096 | .046 | |

## EXPERIMENT 3

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0 | | .041 | 10.612 | 3.371 | 1.313 | -- | |
| 2 | .25 | | .045 | 10.703 | 7.718 | 1.148 | -- | |
| 3 | .50 | | .033 | 10.416 | 10.510 | 1.043 | -- | |
| 4 | .75 | | .053 | 10.874 | 11.482 | 1.006 | .166 | |
| 5 | 1.0 | | .047 | 10.762 | 12.518 | .967 | .173 | |
| 6 | 1.25 | | .047 | 10.751 | 12.865 | .954 | .194 | |
| 7 | 1.5 | | .033 | 10.392 | 14.339 | .898 | -- | |
| 8 | 1.75 | | .034 | 10.431 | 17.623 | .774 | .215 | |
| 9 | 2.0 | | .035 | 10.466 | 24.488 | .514 | .224 | |
| 10 | 2.25 | | .031 | 10.432 | 24.042 | .531 | .254 | |
| 11 | 3.0 | | .030 | 10.309 | 13.992 | .911 | .214 | |
| 12 | 4.0 | | .039 | 10.569 | 15.954 | .837 | .142 | |
| 13 | 5.0 | | .033 | 10.389 | 10.690 | 1.036 | -- | |
| 14 | 6.0 | | .030 | 10.309 | 18.008 | .759 | .363 | |
| 15 | 9.0 | | .039 | 10.564 | 21.440 | .610 | .289 | |
| 16 | 12.0 | | .040 | 10.592 | 21.104 | .600 | .324 | -a |
| 17 | 18.0 | | .043 | 10.669 | 18.887 | .628 | .367 | |
| 18 | 24.0 | | .058 | 10.975 | 11.061 | .794 | .359 | |
| 19 | 36.0 | | .088 | 11.388 | 9.914 | .565 | .262 | |
| 20 | 48.0 | | .137 | 11.831 | 6.756 | .512 | .327 | |
| 21 | 60.0 | | .226 | 12.327 | 4.696 | .380 | .356 | |
| 22 | 72.0 | | .387 | 12.867 | 2.385 | .516 | .342 | |
| 23 | 84.0 | 0.0 | .613 | 13.327 | 1.908 | .269 | .265 | -b |
| 24 | 96.0 | 12.0 | .942 | 13.755 | 1.252 | .261 | .267 | |
| 25 | 120.0 | 36.0 | 1.431 | 14.174 | .997 | .013 | .273 | |
| 26 | 168.0 | 84.0 | 2.021 | 14.519 | .650 | .127 | .245 | |
| 27 | 216.0 | 132.0 | 1.978 | 14.498 | .677 | .100 | .292 | |
| 28 | 264.0 | 180.0 | 2.231 | 14.618 | .574 | .160 | .217 | |
| 29 | 312.0 | 228.0 | 2.053 | 14.535 | .651 | .103 | .173 | |

EXPERIMENT 4

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0 | | .021 | 9.957 | 2.091 | 1.397 | -- | |
| 2 | .25 | | .026 | 10.181 | 4.979 | 1.333 | -- | |
| 3 | .50 | | .018 | 9.787 | 4.979 | 1.333 | -- | |
| 4 | .75 | | .021 | 9.938 | 5.652 | 1.319 | .210 | |
| 5 | 1.0 | | .020 | 9.898 | 6.191 | 1.308 | .224 | |
| 6 | 1.25 | | .026 | 10.158 | 8.546 | 1.257 | -- | |
| 7 | 1.50 | | .023 | 10.035 | 6.796 | 1.295 | .196 | |
| 8 | 1.75 | | -- | -- | 6.864 | 1.293 | -- | |
| 9 | 2.0 | | -- | -- | 7.133 | 1.287 | -- | |
| 10 | 2.5 | | -- | -- | 7.402 | 1.282 | -- | |
| 11 | 3.5 | | -- | -- | 8.142 | 1.266 | -- | |
| 12 | 4.5 | | .017 | 9.758 | 8.680 | 1.254 | .271 | |
| 13 | 5.5 | | .019 | 9.842 | 9.622 | 1.234 | -- | |
| 14 | 8.5 | | .024 | 10.077 | 13.929 | 1.142 | .211 | |
| 15 | 11.5 | | .025 | 10.135 | 13.399 | 1.030 | .206 | |
| 16 | 18.0 | | .025 | 10.123 | 32.552 | .635 | .258 | |
| 17 | 24.0 | | .026 | 10.177 | 26.172 | .795 | .262 | |
| 18 | 30.0 | | .029 | 10.825 | 27.080 | .647 | .308 | |
| 19 | 36.0 | | .029 | 10.289 | 27.232 | .639 | .352 | -a |
| 20 | 48.0 | | .042 | 10.633 | 18.841 | .658 | .367 | |
| 21 | 60.0 | | .072 | 11.177 | 13.373 | .484 | .380 | |
| 22 | 72.0 | | .124 | 11.726 | 6.950 | .580 | .382 | |
| 23 | 84.0 | | .185 | 12.130 | 4.497 | .606 | .337 | |
| 24 | 108.0 | 0.0 | .516 | 13.154 | 2.148 | .331 | .329 | -b |
| 25 | 132.0 | 24.0 | 1.048 | 13.863 | 1.198 | .184 | .361 | |
| 26 | 144.0 | 36.0 | 1.257 | 14.044 | .992 | .193 | .371 | |
| 27 | 168.0 | 60.0 | 1.634 | 14.306 | .835 | .076 | .309 | |
| 28 | 192.0 | 84.0 | 1.920 | 14.468 | .715 | .065 | .335 | |
| 29 | 216.0 | 108.0 | 2.084 | 14.550 | .674 | .035 | .288 | |
| 30 | 264.0 | 156.0 | 2.232 | 14.618 | .642 | .007 | .310 | |
| 31 | 312.0 | 204.0 | 2.229 | 14.617 | .627 | .043 | .311 | |
| 32 | 330.0 | 252.0 | 2.290 | 14.644 | .606 | .053 | .230 | |

