A chemostatic system was developed for the long term studies of population dynamics of chain forming diatoms. Vessel volume rather than flow rate was varied in these systems to obtain different growth rates at steady state in systems with an equal capacity to produce.

Population level responses to high dilution rates were examined for Skeletonema costatum both in long term steady state and under non-steady state conditions. Development of populations were observed from small innocula through steady state dilution rates approaching the maximum growth rate of the population. Populations were characterized in terms of particulate carbon and nitrogen, particle numbers and volumes, particle size distributions, and the relationship of production and specific growth rate to biomass. Qualitative observations were made on cell dimensions, morphology,
and buoyancy.

Four phases of growth were identified in some or all of the systems: selection phase in which population concentrations declined, but significant changes in population characteristics occurred; exponential phase in which populations increased in a uniform and density-independent fashion; transition phase in which adjustments were made in population characteristics; and, finally, steady state in which populations were stable. Selection phase was observed when small inocula were placed in high dilution rate systems or when steady state cultures were transferred to higher dilution rates. Those cultures experiencing selection phase showed changes in physical cell dimensions and in nutrient cell size.

Physical cell dimensions and nutrient cell size and their relationship to growth rate are discussed. The work of Caperon (1968) is examined for similar selectional effects. A method using curves of nutrient per cell versus residence time of a particle in a chemostat is suggested to separate selection and dilution rate effects in dynamic systems. A model summarizing the ideas of nutrient reservoirs is suggested.
Population Dynamics of *Skeletonema costatum* in High Dilution Rate Chemostats

by

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A THESIS submitted to Oregon State University in partial fulfillment of the requirements for the degree of Master of Science

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Date thesis is presented August 30, 1973

Typed by Marjorie Wolski for Percy Lowry Donaghay
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INTRODUCTION

Carbon and nitrogen assimilation by marine primary producers, mainly diatoms and dinoflagellates, form the base of the marine food chain. The magnitude of the primary production, as well as the efficiency with which that production is passed on to subsequent trophic levels, sets limits on the entire ecosystem. The complex interactions of grazers and phytoplankton and their environment are therefore of great significance.

The quantity and quality of phytoplankton production as food is a function of the nutrients and energy available, the specific rates at which they are assimilated, and the specific rates at which they are either transferred to zooplankton by grazing or lost by respiration, excretion, sinking, and advection. Any characteristic of a population that affects one or more of these rate processes will be significant to the dynamics of the population. Study of population dynamics in natural systems is complicated by sampling problems and by the high degree of interaction between, and multiplicity of, significant processes. Controlled dynamic systems allow the simplification of natural systems to a level at which operational mechanisms can be defined. Once understood at one level, additional parameters may
be added, jointly or separately, and their effects, interactions and operation described.

Chemostats have been employed extensively in bacteriology as controlled dynamic systems. Kubitschek (1970) has reviewed their operation and the considerable theory developed. To date, chemostat application to marine phytoplankton has involved studies of species succession (Dunstan and Menzel, 1971; Margalef, 1967; Margalef and Ryther, 1960), and studies of population dynamics under nutrient limitation by nitrogen (Caperon, 1965, 1967, 1968, 1969; Caperon and Meyer, 1972a, b; Larsen, 1973; Thomas and Dodson, 1972), phosphate (Carpenter, 1970; Fuchs, 1969), iron (Davies, 1970), and vitamin $B_{12}$ (Droop, 1966, 1970).

Because the main thrust of the nutrient limitation experiments was the elucidation of nutrient kinetic mechanisms per se, attempts were made to approach the idealized condition of homogeneity required by chemostat theory (Kubitscheck, 1970). As a result, the chemostats developed were often typified by small, highly mixed vessels containing low concentrations of nutrients and small single-celled algae (often flagellates). Several other uses of chemostats, such as food sources for grazer-phytoplankton interaction studies, require high yields of high-growth-rate cells with constant chemical composition and particle size distributions. High dilution rates are often required to satisfy these objectives. The use of chain-forming diatoms in such
systems is often desirable because of their apparently dominant role in nearshore food chains. The development of a high-growth-rate, high yield system using chain-forming diatoms is a preliminary objective here.

Several major problems have been observed in high-dilution-rate chemostats (Caperon, 1968; Williams, 1971). Theories descriptive of population dynamics at low dilution rates tend to break down under high dilution (Williams, 1971; Kubitscheck, 1970). In addition, the success of the initial inoculum and the character of the steady-state population depend on inoculum size. Williams (1971) noted that if inocula were small, the steady-state algal populations were highly stable. In many cases, however, the inocula were washed out of the chemostats. With larger inocula, such washout did not occur but resultant steady states tended to be unstable. I have therefore followed the development of small inocula to steady state at several high dilution rates. The high dilution rates allowed development of maximum growth rates that were comparable to those observed under similar nutrient-light conditions in batch culture.

In developing his model of nutrient-limited productivity in the sea, Dugdale (1967) suggested that dynamic systems could be viewed in terms of (1) the concentrations of limiting nutrients within compartments, (2) the transports of those nutrients between compartments (transport rates), and (3) the nutrient-specific rates of change.
within compartments. In light of the fact that the assimilation of two essential elements, say carbon and nitrogen, might limit a system, it is important to estimate the concentrations, transports, and specific rates of each element independently. Although significant problems exist in field determinations of these parameters, each can be defined independently in a chemostat over long periods of time (see theoretical considerations below). Warren (1971) has shown that in systems with an equal capacity to produce, the relationship between "concentrations" and "transports" (in Dugdale terms) can be used to define whether growth is density-independent or density-dependent. Such definition is a necessary first step toward developing a functional relationship to describe growth in a system.

The capacity of a system to produce (productivity, in Warren's terminology) is a very significant property of a system. Although a theoretical upper limit is placed on carbon production by the flux of solar energy into a parcel of water, the efficiency and extent to which carbon production is actually realized is heavily dependent upon standing stocks of phytoplankton and the availability of limiting nutrients. As used here, the term availability includes not only the concentration of a usable form of a limiting nutrient in a parcel of water, but also the ability of advective and regenerative (grazer and bacterial) processes to replace it. Fluxes of energy and materials both within and into a parcel of water can thus be viewed together as
controlling or setting the upper limit of production by the phytoplankton. This upper limit will be termed the production potential for the given conditions.

In a chemostat system the production potential is determined by the fluxes of energy (light) and materials (nitrogen, in my experiments) through the system. It should be possible by maintaining a constant flux of energy and materials (equal light, and equal flux of nitrogen in ug/vessel/day) into a series of chemostat vessels operating at different dilution rates, to examine the effects of high dilution rates on the production of marine phytoplankton within systems of equal production potential. The advantages of operating systems of equal production potential are two-fold: (1) an extensive body of theory exists for interpreting the results (Warren, 1971), and (2) it should be possible to clearly define the nature of the operational mechanism controlling production during different phases of growth in dynamic systems, and if that mechanism is density-dependent or density-independent. A method for developing systems of equal production potential, but having different dilution rates, is developed under theoretical considerations.
THEORETICAL CONSIDERATIONS

Quantitatively, the net production of phytoplankton in a parcel of water, in the absence of sinking, diffusion, or grazing of cells from that parcel, can be expressed as:

\[ \frac{dp}{dt} = p (a - r) \]  \hspace{1cm} (1)

where: \( p \) = phytoplankton concentration, and \( a \) and \( r \) are respectively the specific coefficients or instantaneous rates of assimilation and respiration (including excretion) in units of \( p \) produced or lost per unit of \( p \) per time \( t \). In chemostat systems where cell diffusion and sinking is controlled by flow rates and proper stirring, "mechanical grazing" (\( g \)) can be imposed simply by controlling continuous cell removal into overflow flasks. Thus, a net gain or loss of \( p \) can be:

\[ \frac{dp'}{dt} = p (a - r + g) \]  \hspace{1cm} (2)

where: \( g \) is in units of \( p \) removed per unit \( p \) per time, and \( p' \) is used in place of \( p \) simply to differentiate net response of \( p \) in Equation 2 from net production in Equation 1. Further, the term \( a - r \) is the specific growth rate \( k \) in units of \( p \) produced per unit \( p \) per time, so that:

\[ \frac{1}{p} \frac{dp}{dt} = (a - r) = k \]  \hspace{1cm} (3)
or alternatively:

\[ P_t = P_0 e^{kt} \]  \hspace{1cm} (4)

where \( P_0 \) and \( P_t \) are concentration estimates at time 0 and time \( t \).

With mechanical grazing, Equations 3 and 4 must be expanded to yield:

\[ \frac{1}{P} \frac{dp'}{dt} = (a - r - g) = (k - g) = \alpha \]  \hspace{1cm} (5)

and

\[ P'_t = P'_0 e^{\alpha t} \]

where \( \alpha \) is the **apparent** specific growth rate, or the coefficient of population increase or decrease subjected to cell removal by "grazing."

In natural systems the \( p \) or \( p' \) term is measured as cell numbers or volumes, or in biomass units (carbon, nitrogen, dry weight, organic weight, or pigment concentration), but we usually get no information on \( k \) or \( \alpha \) because of the lack of sequential, precise estimates of \( p \) or \( p' \). The \( k \) term particularly is difficult to measure over long time periods because suspension of the \( g \) term and of cell diffusion and sinking in enclosed bottles leads to other problems involving cells confined for long periods. The \(^{14}\text{C} \) method of measuring net production of carbon comes close to estimating \( k \) during exponential phytoplankton growth, if units of \(^{14}\text{C} \) uptake per unit of carbon biomass are used (these units have seldom been employed
because of difficulties in measuring living carbon biomass in natural systems). No such method is yet routinely available for the rapid estimation of nitrogen production. The $^{15}$N method of Dugdale and Goering (1967), while adequate at high nutrient levels, may present problems at low levels of ambient nutrient because of inequality of uptake and assimilation (i.e., because of the existence of nutrient reservoirs inside cells).