EXPERIMENT 5

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0 | | .046 | 10.728 | 5.645 | 1.236 | .350 | |
| 2 | .25 | | .036 | 10.491 | 8.942 | 1.116 | -- | |
| 3 | .50 | | .034 | 10.443 | 11.102 | 1.038 | -- | |
| 4 | .75 | | .037 | 10.521 | 12.868 | .974 | -- | |
| 5 | 1.0 | | .033 | 10.413 | 11.098 | 1.038 | .432 | |
| 6 | 1.25 | | .037 | 10.511 | 10.302 | 1.067 | -- | |
| 7 | 1.50 | | .033 | 10.404 | 12.455 | .989 | .432 | |
| 8 | 1.75 | | .037 | 10.513 | 21.218 | .672 | .398 | |
| 9 | 2.0 | | .037 | 10.524 | 11.333 | 1.030 | -- | |
| 10 | 2.50 | | .036 | 10.500 | 12.703 | .980 | -- | |
| 11 | 3.0 | | .039 | 10.569 | 11.273 | 1.032 | -- | |
| 12 | 4.0 | | .033 | 10.404 | 14.344 | .921 | .520 | |
| 13 | 6.0 | | .033 | 10.398 | 15.502 | .879 | .530 | |
| 14 | 17.5 | | .052 | 10.853 | 14.871 | .671 | .459 | -a |
| 15 | 22.5 | | .062 | 11.037 | 9.169 | .871 | .491 | |
| 16 | 29.5 | | .076 | 11.235 | 10.575 | .640 | .577 | |
| 17 | 41.5 | | .123 | 11.716 | 6.679 | .622 | .561 | |
| 18 | 45.5 | | .132 | 11.791 | 7.331 | .472 | .602 | |
| 19 | 49.5 | | .157 | 11.966 | 6.089 | .482 | .587 | |
| 20 | 53.5 | | .195 | 12.183 | 4.873 | .488 | .519 | |
| 21 | 57.5 | | .225 | 12.322 | 4.610 | .405 | .502 | |
| 22 | 61.5 | | .257 | 12.458 | 3.617 | .510 | .540 | |
| 23 | 69.5 | | .342 | 12.741 | 3.206 | .345 | .479 | |
| 24 | 77.5 | | .473 | 13.067 | 2.485 | .265 | .537 | |
| 25 | 89.5 | 0.0 | .734 | 13.507 | 1.703 | .170 | .553 | -b |
| 26 | 101.5 | 12.0 | .988 | 13.804 | 1.227 | .227 | .503 | |
| 27 | 113.5 | 24.0 | 1.179 | 13.980 | 1.138 | .099 | .562 | |
| 28 | 149.5 | 60.0 | 1.659 | 14.322 | .853 | .025 | .580 | |
| 29 | 173.5 | 84.0 | 2.026 | 14.521 | .676 | .071 | .577 | |
| 30 | 197.5 | 108.0 | 2.246 | 14.625 | .589 | .117 | .538 | |
| 31 | 221.5 | 132.0 | 2.350 | 14.670 | .560 | .123 | .551 | |
| 32 | 269.5 | 180.0 | 2.429 | 14.403 | .575 | .043 | .528 | |
| 33 | 293.5 | 204.0 | 2.428 | 14.702 | .571 | .055 | .605 | |
| 34 | 317.5 | 228.0 | 2.421 | 14.700 | .550 | .107 | .632 | |
| 35 | 341.5 | 252.0 | 2.424 | 14.701 | .570 | .058 | .590 | |

EXPERIMENT 6

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0 | | | | 5.255 | 1.380 | .589 | |
| 2 | .25 | | | | 6.644 | 1.364 | .549 | |
| 3 | .50 | | | | 7.238 | 1.358 | -- | |
| 4 | .75 | | | | 8.728 | 1.341 | -- | |
| 5 | 1.0 | | | | 10.118 | 1.325 | .349 | |
| 6 | 1.25 | | | | 8.880 | 1.339 | -- | |
| 7 | 1.5 | | | | 9.903 | 1.327 | -- | |
| 8 | 3.5 | | | | 13.023 | 1.292 | .349 | |
| 9 | 5.5 | | | | 16.131 | 1.256 | .399 | |
| 10 | 24.0 | | .017 | 9.723 | 22.281 | 1.068 | .426 | -a |
| 11 | 28.5 | | .018 | 9.815 | 24.881 | .985 | .467 | |
| 12 | 42.5 | | .030 | 10.322 | 18.009 | .893 | .496 | |
| 13 | 50.5 | | .042 | 10.648 | 13.100 | .888 | .488 | |
| 14 | 66.5 | | .074 | 11.215 | 9.376 | .744 | .538 | |
| 15 | 78.5 | | .123 | 11.717 | 8.053 | .453 | .485 | |
| 16 | 90.5 | | .191 | 12.159 | 5.513 | .388 | .499 | |
| 17 | 100.5 | | .278 | 12.535 | 3.950 | .342 | .534 | |
| 18 | 114.5 | | .463 | 13.046 | 2.684 | .197 | .499 | |
| 19 | 126.5 | 0.0 | .699 | 13.458 | 1.783 | .194 | .589 | |
| 20 | 138.5 | 12.0 | .984 | 13.799 | 1.266 | .195 | .559 | -b |
| 21 | 150.5 | 24.0 | 1.246 | 14.036 | 1.075 | .100 | .592 | |
| 22 | 164.5 | 38.0 | 1.559 | 14.260 | .839 | .132 | .557 | |
| 23 | 188.5 | 62.0 | 2.019 | 14.518 | .708 | .011 | .570 | |
| 24 | 212.5 | 86.0 | 2.239 | 14.621 | .635 | .018 | .601 | |
| 25 | 268.5 | 142.0 | 2.467 | 14.718 | .582 | .003 | .571 | |
| 26 | 309.5 | 183.0 | 2.481 | 14.724 | .557 | .057 | .582 | |
| 27 | 333.5 | 207.0 | 2.509 | 14.735 | .541 | .082 | .599 | |
| 28 | 384.5 | 258.0 | 2.456 | 14.714 | .571 | .037 | .628 | |

A mean value of cell numbers was used to calculate Q and Chl-a for samples 1-9. The mean of X = .011 x $10^6$ cells ml.