In chemostats, nutrients are supplied to a population of cells in a culture vessel at a constant rate, and a mixture of cells and unused nutrient is harvested at the same rate. Under conditions of a constant environment (light and temperature) in which all essential nutrients are in excess supply except one, and that one is still in sufficient concentration such that the rate of supply is greater than the rate of biological uptake, then internal cellular processes will determine the maximum specific growth rate. This maximum rate in the chemostat is equivalent to the growth rate in exponential phase of batch cultures, described by Spencer (1954). If the dilution rate ($\omega$) (fractional rate of replacement of nutrient medium) is less than the specific growth rate of the cells, the population will increase until a steady state is established. In steady state, the total biomass of the population and the rate of cell division will be constant and the growth rate will be both independent of cell concentration and equal to the dilution rate ($k = \omega$). Thus, steady state for a given dilution rate implies that the
specific rates of synthesis of all parts of each cell are constant, and therefore both the cell composition and population composition are constant.

In terms of the production equation with grazing (Equation 2), steady state is expressed as

\[
\frac{dp'}{dt} = (k - \omega) \quad p = 0
\]

(7)

where the dilution rate (\(\omega\)) has been substituted for the grazing rate (g) to which it is comparable in many respects (Eppley et al., 1971b).

Calculated from the flow rate (f, in ml hr\(^{-1}\)) and the culture vessel volume (\(V_o\), in ml) by

\[
\omega = \frac{f}{V_o}
\]

(8)

the dilution rate (hr\(^{-1}\)) controls both the specific growth rate at steady state and the mean residence time (R, in hrs) of the cells in the culture vessel at all times. Residence time is the reciprocal of dilution rate,

\[
R = \frac{1}{\omega}
\]

(9)

and is of considerable significance in determining chemostatic "genetic" selection because it describes the mean (implying a distribution) length of time that an individual cell remains in the system (Powell, 1965).

Unless assumptions are made about a functional relationship
between the specific growth rate \( k \) and the ambient nutrient concentration, a solution to Equation 7 is not possible in terms of nutrient measurements (Kubitschek, 1971). As already pointed out, Michaelis-Menten nutrient saturation kinetics have provided a reasonable model for bacterial growth (see Kubitschek, 1971, for review, also Herbert et al., 1956, 1965), but have generally proved unsatisfactory for algae because of the existence of internal nitrogen pools to which \( k \) is related (Caperon, 1968; Caperon and Meyer, 1972a, b; Williams, 1971). A more biologically reasonable model has been developed by Williams (1971), and it will be expanded in the discussion section to include nutrient pools.

The existence of such internal pools invalidates any critical attempts to relate loss of nutrient from the water to growth of algal cells; however, because the pools are small compared to the total cellular nitrogen (less than 1% of total N is NO\(_3\) according to Thomas and Krauss, 1955), they should not greatly affect concentration estimates \((p)\). Total particulate cell nitrogen and carbon estimates can now be directly measured by gas chromatographic methods (O'Conners, 1969; Menzies, 1972). Because such estimates of concentration are normally made at specific time intervals, the exponential mean concentration of N or C, and therefore the apparent specific growth rate \( \alpha \) in terms of N or C, can be calculated using a least squares fit to Equation 4 assuming exponential growth between any
series of two or more concentration estimates. Substituting these concentration estimates, and values of $\alpha$, into a rearranged form of Equation 7 allows the determination of the specific growth rate as

$$k = \frac{1}{p} \frac{dp}{dt} + \omega = \alpha + \omega. \quad (10)$$

That is, specific growth rate is the sum of the apparent growth rate and the dilution rate.

Production ($P$) as defined by Warren's (1971) production theory (in units of $p$ produced chemostat vessel$^{-1}$ time$^{-1}$) can be calculated as

$$P = B k \quad (11)$$

where $B$ is the exponential mean biomass contained within a vessel of volume $V_o$ during the time interval over which $k$ is computed:

$$B = V_o \rho. \quad (12)$$

It is important to note that the Warren production term ($P$) is not equivalent to net production rate (Equation 1) but is rather an estimator of the total amount of biomass elaborated by the system (culture vessel) even if all the biomass is not present in the system at the end of the time period. A significant advantage of $P$ over other production estimates is that plots of $k$ and $P$ against $B$ are sufficient to define density-dependent relationships within systems of equal production potential; i.e., they are sufficient to describe the nature of
the functional relationship between k and the nutrient concentration in
the water during all phases of growth.

Because at steady state k equals \( \omega \), Equation 11 can be written as

\[ P = B \omega \]  \hspace{1cm} (13)

which through Equations 8 and 12, is equivalent to the yield \( (Y) \) of the chemostat (in mg hr\(^{-1}\)).

\[ P = Y = pf \] \hspace{1cm} (14)

or the total quantity of phytoplankton produced by a chemostat per unit time (Herbert et al., 1956). Equation 11 under non-steady state conditions can be expanded by substituting for k using Equation 10,

\[ P = B \left( \frac{1}{P} \frac{dp'}{dt} + \omega \right) \]  \hspace{1cm} (15)

and then can be further expanded by substituting for B (using Equation 12) and for \( \omega \) (using Equation 8), to give the following:

\[ P = V_0 \frac{dp'}{dt} + pf \] \hspace{1cm} (16)

Thus, during all phases of non-steady state growth, production is divided into two fractions: (1) \( V_0 \frac{dp'}{dt} \), which estimates biomass increase within the chemostat vessel, and (2) \( pf \), which is lost from the vessel as yield. Note that at steady state \( \frac{dp'}{dt} = 0 \) by definition, and Equation 16 exactly equals Equation 14; i.e., steady state
production is all yield and therefore a function only of \( p \) and \( f \).

Furthermore, because steady-state specific growth rate must equal
dilution rate, \( k \) at steady state is a function of both vessel volume and
flow rate (\( k = \frac{f}{V_o} \)). It is thus possible to obtain a set of differing
steady-state \( k \) values by altering either \( V_o \) or \( f \). By using similar
flow rates into vessels of different volumes, similar yields should be
obtainable at different growth rates and both the quantity (defined by
\( P \)) and quality (defined by a physiological state related to \( k \)) should be
independently controlled. In addition the maximum yields (equal to
production potential), made possible by maximum growth rates,
should be obtained at high dilution rates. It should be noted that no
prior knowledge of the physiological basis of the relationship between
growth rate and nutrient concentration in the water is needed in
systems of equal production potential, and no assumptions about that
relationship is required.
METHODS

Cultures of Skeletonema costatum, both chemostat-grown and stock batch, were grown in one of three identical controlled light and temperature chambers (Fig. 1). Even under photoperiod, temperature stability of $16 \pm 1^\circ C$ was maintained in each chamber through the use of clear plexiglass baths in which water was rapidly recirculated through a common, thermostatically controlled cooler. Any deviation of temperature was detected with a maximum-minimum thermometer. If the deviation was greater than $2^\circ C$, the experiment was terminated. Filtered air was circulated through the chamber to cool the lamps and prevent condensation.

Uniform constant light for the chemostats and overflow vessels was obtained by a combination of incandescent and fluorescent lamps aligned above and below each chemostatic system. The walls of the chamber were painted gloss white to increase reflectivity. Although the exact light intensity in the chamber was not directly measureable, an approximate intensity of 600 foot candles was determined by summing the upward and downward light components measured with a Weston light meter.

With chain-forming diatoms in continuous-flow systems, stirring had to be rapid enough to maintain all cells in suspension and to keep the solution homogeneous, but at the same time it had to be
Figure 1. Chemostatic system arrangement with accessory equipment. Parts listed by number in the figure.
Chemostatic system: (1) nutrient reservoir vessel (12 liter pyrex carbouy); (2) pump delivery tubing (glass); (3) metering pump (Sigmamotor$^R$ Model T-8); (4) pump tubing (medical grade tygon); (5) media transfer tubing (glass); (6) culture vessel; (7) volume control tube; (8) overflow tube; (9) overflow vessel; (10) sampling and continuous overflow tube; (11) siphoning starter tubing; (12) waste collector; (13) overflow vessel air vent; (14) heat drawn orifices; (15) oval Teflon coated magnetic stirrer bar.
Light system: (16) light tight, high gloss white chamber walls; (17) chamber lid; (18) 2 fluorescent lid lights; (19) 2 incandescent lid lights; (20) 2 fluorescent stirrer lights.
Stirring system: (21) stirrer motor; (22) pulleys; (23) tygon pulley drive belt; (24) magnet.
Temperature system: (25) water bath; (26) recirculating pump; (27) recirculating tubing; (28) Coke cooler; (29) cooler thermostat control.
slow enough to keep cell breakage to a minimum. Microscopic obser-
vation revealed that minimal breakage of cells occurred at speeds of
less than 80 rpm with oval magnetic stirrers, and such speeds yielded
relatively stable chain length. Therefore, a 50 rpm gear motor was
used to pully-drive four identical magnetic stirrers at 60 rpm in each
chamber, providing highly reproduceable stirring both in time and
between vessels.

Two all-glass chemostat culture and overflow vessels were
located in each chamber and arranged as in Figure 1. With this
arrangement the system could be monitored and sampled from outside
the chamber, and all parts of the system downstream of the metering
pump remained undisturbed while nutrient reservoirs were replaced
as needed. The metering pump permitted the use of up to six systems
at the same, or different flow rates for extended periods up to 70 days
if the nutrient reservoirs and tygon pumping tube were changed at
weekly intervals. Back-flushing and subsequent contamination of the
glass delivery and media-transfer tubing were prevented by heat-
drawing the tubing to a 0.1 mm orifice and capping off with a short
section of 1/32 in. I.D. glass-plugged tygon tubing during system
assembly and reservoir replacement. Further, all glassware was
pretreated before each experiment by thorough washing, rinsing with
3N HCl followed by distilled water, and autoclaving for one hour on
two successive days.
Long-term operation was further facilitated by addressing the following problems of culture media preparation: organics as algal growth affectors or bacterial food sources; nutrient precipitation through degassing during autoclaving; and low nutrient content of source water. Offshore water with low nutrient and phytoplankton concentration was aged and stripped of most organic matter by shaking with activated charcoal for over four days. The water was then gravity-filtered through a 0.8 μm Millipore filter into a 12-liter glass carboy, autoclaved for 40 minutes, and, upon removal, quick-cooled to 40°C by stirring in a water bath. Nutrients were added in proportions given in Table 1, and the carboy was shaken vigorously after each nutrient addition.

After autoclaving separately for ten minutes in a 1 liter graduated cylinder, the pump delivery system was flamed and inserted into the nutrient reservoir. The old tubing from the previous reservoir was removed from the metering pump and the new inserted, the glass stoppers were removed from the tubing on the downstream side of the pump, and, after flaming, the glass media transfer tubing was inserted into the pump tube. This procedure required only five minutes of flow stoppage and permitted reservoir change every nine days.

To insure that physiological adaptation to light and temperature was complete before experiments were started, parent cultures were
Table 1. Culture Medium

<table>
<thead>
<tr>
<th>Major ions: added as stock solutions as one ml liter(^{-1})</th>
<th>Strength used</th>
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</thead>
<tbody>
<tr>
<td><strong>NaNO(_3)</strong> 150 mg.</td>
<td>1.76 mg. at. 3/12</td>
</tr>
<tr>
<td><strong>Na(_2)H(_2)PO(_4) \cdot)H(_2)O</strong> 10 mg.</td>
<td>72.5 (\mu)g. at. 5/12</td>
</tr>
<tr>
<td>Fe sequestrene, sodium-iron salt of ethylene dinitrilo tetra-acetic acid (EDTA), 13% Fe. 10 mg.</td>
<td>23.2 (\mu)g. at. Fe 5/12</td>
</tr>
<tr>
<td><strong>Na(_2)SiO(_3) \cdot)9H(_2)O</strong> 30-60 mg.</td>
<td>107-214 (\mu)g. at. Si 4/12</td>
</tr>
<tr>
<td>Thiourea 1 mg.</td>
<td>4/12</td>
</tr>
<tr>
<td><strong>Vitamins:</strong> Added as vitamin mix</td>
<td></td>
</tr>
<tr>
<td>Thiamin•HCl 0.2 mg.</td>
<td>4/12</td>
</tr>
<tr>
<td>Biotin 1.0 (\mu)g.</td>
<td>4/12</td>
</tr>
<tr>
<td>B(_12) 1.0 (\mu)g.</td>
<td>4/12</td>
</tr>
<tr>
<td><strong>Trace Metals:</strong> added as metal mix</td>
<td></td>
</tr>
<tr>
<td>CuSO(_4) \cdot)5H(_2)O 0.196 mg.</td>
<td>0.079 (\mu)g. at. Cu 4/12</td>
</tr>
<tr>
<td>ZnSO(_4) \cdot)7H(_2)O 0.044 mg.</td>
<td>0.015 (\mu)g. at. Zn 4/12</td>
</tr>
<tr>
<td>CoCl(_2) \cdot)6H(_2)O 0.022 mg.</td>
<td>0.085 (\mu)g. at. Co 4/12</td>
</tr>
<tr>
<td>MnCl(_2) \cdot)4H(_2)O 0.360 (\mu)g.</td>
<td>1.82 (\mu)g. at. Mn 4/12</td>
</tr>
<tr>
<td>NaMoO(_4) \cdot)2H(_2)O 0.03 (\mu)g.</td>
<td>0.052 (\mu)g. at. Mo 4/12</td>
</tr>
</tbody>
</table>

Millipore\textsuperscript{®} filtered sea water to make one liter
grown in exponential phase in the chambers for four weeks before any experiments were begun. In these experiments no attempt was made to maintain axenic cultures for several reasons: (1) previous workers (Davey, 1970) had considerable difficulty in obtaining bacteria-free *S. costatum*, and when successful the cultures showed depressed growth rates, a fact which implied possible symbiotic function of bacteria; (2) bacteria are always present in natural systems, and are therefore an integral part of the response of *S. costatum* to those systems; (3) we were interested in the dynamic population responses rather than nutrient uptake kinetics of a single cell; and (4) bacteria are only present in large numbers during senescence of the *S. costatum* cultures.

Two series of chemostats were run at different dilution rates. The different dilution rates were achieved by varying the culture vessel volume (*V₀*) from 1 to 2 liters while maintaining constant flow rate, *f* (58 ml hr⁻¹). Culture volumes of 1 liter gave dilution rates approximately equal to the exponential growth rates observed in the parent batch cultures. Experiments 1A and 1B were designed to examine dilution rate effects on cell "quality" and quantity yielded, with particular emphasis on particle size distributions. In an effort to assess the cause and extent to which particle size shifts observed in 1A could occur, a second high-dilution chemostat (2A), with two subculture chemostats run at lower dilution rates (2B, 2C), was used.
The characteristics of each of these chemostats is summarized in Table 2.

After the chemostats were placed in the chamber and filled from the nutrient reservoir, and the temperature was equilibrated, inocula of sufficient size to obtain identical initial concentrations were added to 1A, 1B, and 2A, and stirred for four hours. Initial concentrations for 1A and 1B were made similar to that of 2A by first computing the particle size distribution and total particle volume (using methods below) for 2A, and for the parent batch cultures. The parent batch cultures were of such a concentration that 200 and 400 ml were used to innoculate 1A and 1B, respectively. After thorough mixing without water flow, volumes equal to the inocula were removed to accurately assess initial concentrations in 1A and 1B. After removal of these volumes the total cultures of 1A and 1B were 1 and 2 liters, respectively. Flow was then started. Chemostat 2B was begun on day 12 of 2A (called day 0 of 2B) by allowing the overflow of 2A to go into a second chemostat vessel until 150 ml had been collected. Cells were allowed to grow without flow until day 14 of 2A, at which time flow started and the vessel volume reached 1.5 l. After both 2A and 2B had reached steady state and 2B had maintained it for 6 days (day 17 of 2B) the pump delivery tube to 2B broke and 200 ml of culture vessel volume was lost. Rather than refill the system, it was allowed to run at the same flow,
Table 2. Experimental Conditions of the Chemostats

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Innoculum</th>
<th>Vessel Volume (ml)</th>
<th>Mean flow rate $\pm 90%$ C.I. (ml hr$^{-1}$)</th>
<th>Dilution rate ($\omega$) (days$^{-1}$)</th>
<th>$\frac{\omega}{\text{batch growth}} \times 100$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>batch</td>
<td>1000</td>
<td>$58.6 \pm 1.6$</td>
<td>1.4</td>
<td>100%</td>
</tr>
<tr>
<td>1B</td>
<td>batch</td>
<td>2000</td>
<td>$58.6 \pm 3.4$</td>
<td>0.7</td>
<td>50%</td>
</tr>
<tr>
<td>2A</td>
<td>batch</td>
<td>1000</td>
<td>$54.9 \pm 4.3$</td>
<td>1.4</td>
<td>100%</td>
</tr>
<tr>
<td>2B</td>
<td>day 12, 2A</td>
<td>1500</td>
<td>$57.8 \pm 4.5$</td>
<td>0.9</td>
<td>64%</td>
</tr>
<tr>
<td>2C</td>
<td>day 17, 2B</td>
<td>1300</td>
<td>$54.9 \pm 4.2$</td>
<td>1.0</td>
<td>73%</td>
</tr>
</tbody>
</table>
but with the lesser vessel volume of 1.3 liters. This condition was called 2C.

There was an important problem involved in determining vessel volume, and therefore in calculating dilution rate from flow rate. The system was designed such that there was a small pressure head maintained in the chemostat; therefore, any breakage of the pump delivery tube resulted in draindown of the culture volume. Because the effects of the replacement of the draindown were unknown, those experiments in which it was a problem were considered preliminary. Draindown also occurred as a result of evolution of oxygen by the phytoplankton in excess of the solubility of oxygen in sea water (Davey, 1970). This type of draindown presented a serious problem in preliminary two-liter chemostat experiments because it resulted in losses of 50 ml per day. The problem was corrected in 1B by the insertion of a volume-level control tube (Fig. 1). This tube operated by normally maintaining contact with the culture surface and therefore maintaining the pressure head necessary for overflow; however, when draindown began, the contact was broken, and overflow stopped until volume was reestablished. Because of the large diameter of the vessels used, this method allowed control of volume for 2B of 30 ml, but this control could be improved by using a smaller vessel with more constricted culture surface. As a further check on volume, a line was drawn around the vessel at the culture surface and observed
daily to insure volume maintenance; no deviation from that line was observed in 1A, 2A, 2B, and 2C.

Based on the spurious results of preliminary experiments in which culture volume varied, and upon the need for large samples that would be representative of the system but not affect its performance, an external indirect method was used to sample the chemostate. Two hours before a sample was taken, the overflow vessel was shaken and drained into the waste container by siphoning through the sampling tube. The overflow volume required for daily flow-rate determinations was measured from the waste container and divided by the time since the last sample, to obtain flow rate. The sample itself was taken by again draining the overflow vessel after two hours. Aliquots of this sample were immediately processed for Coulter Counter® and particulate carbon (C) and nitrogen (N) determinations. This sampling procedure was suitable for determination of concentrations, because the cells in the overflow vessel were viable; however, such a procedure would not have been adequate for kinetic determinations (i.e., nutrient uptake rates).