EXPERIMENT 7

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0 | | .028 | 10.256 | 1.652 | 1.432 | .605 | |
| 2 | 2.0 | | .022 | 9.984 | 2.102 | 1.434 | .558 | |
| 3 | 4.0 | | .024 | 10.065 | 3.410 | 1.399 | .515 | |
| 4 | 6.0 | | .025 | 10.129 | 4.634 | 1.362 | -- | |
| 5 | 8.0 | | .021 | 10.085 | 5.216 | 1.368 | .803 | |
| 6 | 12.0 | | .028 | 10.234 | 5.309 | 1.331 | .681 | $-a_1$ |
| 7 | 24.0 | | .031 | 10.346 | 5.255 | 1.315 | .645 | |
| 8 | 36.0 | | .033 | 10.413 | 4.785 | 1.319 | .674 | |
| 9 | 49.0 | | .040 | 10.594 | 5.040 | 1.277 | .636 | $-b_1$ |
| 10 | 60.0 | | .041 | 10.620 | 5.756 | 1.242 | .678 | |
| 11 | 72.0 | | .045 | 10.711 | 4.356 | 1.283 | .717 | $-a_2$ |
| 12 | 84.0 | | .053 | 10.880 | 5.145 | 1.204 | .753 | |
| 13 | 96.0 | | .063 | 11.052 | 4.149 | 1.216 | .752 | |
| 14 | 106.0 | | .071 | 11.176 | 4.814 | 1.133 | .738 | |
| 15 | 120.0 | | .090 | 11.407 | 4.397 | 1.081 | .690 | |
| 16 | 130.5 | | .102 | 11.533 | 4.965 | .969 | .765 | |
| 17 | 144.0 | | .128 | 11.761 | 6.010 | .704 | .767 | |
| 18 | 158.0 | | .164 | 12.005 | 6.202 | .457 | .714 | |
| 19 | 168.0 | | .191 | 12.161 | 6.301 | .266 | .731 | |
| 20 | 181.0 | | .239 | 12.382 | 5.589 | .136 | .764 | |
| 21 | 192.25 | | .275 | 12.526 | 4.857 | .132 | .751 | |
| 22 | 216.25 | | .404 | 12.908 | 3.425 | .087 | .740 | |
| 23 | 226.75 | | .454 | 13.025 | 3.204 | .015 | .689 | |
| 24 | 240.25 | | .556 | 13.228 | 2.518 | .070 | .663 | |
| 25 | 254.5 | 0.0 | .680 | 13.430 | 2.061 | .067 | .656 | $-b_2$ |
| 26 | 275.0 | 20.5 | .811 | 13.606 | 1.733 | .064 | .666 | |
| 27 | 296.667 | 42.17 | .843 | 13.645 | 1.538 | .173 | .782 | |
| 28 | 321.667 | 67.17 | .912 | 13.723 | 1.562 | .045 | .927 | |
| 29 | 363.75 | 109.25 | 1.017 | 13.832 | 1.430 | .015 | 1.171 | |
| 30 | 390.25 | 135.75 | 1.075 | 13.887 | 1.323 | .046 | 1.181 | |
| 31 | 441.25 | 186.75 | 1.237 | 14.028 | 1.006 | .225 | 1.356 | |
| 32 | 467.5 | 213.0 | 1.566 | 14.264 | .894 | .068 | -- | |
| 33 | 530.0 | 275.5 | 1.874 | 14.444 | .753 | .058 | 1.064 | |

EXPERIMENT 7 CONTINUED

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a |
|---|---|---|---|---|---|---|---|
| 34 | 581.25 | 326.75 | 1.977 | 14.497 | .599 | .286 | .938 |
| 35 | 636.25 | 381.75 | 2.013 | 14.515 | .662 | .136 | 1.119 |
| 36 | 655.5 | 401.0 | 2.158 | 14.585 | .595 | .186 | 1.049 |
| 37 | 680.0 | 425.5 | 2.165 | 14.588 | .556 | .266 | .905 |
| 38 | 702.0 | 447.5 | 2.188 | 14.598 | .582 | .196 | 1.057 |
| 39 | 720.0 | 470.5 | 2.181 | 14.595 | .577 | .212 | .942 |

Note: $a_1 - b_1$ represents the exponential phase at the first level of irradiance. The irradiance was higher for the rest of the experiment.

EXPERIMENT 8

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0 | | .053 | 10.884 | 11.642 | .863 | .583 | $-a_1$ |
| 2 | 24.0 | | .066 | 11.093 | 9.773 | .842 | .526 | |
| 3 | 36.0 | | .066 | 11.091 | 11.039 | .759 | .641 | |
| 4 | 48.0 | | .087 | 11.378 | 12.932 | .350 | .497 | |
| 5 | 72.0 | | .110 | 11.604 | 8.376 | .564 | .555 | |
| 6 | 96.0 | | .141 | 11.859 | 7.082 | .480 | .617 | $-b_1$ |
| 7 | 120.0 | | .206 | 12.234 | 4.760 | .502 | .559 | $-a_2$ |
| 8 | 145.0 | | .268 | 12.498 | 4.122 | .376 | .752 | |
| 9 | 157.33 | | .354 | 12.778 | 2.324 | .659 | .761 | |
| 10 | 157.83 | | .355 | 12.780 | 2.490 | .598 | .764 | |
| 11 | 159.0 | | .369 | 12.818 | 2.716 | .479 | .748 | |
| 12 | 161.0 | | .396 | 12.888 | 2.795 | .374 | .702 | |
| 13 | 163.0 | | .427 | 12.964 | 2.905 | .239 | .651 | |
| 14 | 165.0 | | .440 | 12.994 | 2.593 | .340 | .636 | |
| 15 | 166.0 | | .447 | 13.009 | 2.522 | .354 | .601 | |
| 16 | 168.0 | 0.0 | .440 | 12.995 | 2.360 | .441 | .614 | $-b_2$ |
| 17 | 170.0 | | .436 | 12.984 | 2.429 | .422 | .670 | |
| 18 | 173.0 | | .464 | 13.047 | 2.481 | .329 | .710 | |
| 19 | 177.0 | | .464 | 13.048 | 2.126 | .495 | .762 | |
| 20 | 181.0 | | .512 | 13.147 | 1.855 | .531 | .728 | |
| 21 | 192.0 | 24.0 | .641 | 13.370 | 1.719 | .379 | .608 | |
| 22 | 216.0 | 48.0 | .863 | 13.668 | 1.431 | .244 | .810 | |
| 23 | 230.0 | | .968 | 13.783 | 1.414 | .110 | .883 | |
| 24 | 232.0 | | 1.015 | 13.831 | 1.374 | .083 | .880 | |
| 25 | 234.0 | | 1.090 | 13.902 | 1.341 | .016 | .811 | |
| 26 | 236.0 | | 1.096 | 13.908 | 1.248 | .110 | .859 | |
| 27 | 238.0 | | 1.147 | 13.953 | 1.213 | .087 | .821 | |
| 28 | 241.0 | 73.0 | 1.135 | 13.942 | 1.170 | .151 | .834 | |
| 29 | 244.0 | | 1.141 | 13.948 | 1.173 | .139 | .847 | |
| 30 | 247.0 | | 1.139 | 13.946 | 1.164 | .152 | .884 | |
| 31 | 250.0 | | 1.145 | 13.950 | 1.074 | .250 | .869 | |
| 32 | 253.0 | | 1.214 | 14.009 | 1.000 | .266 | .849 | |
| 33 | 264.0 | 96.0 | 1.379 | 14.137 | .961 | .154 | .783 | |