Particulate carbon and nitrogen samples were taken using the method described by O'Connors (1969), with slight modification. After filtration on glass-fiber filters, the filters were folded, with the sample on the inside, into 1 mm diameter cylinders. They were then inserted into 2 ml vials, oven dried at 60°C, capped while hot,
and stored until run on an F&M Model 185 Carbon-Hydrogen-Nitrogen Analyzer®. Samples and standards were run as described by O'Connor, but an additional standardizing check was made to correct for filter-blank and volume-filtered errors. Because the size of the samples varied both in volume and concentration over a wide range, small blank errors had a disproportionate effect on the small samples. The check involved taking varying volumes from a chemostat sample, converting these subsamples to ugC and ugN per sample (using an acetanelide standard regression slope and intercepts), and regressing these values on the volume of the subsample. The intercepts from these regressions were used to adjust the acetanelide intercepts for blank errors. Using the blank-adjusted values, ugC/ml, ugN/ml, and C/N ratios were computed, and regressions against subsample volumes repeated. Because the slopes of these secondary regressions were not significantly different from zero, the new blank-adjusted intercepts were used for calculation of particulate C and N arising from filtering and from sample volumes used (Fig. 2). Such error estimates were minimal estimates because additional sampling error arose in obtaining representative chemostat samples through the overflow vessel.

Particle size distributions, total particle numbers and total particle volumes were determined using a Coulter Counter® Model B with attached Model J Particle Size Distribution Plotter. The
Figure 2. Regression for blank correction of CHN data. Acetanilid standard regression used for peak height conversion to ugC and ugN:

\[
\text{ugN} = 3.2685 + 0.0644 \text{ peak height in cm.}
\]

\[
\text{ugC} = -4.6949 + 1.5377 \text{ peak height in cm.}
\]

Regression equation for ugC and ugN per sample versus sample volume:

\[
\text{ugN/(sample)} = \triangle = 1.0473s - 0.6013 \quad r = 0.9991
\]

\[
\text{ugC/(sample)} = \square = 7.6406s + 1.8580 \quad r = 0.9993
\]

\[
\text{C/N ratio} = \bigcirc = 0.00s + 7.7121 \quad r = 0.9999
\]
methodology used was that described by Sheldon and Parsons (1967), with modifications developed by O'Connor (1973) to avoid coincidence events and with my own data-processing procedure. Coincidence occurred when two or more particles passed through the counting aperture simultaneously and were counted as one particle of a size equivalent to their combined volume. Such an event resulted in the underestimation of particle numbers, and in the shift of the particle size distribution in an indeterminate manner (thus destroying its utility). Because the probability of coincidence increased with the number of particles and the size of the aperture, but was actually very low up to some threshold (after which it increased markedly), a zero coincidence threshold was determined by counting a series of dilutions of ragweed pollen (also Ditylum brightwelli, Chaetoceros discipiens, and Thallasiosira nordenskioldii) and plotting the number of particles counted against the dilution. A sharp break in the slope was noted at 1000 particles ml\(^{-1}\) for the 280 \(\mu\) aperture, and this break denoted the departure of the actual counts from a calculated coincidence-free line (based on the extrapolation of low-concentration samples). The value of 1000 particles ml\(^{-1}\) was used as the threshold, and all samples were diluted to less than this concentration before counting. Dilutions were made in a graduated cylinder by adding enough Millipore\textsuperscript{3}-filtered sea water (of the same nutrient and temperature conditions) to the sample to make the total up to 200 ml,
and at a concentration near to, but less than, the coincidence threshold. Thorough mixing without cell or chain disruption was obtained by three gentle pourings between the counting sample holder and the graduate cylinder. All samples were counted with the 280 µ aperture, but with sensitivity settings altered so as to obtain each entire distribution in one setting.

The sensitivity, and therefore the ability of the Coulter Counter® to more finely partition the particle size distribution observable with any aperture, is controlled by the current across the aperture (aperture current switch) and by the electronic amplification within the counter (amplification switch). The settings on the instrument front are given as reciprocals; i.e., an 8-fold amplification is given as 1/amplification or 1/8. Sensitivities are therefore given below as the product of 1/amplification and 1/aperture current; i.e., 1/1 X 1/8 or 1 X 1/8.

Counting of a distribution may be done manually at a particular sensitivity by placing the lock switch in the lock mode, and the upper threshold on 40 and moving the lower threshold selector in 4 threshold unit increments from 0 to 100. Replicate counts are made at each of these lower threshold settings. By this procedure the particle size spectrum is divided into 25 windows 4 threshold units wide which are equivalent in position on the spectrum to the 25 plotter windows.

In experiments 1A and 1B the total number of particles in a
distribution were determined by setting the lock switch in the separate position, the lower threshold at 8, and the upper threshold at 100, and counting the number of particles at one sensitivity. When particle size distributions were constant such manual particle counts provided a rapid estimate of biomass; when not, the manual counts were used to insure proper dilution. For the experiments in which shifts were large (2A and 2B), manual counts were taken at the sensitivity in which most of the particles were found.

Three replicate plots also were taken at sensitivity 2 X 1/8 for 1A and 1B. Sensitivity of 2 X 1/8 was optimum for the center of the particle distribution. Accessory distributions were taken at higher sensitivity (1 X 1/8, 1/2 X 1/8) to insure that the majority of the distribution was obtained, but these were not used in the calculations below. For experiments 2A, 2B, and 2C, the larger size shifts required estimates at sensitivities of 1 X 1/8, 1/2 X 1/8, and 1/4 X 1/8 before visual comparison could be made. Because of the low cell concentration in 2A, insufficient sample was available for replicate plots at any one sensitivity setting.

Because particle volume (measured in $\mu^3$) was a better estimator of biomass than numbers (Sheldon and Parsons, 1967), conversions of number distributions to volume distributions and total volume ($V_T$) were desirable. However, there was often insufficient time and sample for manual distributions, and the plotter conversion method of
Sheldon and Parsons (1967) proved too inaccurate; therefore, a conversion method was developed. Theoretically, with any aperture there is a constant conversion factor between plotter peak height and manually determined particle numbers, if constant flow is maintained through the aperture. Constant flow was necessary because in the plotter mode the instrument counted the particles in a measured interval of time.

Determination of the plotter conversion factor for the 280 μ aperture involved making four replicate plots and four replicate manual counts (using four threshold windows as described above) at sensitivities of 1 X 1/8 and 1/2 X 1/8, using ragweed pollen (identical results were achieved with S. costatum instead of ragweed pollen). By computing means of peak heights and particle numbers in each principal window (defined as those windows with coefficients of variations of less than 10%), and then regressing manual counts on peak-height units, a conversion factor of 8 was determined (Fig. 3a). The validity of this conversion factor was checked by multiplying plotter peak heights by the factor, converting manual and plotter data to volumes, and plotting the volume distributions for comparison (Fig. 3b). Excellent fit was obtained.

Using the conversion factor derived above, daily plotter peak heights were converted to particle numbers (ml of culture)$^{-1}$ window$^{-1}$ ($N_N$) as follows:
Figure 3. Plotter conversion factor
(a) Regression of peak heights on manual counts for the 280 μ aperture where □ = sensitivity of 1/2 x 1/8 and ○ = sensitivity of 1 x 1/8.
(b) Verification of conversion factor by overlay of volume distributions determined using manual (○) and plotter (□) modes.
where \( x \) = individual peak heights, \( n \) = number of replicate plots, \( v_s \) = sample volume of Coulter Counter\textsuperscript{®} (2 ml), \( d \) = dilution factor of sample, and \( c \) = conversion factor of 8. Particle volume distributions \( (V_N) \) and total particle volume \( (V_T) \) were computed by the standard methods described by Sheldon and Parsons (1967) that were mathematically formulated by us as follows:

\[
V_N = N_N(2N - 1)mvw
\]

and

\[
V_T = \sum_{N=1}^{N=f} V_N
\]

where \( V_N \) = particle volume in window \( N \) (measured in \( \mu^3 \)), \( N_N \) = particle numbers \( ml^{-1} \) window \( -1 \) calculated above, \( m = 1/amplification \), \( v \) = volume conversion factor, \( w = 1/2 \) the window width in threshold units, \( V_T \) = total particle volume in the distribution (measured in \( \mu^3 \)), and \( i \) and \( f \) are the initial and final windows of the distribution, respectively. Total particle numbers \( (N_T) \) in the plotter mode were determined by summing the particle numbers in the same fashion as particle volume, and over the same interval.

Particle number and volume distributions were treated in several ways: (1) as distributions in plotter peak-height units converted to a common dilution (i.e., 20:1), principally for immediate
comparison of time series of particle size shifts; (2) as particle number distributions providing the same information for replicate samples (1A and 1B); (3) as volume distributions, valuable for defining steady-state distributions available for potential grazer experiments; and (4) as %N T distributions for defining starts and ends of phases. The %N T distributions were calculated as

\[ \%N_T = \frac{N}{N_T} \times 100 \] (100)

Using the general theory described earlier, apparent specific growth rates (\( \alpha \)) and exponential mean concentrations (\( \bar{p} \)) were calculated for each pair of successive daily \( p \) estimates of \( C \) and \( N \) using the Wang 500 calculator and exponential-fit program. The specific growth rates (\( k \)), biomass in the chemostat vessel (\( B \)), and production (\( P \)) were calculated for each pair using equations 10, 12, and 11, respectively, and using both a mean flow rate and a flow rate for each interval to calculate the dilution rate (\( \omega \)). Subsequently, 90% confidence limits were placed on each of these terms in steady state, and for all values of \( k \).
RESULTS

After inoculation of cells from an exponential-growth batch culture into a chemostat, four population growth phases were defined on the basis of $\alpha$: selection (lag) phase where $\alpha < 0$; exponential phase where $\alpha > 0$; transition phase where $\alpha$ was decreasing and often variable; and steady-state phase where $\alpha = 0$. Selection phase occurred only when inocula were placed in chemostats (1A and 2A) in which the dilution rate was approximately equal to the maximum growth rate for the population under batch conditions. End points for each growth phase, when they could be determined, are indicated by arrows on the time course of development of the population in terms of $N$, $C$, $V_T$, and $N_T$ (Fig. 4a, b, c, d). It is obvious that the length and characteristics of each phase varied with each experiment, and these variations are made explicit in Table 3. Each phase is characterized by different particle size distributions, concentration parameters, and growth and production relationships, which will be considered in order below.

Development of Particle Size Distributions

Particle number distributions have been summarized in Fig. 5 as a time series. All days are included for Experiments 1A and 1B, but only representative days for Experiments 2A and 2B. Steady-state
Figure 4. Time changes in particle concentration estimates for N (4a), C (4b), for all experiments, and for VT (4c) and NT (4d) for experiments 1A and 1B.

Experiments designated by

- **1A**: \(\theta\)----\(\bullet\)
- **1B**: \(\bigcirc\)----\(\bigcirc\)
- **2A**: \(\square\)----\(\square\)
- **2B**: \(\triangle\)----\(\triangle\)
- **2C**: \(\triangledown\)----\(\triangledown\)

Arrows (†) indicate start or end of phase. Straight lines are drawn through phases where \(\alpha\) is constant: selection, exponential, and steady state. Lines connect data points in transition phase.
Table 3. Phases of Growth

The three successive numbers under each chemostat for each measurement parameter are, in order: first day of exponential phase, last day of exponential phase, and first day of steady state.

<table>
<thead>
<tr>
<th>Measurement Parameter</th>
<th>Fig.</th>
<th>Chemostat Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1A</td>
</tr>
<tr>
<td>N</td>
<td>4a</td>
<td>2-15-16</td>
</tr>
<tr>
<td>C</td>
<td>4b</td>
<td>3-15-16</td>
</tr>
<tr>
<td>V_T</td>
<td>4c</td>
<td>2-15-16</td>
</tr>
<tr>
<td>N_T</td>
<td>4d</td>
<td>2-15-16</td>
</tr>
<tr>
<td>N_N</td>
<td>5b</td>
<td>2-15-16</td>
</tr>
</tbody>
</table>

* See text for explanation of choice of day 09 for 1B and for explanation of the results on day 11.
Figure 5. Time series for particle number distributions. Distributions correspond to the day of experiment listed in the upper right of each distribution. All days are given for 1B, representative days for other experiments. Steady state distributions are given as sequence after the mark SS for 1A, 1B, 2B, 2C. Areas under the curves are proportional to total particle number.
Figure 5f. Log-log transform plot of $V_T$ on $N_T$ showing the shift to smaller particles in selection phase of 2A (marked $T_{sp}$).
distributions, plus 90% confidence intervals about each mean number in each size class (window), are given for all experiments in Fig. 6.

The predominant features of these distributions through time are particle size shifts, first toward smaller particles in selection and exponential phase, then often back toward larger sizes at steady-state. During selection phase in 2A, for example, the smaller particles began to predominate as evidenced by the small peak near the end of the phase (Day 12 of 2A, Fig. 5). However, there was a concomitant loss of larger particles throughout this phase. Although the magnitude of the shift to small particles and loss of larger particles is not obvious in Fig. 5, it was sufficient to require changing the Coulter Counter® sensitivity (by a factor of 2 or 4) in order to measure the whole distribution. Such a sensitivity change meant that there was a reduction of distribution width by a factor of 2 or 4. The nature of the particle size shifts in 2A can be more clearly seen in the log transform of the plot of $V_T$ on $N_T$ (Fig. 5e). It is clear that during the time required for selection phase ($T_{sp}$), total particle number increased while total volume remained constant, and that this shift to smaller particles continued until exponential phase commenced, either in subculture 2B or in 2A after Day 14. In exponential phase of all experiments but 1A, the small particles dominated the distribution. However, shifts in particle size had stopped or greatly slowed in exponential phase, as indicated by the linearity of
Figure 6. Steady state particle number distributions. Symbols are as in Figure 4. Areas under the curves are proportional to total particle number. Confidence limits for these means are given in insert.
the log-log plots of $V_T$ on $N_T$ (Fig. 5e).

In transition phase, small-particle division slowed markedly, while larger particles increased in abundance. Such a reverse shift back to larger particles eventually resulted in a stable distribution at steady state, after which no further shifts occurred (Fig. 5). This reverse particle size shift was a dominant feature in low (1B) and intermediate-flow (2B) systems, occurring to such an extent in these systems (particularly 2B) that the actual number of particles in smaller size classes decreased while larger particles increased.

Although 1A had no perceptible transition phase in terms of $C$, $N$, $V_T$, and $N_T$ (Fig. 4a, b, c, d), small particles decreased from Day 12 until steady state at Day 15 (Fig. 5).

The magnitude of the reverse shifts was a function of the degree of cell selection during the selection phase. In the high-dilution-rate chemostat 2A, during the lengthy transition phase small particles continued to increase with very little shift back to larger particles as steady state was approached (Days 24, 26, Fig. 5c). Little reverse size shift occurred because only small particles were present, a direct result of the loss of all large particles during selection phase. Such loss cannot be attributed to flow rate because no loss was evident in the other high-dilution-rate system (1A).

Steady-state distributions are remarkably stable in contrast to those of the other phases. In the sequence of steady-state cell
number distributions for 1A, for example (Fig. 5a), no trends are obvious; however, in 1B (Fig. 5b), when the size distribution does change slightly (as on Day 15), the change is associated with an increase in total volume (Fig. 4c). The greater variability of steady-state volumes in 2B is probably sampling variability associated with single replicate determinations. Such variability is noticeable in comparison to steady-state distributions of 1B and 1A (Fig. 5).

In the overlays of \( N_N \) and \( V_N \) steady-state distributions for all experiments, it is clear that the distribution for each experiment is unique (Figs. 6 and 7). If we compare 1A and 1B, which differ principally in flow-rate effects, the volume distributions are reasonably similar in width and general shape, but there is considerably more area under the curve of 1B than 1A (Fig. 7). In comparing 1A and 2A, however, which differ only in selection phase length (\( T_{SP} \)), the size distribution of the system with longer \( T_{SP} \) (2A) is shifted strongly to the left and is much narrower and more concentrated at the modal size. This trend is most obvious in the particle size distributions (Fig. 6); however, less than 10% of the volume distributions of 1A and 2A overlap (Fig. 7), and totally different sensitivities of the Coulter Counter had to be used to obtain the distributions. Intermediate \( T_{SP} \) acts to shift the 2B distribution to a position intermediate between 2A and 1B. When 2B and 2C are compared, a slight shift to smaller sizes in 2C is evident, implying that changing to a higher
Figure 7. Steady state particle volume distributions. Bars represent 90% confidence intervals. Symbols are as in Figure 4. Areas under the curves are proportional to total particle volume.
dilution rate is sufficient to generate a selection-phase-like shift in steady-state particle size distributions (Figs. 6 and 7).

It should be noted that particle size distributions reflected both cell dimensions (diameters and lengths) and chain lengths. It was therefore possible that some of the distribution shifts to smaller particle sizes arose from chain breakage, perhaps during log-growth-phase. However, steady-state differences appeared to be the result of cell size changes almost entirely, because microscopic observation revealed not only that long-$T_{SP}$ cells were smaller, but that the chains were actually slightly longer in terms of number of cells.

Development of Concentration Parameters

Selection Phase

The length of the selection phase differed greatly in high-dilution-rate systems on the basis of both carbon and nitrogen (compare 1A and 2A, Table 3). In these systems the dilution rate ($\mu$) was the same and approximately equal to the specific growth rate ($k$) in the parent culture ($k = 1.4 \text{ day}^{-1}$ using Equation 3). Also in these systems initial C/N ratios were high (10-15); however, initial growth rates were differentially suppressed for carbon and nitrogen so that particulate carbon decreased while nitrogen remained nearly constant (in Fig. 4a compare slopes of C to N for 1A and 2A in selection
Comparison of selection-phase specific growth rates for experiments 1A and 2A showed that the nitrogen rates exceeded those of carbon (Table 4). These different rates with respect to C and N continued until C/N ratios reached a minimum value of 4 to 5, after which exponential growth phase occurred (Fig. 4a, b).

These results indicate that populations 1A and 2A are capable of higher specific growth rates than those achieved in selection phase, but that in some fashion, high dilution rate suppressed these rates during selection until sufficient shifts in population C/N ratios had occurred for the high-dilution-rate environment to be better utilized.

The processes governing shifts in C/N ratios and particle size distributions were termed selection rather than "adaptation" because adaptation is usually thought of in terms of altering an individual cell's internal composition before cell division can occur. Selection is thought of in terms of selecting from a population those cells best suited to grow under a given set of environmental conditions. In our system, selection phase lasted for up to 14 days (2A, Fig. 4a); however, cells were required to divide every 18 hours to maintain the population during those 14 days, a fact which precludes use of the term adaptation as defined above. Population level selection is also characterized by irreversibility. Such irreversibility is demonstrated by the shift in particle size in relation to total cell volume in 2A.
<table>
<thead>
<tr>
<th>Growth Phase</th>
<th>Experiment</th>
<th>Specific growth rate $k \pm \text{standard deviation (day}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N-based</td>
</tr>
<tr>
<td>Selection Phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td></td>
<td>1.30 ± 0.10</td>
</tr>
<tr>
<td>2A</td>
<td></td>
<td>1.36 ± 0.09</td>
</tr>
<tr>
<td>Exponential Phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td></td>
<td>1.65 ± 0.15</td>
</tr>
<tr>
<td>1B</td>
<td></td>
<td>1.22 ± 0.21</td>
</tr>
<tr>
<td>2A</td>
<td></td>
<td>1.72 ± 0.16</td>
</tr>
<tr>
<td>2B</td>
<td></td>
<td>1.23 ± 0.11</td>
</tr>
</tbody>
</table>
(Fig. 5e), and the subsequent maintenance of this set relationship through exponential growth. The set relationship is passed on to sub-populations at different dilution rates (2B, for example) in a magnitude proportional to the length of $T_{SP}$ experienced.