EXPERIMENT 8 CONTINUED

| Sample No. | 1 Time | 2 Time | 3 X | lnX | 4 Q | 5 S | 6 Chl-a |
|---|---|---|---|---|---|---|---|
| 34 | 312.0 | 144.0 | 1.813 | 14.411 | .713 | .186 | .745 |
| 35 | 336.0 | 168.0 | 1.946 | 14.481 | .699 | .118 | .763 |
| 36 | 360.0 | 192.0 | 2.053 | 14.535 | .647 | .151 | .733 |
| 37 | 384.5 | 216.5 | 2.229 | 14.617 | .646 | .038 | .734 |
| 38 | 408.5 | 240.5 | 2.269 | 14.635 | .649 | .004 | .759 |
| 39 | 432.0 | 264.0 | 2.426 | 14.702 | .590 | .046 | .722 |
| 40 | 456.0 | 288.0 | 2.505 | 14.734 | .576 | .035 | .736 |
| 41 | 480.0 | 312.0 | 2.600 | 14.771 | .567 | .004 | .738 |
| 42 | 504.0 | 336.0 | 2.550 | 14.752 | .565 | .036 | -- |
| 43 | 528.0 | 360.0 | 2.532 | 14.744 | .582 | .004 | .756 |

Note: $a_1 - b_1$ represents the exponential phase at the first level of irradiance. The irradiance was higher for the rest of the experiment.

## Appendix IVB

## Discussion of Chemostat "Steady State"

One aspect of the design of the batch-culture experiments in this study was to start the cultures with an inoculum with a known "prehistory". This was to be accomplished by using an aliquot taken from a chemostat. Furthermore, inocula taken at the same dilution rate would provide algae in the same physiological state, which would allow the batch cultures to start with the same initial conditions (of biomass and cell quota). It was not possible to meet the above criterion because the biomass and cell-quota of phosphorus (the chemostats were phosphorus limited) did not, over a period of 2-3 months, stay in steady state.

Over the period of a few months at the same growth rate, $D = .32\ day^{-1}$, the concentration of cells slowly decreased, the phosphorus cell-quota increased as did dissolved phosphorus. The values of cell quota and dissolved phosphorus eventually reached levels which would <u>apparently</u> indicate that phosphorus was not limiting. The measures from the chemostat, sampled at 1-3 week intervals, are given below.

| TIME → | | | | | | | |
|---|---|---|---|---|---|---|---|
| $X$ ($10^6$ cells $ml^{-1}$) | 2.201 | 2.010 | 1.726 | 1.551 | 1.235 | .969 | .603 | .267 |
| $Q$ (nM P $(10^6$ cells$)^{-1}$) | .64 | .69 | .78 | .86 | 1.05 | 1.12 | 1.84 | 4.00 |
| $S$ (nM P $ml^{-1}$) | .031 | .053 | .094 | .106 | .143 | .354 | .330 | .372 |

It seems that over a period of time the algae adjust to the growth conditions and become, as Droop (1974, 1975)

described, "slow-adapted". The efficiency of the growth mechanism relative to phosphorus cell-quota decreased to the point where a growth rate of .32 $day^{-1}$ was maintained at a cell quota of 4.00 nM P $10^6$ cells - a level which would usually indicate that P is not limiting.

The change in the population was probably not a genetic change because the algae began to grow at a reproducible, exponential growth rate ($\mu_m$ at the beginning of the batch culture experiments) within twenty hours after being added to the batch-culture medium (by the time the cells had doubled once, the growth rate was at a constant maximum).

I can only guess why this phenomenon is not often observed, or how the problem can be overcome. Most chemostats are started with a small inocula which is allowed to grow until the culture is in steady state, or the reactor is sampled, leaving a small amount of culture, and the volume is raised so the algae can grow at a new dilution rate. In both cases the cultures have grown unrestricted, for at least a short period, until a steady state is observed. By changing the dilution rate regularly, the insidious change in the growth rate/P-quota relationship is not observed.

## Appendix IVC

## Interpretation of Size-Ratio Information from the Light/Dark Experiment (No. 8)

In Figure 18 are plotted values of a cell-size index, calculated from samples taken at 1000 hours each day, versus time. The data are given below.

| Sample No. | Index | Sample No. | Index | Sample No. | Index |
|---|---|---|---|---|---|
| 16 | 2.53 | 34 | .84 | 39 | .26 |
| 21 | 2.29 | 35 | .72 | 40 | .25 |
| 22 | 2.16 | 36 | .52 | 41 | .20 |
| 28 | 1.65 | 37 | .36 | 42 | .22 |
| 33 | 1.64 | 38 | .32 | 43 | .28 |

The index is a ratio of small cells to large cells and was calculated taking the counts per minute (CPM) from a signal-window setting (on the particle counter) representing cells with sizes ranging from 8% to 30% of the total window and dividing this number by the counts per minute representing cells from 30% to 100% of the total window. The lower threshold of 8% was chosen to minimize electrical noise, and the middle value (30%) was arbitrarily selected.