Selection-phase phenomena were only observed when innocula were initially placed in systems of high dilution rate (1A, 2A) or transferred from intermediate to higher dilution rate (2C). If the innocula were placed in low-dilution-rate systems (1B) or transferred from a high- to an intermediate-dilution-rate system (2B), no selection phase was observed in terms of $C$, $N$, $V_T$, and $N_T$ (Fig. 4a, b, c d), and exponential growth commenced immediately.

**Exponential Phase**

Exponential phase was a period of rapid increase in all concentration parameters ($N$, $C$, $N_T$, $V_T$), with a high degree of constancy observed in all population responses within any given experiment (Fig. 4a, b, c, d). The apparent growth rates ($\alpha$) in all experiments (i.e., the slopes of all the exponential phases in Fig. 4a, b, c, d) were functions of both the dilution rates ($\omega$) and the increases in $k$ resulting from selection-phase effects. The effects of selection phase on exponential $k$ values are obvious if we compare selection-phase $k$ values in experiments 1A and 2A with the exponential $k$ values of these same experiments and with exponential $k$ of a zero-$T_{SP}$ experiment,
1B (Table 4). It is obvious that exponential \( k \) for both C and N in the \( T_{SP} \) experiments (1A, 2A) were significantly greater than the selection-phase \( k \) values in those same experiments, and significantly greater than the exponential \( k \) of the zero-\( T_{SP} \) experiment, 1B. The 2B experiment behaved as a zero-\( T_{SP} \) experiment in terms of exponential \( k \) (Table 4).

Throughout exponential phase in any given experiment the apparent growth rates were equal regardless of whether they were in terms of C, N, \( N_T \), or \( V_T \) (Fig. 4a, b, c, d). Even though over a range of concentration levels individual points exhibited variability about a mean, when C, N, \( N_T \) and \( V_T \) were plotted against one another (Fig. 8a, b, c, d), it was obvious that in Experiment 1A or 1B during exponential growth phase these estimators were very tightly coupled. In experiments 2A, 2B, and 2C, the variability in the \( V_T \) estimates from single replicate plots obscured the relationship between \( V_T \) and C and \( V_T \) and N. Therefore, only 1A and 1B are plotted in Figs. 8c and 8d.

Although the regression slopes for Experiments 1A and 1B alone were not significantly different from the mean regression of all values, 1B tended to show slightly less C and N per unit \( V_T \) than 1A (Fig. 8c, d). This trend suggested that perhaps the selection event during the short selection phase of 1A was an increase in the N and C per unit volume (relative to unselected 1B), rather than a
Figure 8. Interrelationship plots.
(a) Relationship of particle volume ($V_T$) to particle number ($N_T$) for all experiments.
(b, c) Relationship of carbon (8b) and nitrogen (8c) to particle volume ($V_T$) for Experiments 1A and 1B.
(d) Relationship of nitrogen to carbon for all experiments. Symbols for all plots are as in Fig. 4; steady state values enveloped.
dramatic shift in cell size such as observed in the long selection phase in 2A. The plots of C vs. N (Fig. 8d) were reasonably linear during exponential phase for any one experiment, and showed no discernable differences between experiments. Despite sharp changes in the C/N ratios noted in selection phase, these changes did not carry over into the exponential phase.

**Transition Phase and Steady State**

The exponential phase continued until nitrate became limiting and specific growth rate decreased. This period of decreasing growth rate, leading to the establishment of a steady state between nutrient flow and growth, was called the transition phase. The relationships among cell volume, size distributions, carbon and nitrogen, all of which were so tightly coupled in exponential phase, became uncoupled in transition phase and steady state (Fig. 8a, b, c, d). The degree of uncoupling was some function of $T_{SP}$ and dilution rate. One experiment (1A) did not have a discernible transition phase (Fig. 4a, b, c, d).

The relationship between particulate C and N during transition phase in 2B was particularly interesting. Apparently nitrogen growth rate asymptotically adjusted to equal the dilution rate (that is reached steady state) between Day 19 and Day 22; however, carbon synthesis continued at the exponential rate over the same time increment, to give a carbon "overshoot" (Fig. 4a, b). In operational terms this
overshoot resulted in an increased C/N ratio, with the cells losing their buoyancy and tending to fall out of suspension. Judging by the tendency of the cells to stick together and to form films on the culture vessel walls (unique to the transition phase), dissolved organic material was also released. At this point the system had to be watched and carefully swirled, or "fallout" and subsequent bacterization would have destroyed the system. After fallout was prevented, a steady state was established in which buoyancy was not a problem in the culture vessels. Carbon and nitrogen concentrations in steady state became a function of dilution rate. Although carbon overshoot was not observed in the other experiments, fallout problems during transition phase were encountered with the other low-dilution-rate system (1B). Such problems were never observed in the high-dilution-rate systems (1A, 2A); in fact, in 2A fallout was never observed even in the overflow vessel.

While the end of the exponential phase was easily and singularly definable by a sharp break in the rate of increase of each of the concentration parameters (except where carbon overshoot occurred as noted above) (Fig. 4a, b, c, d), the initiation of steady state was less well defined. For example, in Experiment 1B initiation of steady state was defined differently by two out of the five measurement parameters (Table 3). The problem of steady-state definition was further complicated by the possibility that failure to completely
remove fallout particles either before presample draining or during sampling could have biased the concentration estimates (causing overestimation or underestimation of concentration, respectively). It was thus necessary in 1B to define onset of steady state from the total population response. Because the particle size distributions are free from any bias arising from concentration estimate errors, and are distinctive in form for each phase (Fig. 5), the termination of the particle size shift back to larger particles, and the beginning of the typical steady-state distributions on Day 9, implicated Day 9 as the borderline between transition and steady state in Experiment 1B. Likewise in 1B, the similarity of steady-state distributions on Day 11 (Fig. 5) to the other steady-state distributions implied that low concentration estimates on Day 11 (Fig. 4) arose from fallout; i.e., from sampling problems rather than from a continuation of transition phase. Because Day 11 contributed up to 50% of the variance in steady-state means (Table 5), the steady-state means and production relationships (below) were calculated with Day 11 included (1B) and not included (1B*).

In any given steady state, concentration estimates were relatively non-variable and showed no trends toward increase or decrease throughout the phase; i.e., \( \alpha = 0 \) (Fig. 4a, b, c, d). However, steady-state values were distinctive between experiments (Table 5).

The relationships of steady-state concentrations of \( N \), \( C \), \( V_T \),
Table 5. Steady State Concentration Estimates ± One Standard Deviation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>ugN ml⁻¹ ± s. d.</th>
<th>ugC ml⁻¹ ± s. d.</th>
<th>10⁶VT ml⁻¹ ± s. d.</th>
<th>NT ml⁻¹ ± s. d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>0.953 ± 0.075</td>
<td>7.104 ± 0.981</td>
<td>73.01 ± 1.242</td>
<td>6834 ± 419</td>
</tr>
<tr>
<td>1B</td>
<td>1.687 ± 0.155</td>
<td>10.730 ± 1.148</td>
<td>141.20 ± 18.719</td>
<td>13605 ± 2144</td>
</tr>
<tr>
<td>1B*</td>
<td>1.716 ± 0.122</td>
<td>11.010 ± 0.644</td>
<td>143.20 ± 18.854</td>
<td>13900 ± 1976</td>
</tr>
<tr>
<td>2A</td>
<td>0.471 ± 0.051</td>
<td>4.152 ± 0.522</td>
<td>42.35 ± 2.849</td>
<td>23914 ± 2119</td>
</tr>
<tr>
<td>2B</td>
<td>1.312 ± 0.082</td>
<td>11.494 ± 0.907</td>
<td>94.96 ± 17.006</td>
<td>18443 ± 2930</td>
</tr>
<tr>
<td>2C</td>
<td>0.987 ± 0.057</td>
<td>7.619 ± 0.141</td>
<td>80.94 ± 1.158</td>
<td>19983 ± 726</td>
</tr>
</tbody>
</table>
and \( N_T \) to residence time of particles in the chemostat vessels are plotted in Fig. 9a, b, c, d, respectively. Although there is a general trend of increasing concentration with increasing residence time (excluding \( N_T \) of Experiments 2A, 2B, and 2C), two data groups can be clearly separated based on the length of the selection phase \( (T_{SP}) \). For C, N, and \( V_T \), the populations with a history of long \( T_{SP} \) (2A, 2B, and 2C) increased more rapidly with respect to increasing residence time than did the populations with short (or zero) \( T_{SP} \) (1A and 1B). In comparing the populations differing only in \( T_{SP} \) (1A and 2A), the steady-state concentrations of N, C, and \( V_T \) in the population with longer \( T_{SP} \) (2A) were about one-half those found in the population with shorter \( T_{SP} \) (1A). Because these were steady-state levels in 1A and 2A, \( T_{SP} \) must have so affected the dynamic relationships of nutrient to the cells that such relationships carried through all growth phases.

It is important to note that the strong suppression of steady-state concentrations in 2A, and the weaker suppression in 1A, were dynamic suppression effects that is; when the dilution rate was stopped at the end of Experiments 1A and 2A, the particulate N concentrations increased to the same final state level (equivalent to the level at stationary phase of batch culture) obtained in the no-selection-time culture (1B). Such is the nature of chemostats operated at equal production potential.
Figure 9. Residence time functions. Steady state means (with 90% confidence intervals as bars) are plotted as functions of residence time in the system (1/Ω) for ugN (9a), ugC (9b), VT (9d). Symbols as in Figure 4.
From the residence-time plots (Fig. 9a, b, c, d), dilution rate appeared to have a capability similar to $T_{SP}$ to affect steady-state concentrations (note that concentrations in 1A were always lower than those in 1B, for example). However, selection time and dilution rate were possibly confounded in 1A and 1B because 1A had a 2-day $T_{SP}$ and 1B had no $T_{SP}$. Conceivably the two days of selection in 1A could have created the difference in steady-state responses between 1A and 1B (Fig. 9a, b, c, d) even in the face of very different dilution rates for the two experiments. A separation of dilution-rate effects from $T_{SP}$ is suggested in the discussion.

In sharp contrast to the $N$, $C$, and $VT$ data, the $N_T$ data with a history of long $T_{SP}$ (from Experiments 2A, 2B, and 2C) formed a decreasing relationship to residence time (Fig. 9d). This relationship was a direct result of the particle size shifts associated with longer $T_{SP}$, leading to great increases in numbers of small particles. Thus, $T_{SP}$ not only appeared to depress concentration values ($C$, $N$, $VT$) below their steady-state potentials at given dilution rates or residence times, but at the same time reduced particle size to such an extent that a net increase in $N_T$ occurred in highly selected cultures (2A, 2B, and 2C). The increases in $N_T$ appeared to be proportional to $T_{SP}$, because the increases occurred in the order of longer $T_{SP}$; i.e., 1B:2B:2C:2A.
Production, Growth, and Biomass

Relationship of production (kB) and specific growth rate (k) to biomass (B) for Experiments 1A and 1B are plotted for both carbon and nitrogen in Fig. 10. During exponential phase, a linear relationship is defined between production and biomass for both carbon and nitrogen (Fig. 10b, d). A log-log transform of the data on nitrogen production versus biomass, to expand the low production-low biomass data in Fig. 10b, shows that linearity applies over the entire range of exponential-phase values (Fig. 10e). This linearity means that although there is variability in k during exponential phase (Fig. 10a), the variability is random and k is density-independent. The high correlation coefficients of the regressions of P on B over the entire range of values (see Fig. 10), coupled with the obvious linearity of the exponential phase log-log transforms, indicate that variability in k is the result of my method of calculating k from sequential pairs of data.

The effects of selection phase on exponential growth can be seen (Fig. 10e) to lead to higher k in 1A and 2A than in 1B because the nitrogen production per unit biomass in 1A is shifted to a higher level than that in 1B. The higher k for 1A is also reflected in Fig. 10a, and in the steeper slopes for 1A in the plots of P versus B (Fig. 10b).

In Fig. 11a, b, c, d steady-state C and N production and specific
Figure 10a, b. Growth rate and production as functions of biomass for nitrogen in all phases of growth of Experiments 1A and 1B. Symbols as in Figure 4. Steady state values are enveloped.
Figure 10c, d. Growth rate and production as function of biomass for carbon in all phases of growth for Experiments 1A and 1B. Symbols as in Figure 4. Steady state values are enveloped.
Figure 10e. Log-log plot of production as a function of biomass for nitrogen for Experiments 1A, 1B and 2A in selection and exponential phases of growth. Symbols as in Figure 4. Selection phase values are enveloped.

Regression equations for lines are:

1A \[ P = -7.725 + 1.704 B \quad r = 0.9949 \]
1B \[ P = -28.541 + 1.304 B \quad r = 0.9959 \]
2A \[ P = -6.677 + 1.768 B \quad r = 0.9721 \]
Figure 11a, b. Steady state growth rate and production as functions of biomass for nitrogen. Symbols as in Figure 4.
Figure 11c, d. Steady state growth rate and production as functions of biomass for carbon. Symbols as in Figure 4.
growth rate are plotted against C and N biomass at steady state for all experiments. Because all of the chemostats had equal production potential, any factor affecting nutrient-cell relationships should affect the steady-state production observed. A density-dependent curvilinear relationship can be drawn through the C and N steady-state production data (Fig. 11b, d), indicating that increasing \( T_{SP} \) and/or increasing dilution rate will suppress the production-biomass response (i.e. will move the mean values back down the curves) from the no-\( T_{SP} \), low-dilution-rate conditions (1B). We can compare the relative positions of 1A and 2A along the curves to see \( T_{SP} \) effects, and the relative positions of 1A and 1B to see mainly dilution rate effects. Because the specific growth rate at steady state is controlled by the dilution rate (\( \omega \) by definition), and production is limited to some value equal to or less than the production potential, biomass is the free oscillator that must adjust so that the steady state can exist. The biomass-adjustment process occurs most dramatically in the transition phase. For example, in Experiment 1B, production reaches a value near that of steady state on the last day of exponential phase (Fig. 10b, d), at which time \( k \) drops to the steady-state value (Fig. 10a, c). The \( k \) value (\( \omega \)) remains relatively low (though variable because of method of calculation) through transition and steady state, but biomass slowly increases from transition to steady state to give an increasing production rate (\( k_B \)) up to steady state (Fig. 10b, d).
Once at steady state, biomass does not increase further, and both $k$ and production values form a clump about the maximum biomass. It is very important to note that for any experiment the steady-state points on the production-biomass curves lie to the right of the maximum exponential-phase value, but at approximately the same numerical value of $P$; hence, adjustment from exponential phase to steady state occurs by an increase in biomass in the face of a uniform specific growth rate. The mean size of individual cells (and chains) must increase during transition phase. This individual cell size increase can be noted in the time series of particle size distributions (Fig. 5). Because the steady-state values in P-B curves often lie so far displaced from the linear relationship defined during exponential phase, it is neither possible nor correct to try to fit one curvilinear relationship through all points (in Fig. 10b, d, for example) and thereby imply a single density-dependent function for all phases of growth.
Particle Size Shifts and Cell Dimensions

Particle size shifts can result from a variety of sources. Mechanical perturbations such as air bubbling (Lundy, 1973), grazing (O'Connors, 1973), or excessive stirring or shaking can lead to shorter chains and therefore a shift to smaller particle sizes. Preferential grazing of larger particles by copepods (Thomson, 1950; Strickland, 1961; Mullin, 1963) might be significant and might on occasion be distinguished from mechanical disruption by the decrease of larger size classes over time without concomitant increase in small particles. Most importantly in terms of my work, changes in particle sizes might reflect cell size shifts.

Particle sizes are functions of the physical dimensions of cells in terms of lengths and diameters as well as chain lengths. Considerable care must be exercised in interpreting particle size shifts as cell dimension shifts unless optical cell size measurements are made and stable chain length can be maintained.

Natural populations of most diatoms exhibit a wide range of cell diameters. Because new frustules are formed inside the valves of each parent cell, mean cell diameter for the population decreases with each cell division. Once a certain critical dimension is reached, division is prohibited by intracellular space requirements, and an
auxospore is formed which allows the cell to regain its maximum size. Castellvi (1969) has observed this cycle to require about two months in a natural population of Skeletonema costatum off Spain. I have observed the formation of large auxospore-like bodies in Experiment 2A, in transition phase. However, the larger cells resulting from these auxospores were never able to dominate the steady-state population in Experiment 2A. Fritsch (1948) has noted more rapid division rates of large cells just derived from auxospores, but these rates might not be maintained.

Cell size is very strongly related to specific growth rate over short time intervals. In describing the relationships, however, it is necessary to distinguish between changes in the physical cell dimensions (length and diameter) and changes in nutrient cell size (nutrient per cell). Such a distinction is required because of the fact that physical size of a cell often decreases with increasing growth rate, but the nutrient required per cell increases. The physical diameter of any one diatom cell is rather rigidly determined by its frustule, but its length can vary over a wide range. Such length variability can occur through movement apart of the two valves of the frustule as the cell increases its cytoplasmic mass preparatory to division. At the population level, both cell diameters and lengths can vary.

Cell dimensions vary as a result of the differential responses of organic syntheses and cell division, under given environmental
conditions. For example, the photosynthetic optimum for *Skeletonema costatum* occurs at 10°C, but the cell division rate reaches a maximum at higher temperatures. As a result, small cells are observed at low light and high temperatures, while large cells predominate at high light and low temperatures (Curl, unpublished; Jitts et al., 1964). Nutrient source and concentration can also be significant. Strickland et al. (1969) found that *Ditylum brightwellii* cells grown in high NO$_3$ levels were smaller and had higher growth rates than deep-tank cultures grown in lower, but still saturating, nutrient levels.

The increase in specific growth rate with decreasing cell dimensions has been observed both within species (Eppley & Sloan, 1966; Werner, 1970; Williams, 1965; Paasche, 1960; Ketchum and Redfield, 1949) and between species (Eppley et al., 1969; Maguire and Neill, 1971; and Malone, 1971). Two mechanisms have been suggested. As cell dimensions decrease, the surface-to-volume ratio greatly increases (Malone, 1971; Odum, 1956; Sayo and Takesue, 1965; Williams, 1965). As a result, the uptake of light (Paasche, 1960) and nutrients (Eppley et al., 1969) should be greatly enhanced. Increased specific growth rate might also result if, as the cell becomes physically smaller, there is a smaller structural fraction produced (such as cell walls and membranes) but the same amount of synthetic machinery is retained in the cell. Reduction in the size of the cell vacuole as cell size decreases would lead to a smaller
structural fraction without concomitant decrease in cell cytoplasm (Jorgensen, 1964). I have observed that the small *Skeletonema costatum* cells in Experiment 2A tended to have very small vacuoles, but at the same time a decrease in chloroplasts and cytoplasmic structures was also observed.

It must be pointed out that the reciprocal relationship between cell dimensions and $k$ does not hold for bacteria (which apparently are constant in size) (Kubitschek, 1971) or for *Chlorella* in chemostats (Williams, 1971). However, it does apply to a wide variety of marine diatoms and possibly flagellates.

**Dilution Rate and Nutrient Cell Size**

The principal effect of dilution rate in algal chemostats is on nutrient cell size. Nutrient cell size is usually defined in terms of $q$: the amount of limiting nutrient required per cell to maintain a specific steady state growth rate under a given set of environmental conditions. A minimal value of nutrient cell size ($q_{\text{min}}$) is determined in stationary phase of a batch culture when all the limiting nutrient has been converted into cell protoplasm and cell division has stopped.