The values of the size index seem to indicate that the cells got larger as the experiment progressed into the stationary phase. I could observe the size distribution on the Coulter Counter oscilloscope and it was obvious that the average cell size was just above the middle range (30%). Therefore, from sample 37 to the end of the experiment, another pair of counts were made, with ranges of 8-40% and 40-100%, providing a measure of cells in the 30-40% range. The data is given in Tables 17a and b.

By the end of the experiment, the cells in the 30-40% range made up one third of the total population. The sizes of a single-celled population are usually normally distributed so the mean size of the population is probably between the 30-50% ranges. A complete size distribution would be very informative, providing data for comparison of size distribution within a day relative to growth rate, and the changes in distribution from day to day until the experiment ended.

Table 17.

a. Counts per minute (CPM) within different windows (size classes).

| Sample Number | 1 8-30% | 2 8-40% | 3 30-100% | 4 40-100% | 5 30-40 | 6 $CPM_1$ $(\frac{1+2+3+4}{2})$ | 7 $CPM_2$ 8-100% |
|---|---|---|---|---|---|---|---|
| 37 | 3175 | 8050 | 9168 | 4650 | 4698 | 12523 | 12825 |
| 38 | 2943 | 8189 | 9869 | 4894 | 5111 | 12950 | 13048 |
| 39 | 2774 | 8517 | 11227 | 5059 | 5956 | 13789 | 13916 |
| 40 | 2495 | 7393 | 11497 | 6821 | 4787 | 14103 | 14350 |
| 41 | 2429 | 7493 | 12405 | 7610 | 4930 | 14969 | 14870 |
| 42 | 2479 | 7233 | 11859 | 7013 | 4800 | 14292 | 14597 |
| 43 | 2989 | 7853 | 11240 | 6426 | 4838 | 14254 | 14497 |

b. Percent CPM within different windows relative to total CPM (column 7 above)

| | 1 8-30% | 2 30-40% | 3 40-100% | 4 8-100% |
|---|---|---|---|---|
| 37 | 25 | 37 | 36 | 98 |
| 38 | 23 | 39 | 38 | 100 |
| 39 | 20 | 43 | 36 | 99 |
| 40 | 17 | 33 | 48 | 98 |
| 41 | 16 | 33 | 51 | 100 |
| 42 | 17 | 33 | 48 | 98 |
| 43 | 21 | 33 | 44 | 98 |

## Appendix V

## The Results of Fitting Models to $\mu_m$ and $\mu_m'$ vs Light Data

The equations for ten models of maximum growth rate vs irradiance (the first eight are as presented by Jassby and Platt, 1976) are given below.

| Model Number | Equation | Source |
|---|---|---|
| 1 | $\mu_m = \alpha I,\ I < \mu_{opt}/\alpha$<br>$\mu_{opt},\ I > \mu_{opt}/\alpha$ | Blackman (1905) |
| 2 | $\mu_m = \mu_{opt}\ \alpha I/(\mu_{opt} + \alpha I)$ | Baly (1935) |
| 3 | $\mu_m = \mu_{opt}\ \alpha I/[(\mu_{opt})^2 + (\alpha I)^2]^{1/2}$ | Smith (1936) |
| 4 | $\mu_m = \alpha I e^{(-\alpha I/\mu_{opt} e)}$ | Steele (1962) |
| 5 | $\mu_m = \alpha I e^{(-\alpha I/\mu_{opt} e)},\ I \leq \mu_{opt} e/\alpha$<br>$\mu_{opt},\ I > \mu_{opt} e/\alpha$ | Jassby and Platt (1976) |
| 6 | $\mu_m = \mu_{opt}\ [1-e^{(-\alpha I/\mu_{opt})}$ | Webb et al. (1975) |
| 7 | $\mu_m = \alpha I - (\alpha I)^2/4\ \mu_{opt}$ $\quad I \leq 2\ \mu_{opt}/\alpha$<br>$\mu_{opt}$ $\quad I > 2\ \mu_{opt}/\alpha$ | Platt et al. (1975) |
| 8 | $\mu_m = \mu_{opt}\ \tanh\ (\alpha I/\mu_{opt})$ | Jassby and Platt (1976) |
| 9 | $\mu_m = \mu_{opt}\ (1 - {}^{k}I/_{I})$ | Droop (1968) |
| 10 | $\mu_m = \mu_{opt}\ \left(\frac{I - I_o}{K_I + I - I_o}\right)$ | Caperon (1968) |

where: $\mu_m$ = maximum growth rate ($day^{-1}$)

$\mu_{opt}$ ($\mu'_{opt}$) = optimum growth rate ($day^{-1}$)

$\alpha$ = "slope" parameter ($W^{-1}\ m^2\ day^{-1}$)

$I$ = irradiance ($W\ m^{-2}$)

$k_I$ ($I_o$) = subsistence quota for light ($W\ m^{-2}$)

$K_I$ = half-saturation constant for light ($W\ m^{-2}$)

$r$ = respiration rate ($day^{-1}$)

To incorporate respiration in the above models (1-8) the term $-r$ ($day^{-1}$) is added to each equation. In equations 9 and 10, the subsistence quota for light, $k_I$ or $I_o$, are interpreted as respiration terms (they represent the compensation point, the light level at which due to respiration, growth rate equals zero).

The estimates of the parameters in the models fit to the $\mu_m$ vs irradiance data (in Table 7) are given in Table 18. The models with and without a respiration parameter were fit to the data. Since light inhibition was significant, the preferred model is number 4 (Steele's, 1962) with a respiration term. The same equation without respiration does not give a significantly worse fit, but the data indicates that there is a compensation level of irradiance.

In Table 19 are given the estimates of the parameters in the models fit to the $\mu'_m$ vs irradiance data. Once again, the data supported the use of a respiration term, but there was no significant light-inhibition of growth rate. Model 9 is the preferred model for a variety of reasons. Models one and seven were rejected because they are

discontinuous functions (also, the good fit of model one may be fortuitous - see section IV E 2). Models 3, 6, and 8 were rejected because the values of respiration were unrealistically high (relative to growth rate). Also, since model 9 has only two parameters (the rest have three), none of the models provides a significantly better fit to the data when the variance ratios are compared (as in section IV D 1).

It is interesting to note that the estimates of $K_I$ and $I_o$ in Caperon's model (number 10) are nearly equal. Thus, model 10 becomes equivalent to model 9 (as noted in the introduction, section I F).

Table 18. Estimates of various parameters in models for $\mu_m$ vs irradiance fit to the batch-culture data. The sum of squared error (SSE) for each model is given for comparison.

$\mu_m$ vs I

| Model Number (including respiration) | SSE ($day^{-2}$) | $\alpha$ ($W^{-1}\ m^2\ day^{-1}$) | $\mu'_{opt}$ ($day^{-1}$) | r ($day^{-1}$) | $k_I$, $I_o$ ($W\ m^{-2}$) | $K_I$ ($W\ m^{-2}$) |
|---|---|---|---|---|---|---|
| 1 | .040 | .026 | .917 | .011 | | |
| 2 | .138 | .327 | 2.063 | 1.00 | | |
| 3 | .101 | .070 | 1.347 | .388 | | |
| 4 | .046 | .041 | 1.164 | .110 | (preferred model) | |
| 5 | .094 | .036 | .912 | .003 | | |
| 6 | .084 | .100 | 1.428 | .499 | | |
| 7 | .056 | .047 | 1.112 | .197 | | |
| 8 | .073 | .050 | 1.175 | .248 | | |
| 9 | .143 | | .998 | | 7.70 | |
| 10 | .138 | | 1.029 | | 7.12 | 9.52 |

| Model Number (no respiration) | SSE ($day^{-2}$) | $\alpha$ ($W^{-1}\ m^2\ day^{-1}$) | $\mu'_{opt}$ ($day^{-1}$) |
|---|---|---|---|
| 1 | .040 | .025 | .905 |
| 2 | .209 | .056 | 1.125 |
| 3 | .129 | .034 | .977 |
| 4 | .058 | .035 | 1.023 |
| 5 | .093 | .036 | .913 |
| 6 | .134 | .042 | .946 |
| 7 | .074 | .033 | .921 |
| 8 | .094 | .032 | .935 |

Table 19. Estimates of various parameters in models for $\mu'_m$ vs irradiance fit to the batch-culture data. The sum of squared error (SSE) for each model is given for comparison.

$\mu'_m$ vs I

| Model Number (including respiration) | SSE ($day^{-2}$) | $\alpha$ ($W^{-1}\ m^2\ day^{-1}$) | $\mu'_{opt}$ ($day^{-1}$) | r ($day^{-1}$) | $k_I$, $I_o$ ($W\ m^{-2}$) | $K_I$ ($W\ m^{-2}$) |
|---|---|---|---|---|---|---|
| 1 | .102 | .030 | 1.308 | .172 | | |
| 2 | .178 | .215 | 2.389 | 1.00 | | |
| 3 | .126 | .089 | 1.995 | .804 | | |
| 4 | .161 | .036 | 1.294 | .203 | | |
| 5 | .166 | .037 | 1.177 | .037 | | |
| 6 | .114 | .116 | 2.021 | .871 | | |
| 7 | .099 | .049 | 1.509 | .373 | | |
| 8 | .108 | .057 | 1.653 | .506 | | |
| 9 | .150 | | 1.267 | | 10.81 | (preferred model) |
| 10 | .149 | | 1.287 | | 10.61 | 11.91 |

| Model Number (no respiration) | SSE ($day^{-2}$) | $\alpha$ ($W^{-1}\ m^2\ day^{-1}$) | $\mu'_{opt}$ ($day^{-1}$) |
|---|---|---|---|
| 1 | .118 | .024 | 1.136 |
| 2 | .281 | .047 | 1.490 |
| 3 | .198 | .031 | 1.239 |
| 4 | .152 | .034 | 1.210 |
| 5 | .173 | .034 | 1.124 |
| 6 | .215 | .039 | 1.194 |
| 7 | .146 | .030 | 1.143 |
| 8 | .167 | .029 | 1.171 |

# Is is possible to distinguish best models for primary production processes?

## Appendix VI

## Comment on fitting models to data

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Mathematical models are basic tools in production ecology and ecosystems modelling. We are concerned that, when several models are available, selection is sometimes made on an erroneous basis. Thus, a variety of models have been proposed to describe 1) photosynthesis-light and 2) nutrient-growth relationships for algae. Jassby and Platt (1976) have recently listed and compared a number of models for the photosynthesis-light relationship, and Nyholm (1977) has done the same for nutrient-growth. Each author arrives at the conclusion that there is a best model. We can show that, given realistic levels of experimental error, most of these models are so similar that they cannot be discriminated on the basis of their ability to fit real or simulated data. We dc this using simulated data, on the grounds that if models cannot be distinguished with perfectly understood data, there must be doubt about positive results obtained with data of less well-known statistical properties.

In Figures 35a and 36a are graphs of the various models drawn using parameter values chosen by the relevant authors. Their estimation techniques are, however, questionable, and involve the implicit or explicit assumption that one or more parameters have a real physiological meaning. Jassby and Platt (Figure 35a) estimated only one parameter ($P^B_{max}$) independently for each model, using a common estimate for the other parameter ($\alpha$). Nyholm (Figure 36a) used a variety of techniques to obtain his estimates. We contend that, to compare models, the same estimation techniques be used for all models, and that parameters within a model must be es-

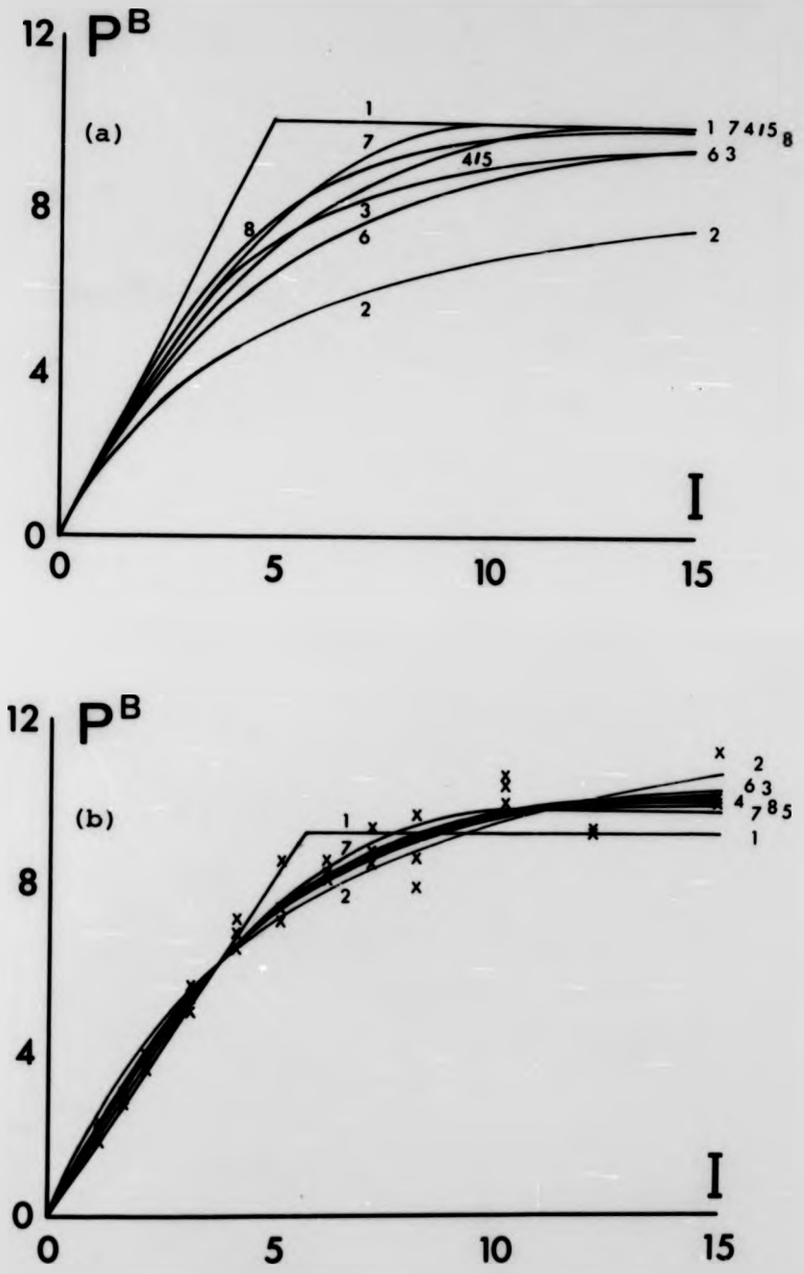


Figure 35. Curves produced by several models of photosynthesis ($P^B$) vs irradiance (I). (a) The values of the parameters in the models are the same. (b) The models fit to simulated, noise-corrupted data - the parameters in each model were independently estimated.

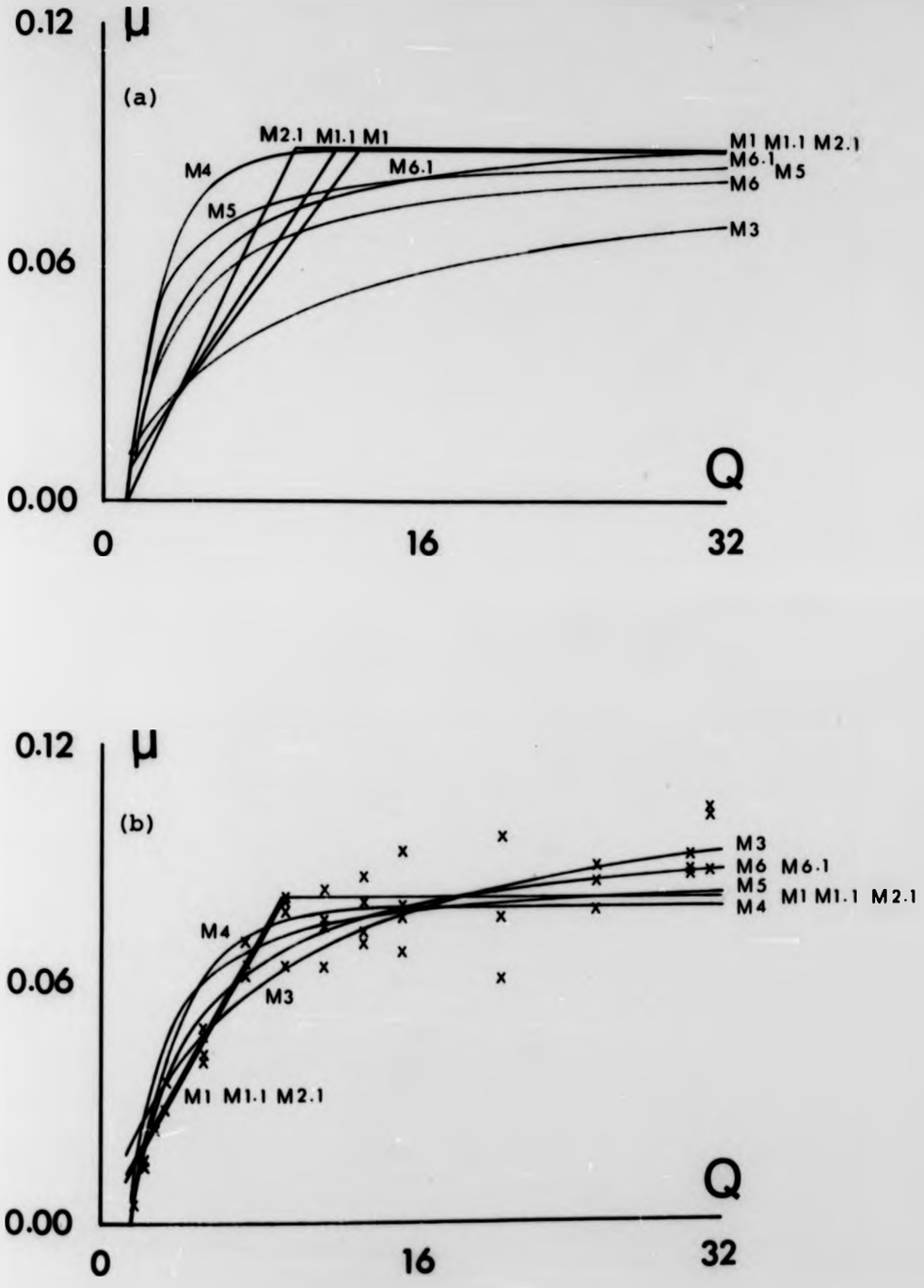


Figure 36. Curves produced by several models of growth rate (μ) vs cell quota (Q). (a) The values of the parameters in the models were calculated by various estimation techniques. (b) The models fit to simulated, noise-corrupted data - the parameters in each model were estimated using the same procedure.

timated simultaneously and independently.

Many suitable nonlinear parameter estimation techniques are available: these include various library programs available at most computer centers and a comprehensive package designed by Bard (1967b). Figures 35b and 36b show the result of independently fitting each model to 48 points generated by the relevant "preferred" model (the model that, according to Jassby and Platt or Nyholm, fits best). The points were noise-corrupted with a random error having a mean of zero and a standard deviation equal to expected measurement error. This was 8.5% for $P^B$, photosynthesis per unit chlorophyll, based on errors given by Strickland and Parsons (1972), and 10% for cell nutrient content, Q, based on data for replicate measurements in chemostat experiments described by Droop (1968, 1974), Eppley and Renger (1974) and Paasche (1973a).

It is obvious from Figures 35b and 36b that a subjective choice of best model is very difficult: most curves fit well because the parameters defining their initial slope and upper limit were varied independently and simultaneously during the fitting process. The goodness of each model's fit can be assessed objectively using the sum of squares of the differences between observed and model-predicted values (SSE). Testing of the model with smallest SSE against the others shows no differences significant at $p \leq 0.05$ between non-linear models. (That is, only the two-step linear models, 1 in Figure 35b and m1, M1.1 and M2.1 in Figure 36b, are shown to fit the data significantly more badly). As it is impossible to distinguish between the

models using data generated from one model, it is logically impossible to distinguish the models using experimental or field data, unless this can be collected with errors appreciably smaller than usual.

Jassby and Platt (1976) and Nyholm (1977) came to erroneous conclusions because of the estimation techniques they used. We wish to bring to the notice of ecologists using models of algal physiological responses the need to avoid spurious selection of the best model through incorrect methods for parameter estimation. In circumstances such as we have discussed (and which should always be investigated, using simulated data, before experimental work is begun), we suggest that the following criteria be used in addition to goodness of fit when choosing the best from a set of similar models. These criteria are 1) simplicity (smallest number of parameters), 2) elegance (least complexity and greatest ease in calculation), 3) catholicism (ability to fit data from a range of circumstances) and 4) conservatism (preference for long- or widely-used models). A criterion that we think should not be used by ecologists is that of the physiological significance of model parameters, as this poses a number of complex problems.

Copies of simulated data sets and results are available.

(This concludes the manuscript; further discussion follows).

Jassby and Platt (1976) concluded that one model from a set of eight models was a superior representation of 185 sets of photosynthesis vs light data. They based their conclusions on two, sometimes conflicting, criteria for choosing the best model:

> 1) The model's abilities to describe data - measured in terms of the sum of squared error (SSE, a model's prediction minus the observed measure of photosynthesis). The model with the smallest SSE is the best.
>
> 2) A model whose parameters have physiological significance is considered superior.

They could not fit the models to the data by using an objective parameter estimation procedure; so, they first chose $\alpha$, the initial slope of the photosynthesis/light curve, and then estimated $P_m^B$, the maximum level of photosynthesis. Lederman and Tett argue (in reference to criterion number two) that the models are simply empirical equations in which there are parameters that, for the sake of convenience or conceptualization, may be thought of as also having a biological reality. (For example: $P_m^B$, a parameter in an abstract model, should not be confused with the maximum observed rate of photosynthesis, a biological reality). These parameters, however, may not have a reality outside their function in the equations. For this reason, it may be better to make comparisons between models based on their abilities to describe data. It is shown, through use of an objective parameter estimation procedure and a simulated data set (with a known distribution or error), that most of the models offer very similar predictions.

A similar interpretation can be made of the abilities of the models presented by Nyholm (1977) - his models were for steady-state growth rate vs cell quota in algal chemostats. The reason why Nyholm was able to select one model as best is because he used different estimation procedures with different models. In fact, by using the same, objective estimation-procedure on all the models, it becomes obvious that some of the models are algebraically equivalent.

It can be dangerous to compare models when the estimation procedure and the models are not understood. A study using simulated data and the various models (as carried out by Lederman, 1974) can show whether a proposed, experimental study is theoretically plausible. Lederman and Tett raise this point in the comment presented above.

Moreover, further evidence that most of Jassby and Platt's models are very similar can be provided by fitting the models to their set of field data (Irwin et al., 1975) - the use of real data provides superfluous evidence and would be inconclusive without a study using simulated data. The fit of the eight models to the 185 data sets demonstrates that the conclusions, based on simulated data, were correct (Table 20). Using the objective estimation procedure, a different model from Jassby and Platt's preferred one gave a smaller sum of squared error (but the difference is insignificant - as the theoretical study predicts).

Table 20. Sum of squared errors for the light models presented by Jassby and Platt (1976) and fit to the 185 data sets from their field study. The models were all modified to include a respiration term (as given in Appendix V). Variance ratios relative to model 6 are given, along with the levels of significance.

| Model number | Sum of squared error | Variance ratio ($F_{185, 185}$) | $\alpha$ |
|---|---|---|---|
| 1 | 780.99 | 1.454 | .01 |
| 2 | 696.22 | 1.296 | .05 |
| 3 | 550.65 | 1.025 | n.s. |
| 4 | 2042.2 | 3.802 | .001 |
| 5 | 557.26 | 1.037 | n.s. |
| 6 | 537.19 | 1.000 | - |
| 7 | 563.17 | 1.048 | n.s. |
| 8* | 543.54 | 1.012 | n.s. |

*The model selected as best by Jassby and Platt.