According to bacterial kinetic theory, steady state growth rates should be a hyperbolic function of nutrient concentrations in the media and $q$ should be constant ($q = q_{\text{min}}$). However, in algal chemostats,
ambient nutrient levels can remain near zero with increasing dilution rate, but at the same time, cell numbers decrease. As a result, $q$ increases with increasing dilution rate and the specific growth rate becomes some hyperbolic function of $q$ rather than ambient nutrient concentration (Caperon, 1968; Caperon and Meyer, 1972a, b; Droop, 1969; Fuhs, 1969; Larsen, 1973; Thomas and Dodson, 1972; Williams, 1971). The increase in $q$ required with each increase in steady state growth rate (that is, $q - q_{min} = q_r$) has been variously called a nutrient reservoir (Caperon, 1968), a cellular synthetic fraction (Williams, 1971), or a physiological state (Thomas and Dodson, 1972).

Several lines of evidence suggested the existence of a nitrogen reservoir before the chemostat experiments of Caperon (1968). Ketchum (1938a, b) first suggested a variable $q$ and a decoupling of nutrient uptake from and growth, based on 1) continued cell division after nutrient depletion from the culture media, and 2) large capacities for uptake by nitrogen starved cells. Rhode (1948), observing similar events in natural systems and high growth rates in nutrient poor waters, suggested that growth rate of algae was some function of intracellular reservoirs rather than environmental levels. The existence of such reservoirs is possible because maximum uptake rates are sometimes 10 to 20 times greater than maximum growth rates (Eppley and Coatsworth, 1968; Fitzgerald, 1968; Syrett, 1962).
The exact physical-physiological form of nitrogen reservoirs under varying conditions is unclear. However, two discrete fractions of $q_r$ probably exist: 1) a reservoir of inorganic, unassimilated, intracellular nitrogen (NO$_3^-$, NO$_2^-$, NH$_3$), which we can call $q_r^1$, and 2) some additional cellular machinery (enzymes, RNA, amino acid pools) over and above levels found in a minimal cell, which we can call $q_r^2$.

Inorganic intracellular pools of NO$_3^-$, NH$_3$, and NO$_2^-$ ($q_r^1$) have been both implied and measured under a variety of conditions (Eppley and Coatsworth, 1968; Eppley and Rogers, 1970; Lundy, 1973; Thomas and Krauss, 1955). These pools are sufficiently large to give a concentration factor of 4000 over ambient levels. Intracellular NO$_3^-$ concentrations have been suggested through evaluation of NO$_3^-$-reduction kinetics which require NO$_3^-$ concentrations 100 times higher than uptake kinetics in order to maintain equivalent rates (Eppley and Rogers, 1970; Eppley and Coatesworth, 1968; Roelofs, 1971).

Inorganic nitrogen reservoirs appear to be highly labile (Lundy, 1973; Zakar, 1973). As a result, differentiation from ambient levels in the media has only recently been achieved in batch culture (Lundy, 1973). Therefore, no information on reservoir sizes is directly available in chemostatic systems. Based on Lundy's (1973) observation that most (but not all) of the inorganic pools are released on
membrane filtration, I have assumed that my particulate N measurements, made on membrane filtered particles, are estimates of total cellular nitrogen exclusive of inorganic pools; i.e., they are estimates of $q_{\text{min}} + q_{r2}$. Under such an assumption, any difference between high- and low-dilution-rate particulate nitrogens at steady-state in chemostats of equal production potential (Fig. 9) must be interpreted as the additional amount of inorganic nitrogen (both ambient and intracellular) required at steady-state to maintain the specific growth rate of the high-dilution-rate population.

Evidence is more indirect for an organic fraction of nutrient reservoirs that exists as additional synthetic machinery over and above that required to maintain a minimal cell. However, it has been established that when nitrogen deficient cells are resupplied with NO$_3^-$, large increases occur in various intracellular organic nitrogen fractions such as free amino acids, enzyme systems, RNA, and chlorophyll (Fogg, 1966; Parsons et al., 1960; Thomas and Krauss, 1955). Similar changes have been shown for bacteria in chemostatic systems (Herbert, 1961; Kubitschek, 1970). Ratios of various macroscopic cell properties ($C$, $N$, $V_T$, chlorophyll $a$, $^{14}$C production) have been shown to be closely related to growth rate both in batch culture (Lundy, 1973) and in chemostatic steady-state (Caperon and Meyer, 1972a; Larsen, 1973; Thomas and Dodson, 1972).

Analyzing different steady-state conditions in different experiments,
Larsen (1973) has observed a linear relationship between steady-state growth rate and C/N ratio. Although significant shifts in C/N ratios did occur in my experiment 2A during selection phase, C/N ratios throughout other phases in all experiments generally remained constant and typical of actively growing cells. Despite the general lack of change in C/N ratio, shifts in production-biomass curves, reduced bouyancy, and changes physical demensions of cells indicated that significant changes in physiological state occurred just before populations entered steady-state. Reduced bouyancy, for example, is strongly correlated with reduced growth rate for some species (Eppley and Strickland, 1968; Smayda, 1972) but not for others (Larsen, 1973).

Selection of Individuals in a Population

In order to fully understand population level responses in dynamic systems, and to help understand the interaction between selection and dilution rate, it is necessary to explore the results of variability in response of an individual cell, and of populations of individuals, to environmental stimuli. Such variability may occur in time for one individual or between individuals at one time. Each individual cell, and each population, has a series of responses to environmental stimuli and a series of physical properties, such as cell size, that might strongly affect those responses. In terms of
selection, the responses of interest are those that affect $\alpha$, either through change of $k$ or reduction of losses by grazing, sinking, or advection. Those responses or properties to which $\alpha$ is functionally related are defined as characteristic (c) of that population or individual. Some examples of characteristics are cell size, parameters of light-photosynthesis saturation curves, and parameters of nutrient-growth saturation curves.

The relative variability of some characteristic c at both the individual and population level is suggested in Fig. 12a. Under a relatively constant set of environmental conditions (light, temperature, etc.), the variation in response of an individual cell is based on that cell's previous experience, physiological state, and position in the cell-division cycle or auxospore cycle. Because the variability of the population is the summation of the variability within all the individuals plus the variability among individuals, the range of responses characteristic of the population is always greater than the range for the individual. Over a wider range of environmental conditions, with sufficient time for complete physiological adjustment, the response pattern in Figure 12a still applies, but over a wider range of c values.

With these ideas in mind, it is possible to define adaptation of an individual cell as a shift in the mean response of some c, even though all other responses remain possible. For example, chlorophyll "a" per cell can vary over a wide range in response to light-nutrient
Figure 12. Frequency of occurrence of characteristic \( c \) for individuals and populations (12a) plus possible population selection patterns on a single characteristic gradient. Note: \( \omega \) may be set equal to \( k \) at some value of \( c, c' \), by determining the maximum growth rate under similar environmental conditions in batch culture and the mean value of \( c, \bar{c} \), under those conditions, then starting a chemostat with \( \omega \) equal to the batch culture \( k, \bar{k} \).
conditions, and this variation is likely to affect $k$ and hence $\alpha$. At the population level, adaptive changes in chlorophyll "a" in all the cells might give distribution III in Figure 12b. In this case, a strong distributional shift from distribution I occurred, but all possible $c$ values were still "retained" in distribution III.

Population selection might be defined as the "non-returnable" shift of the population toward higher or lower $c$, in which individual cells above or below some level of $c$ are lost from the population (distribution II, Fig. 12b). This process requires a non-random loss of population members along the $c$ axis. Selection can be of two basic types: genetic selection and phenotypic selection. Under genetic selection a distribution of genetic potential must originally exist in the population and some part of this distribution must be lost. Selection might occur at a purely phenotypic level in populations of uniform genetic composition (clones) if, and only if, the phenotypic variability of the population is sufficiently broad to allow part of the population to be favored under particular conditions. Thus, large cells might be selected against, and small cells favored under a given set of conditions, even though the genetic make-up of all the cells is identical.

In the restricted case of chemostats, selection must occur through enhancement of $k$; therefore, any characteristic selected for must in some way lead to higher $k$. The selective pressure exerted in any chemostat system is a direct function of the difference between
the dilution rate ($\omega$) and $k$. If in Figure 12b we associate increasing characteristic $c$ with a decreasing $k$, and set $\omega = k$ at some value $c'$, then the part of the population above that point will be eliminated and distribution II will result. However, distribution II has less variability than distribution I and therefore less capability to respond to those environments favoring values of $c$ larger than $c'$. If on the other hand, large values of $c$ are favored, but $k$ values at the smaller values of $c$ still are greater than $\omega$, then a type III distribution will arise with small $c$ values still represented in the population distribution.

**Interactions Between Selection and Dilution Rate**

Throughout the Results section, I have described effects of selection on the population response, and the confounding of these effects with those of dilution rate. I have suggested that reduction of cell dimensions and irreversible suppression of steady-state biomasses were the results of selection processes. These processes might be responsible for some of the variability in response and deviations from theory reported for high-dilution-rate chemostats (Caperon, 1968; Thomas and Dodson, 1972; and Williams, 1971). However, only Caperon's (1968) data have been reported in sufficient detail to allow analysis of population selection in prior studies.

Caperon's data are of particular interest because he ran a series of experiments in which dilution rate was increased in a stepwise
Each experiment was allowed to proceed to a steady state under a given dilution rate, then the chemostat flow was turned off and the population was allowed to reach final concentration in terms of cell numbers (final state). After final state was achieved, a higher dilution rate was imposed on the population, and a new steady state was achieved, then final state again. This procedure was followed through increasing dilution rates. In Figure 13a, I have replotted steady state cell numbers from Caperon's Run 2 as functions of dilution rate. The final-state numbers of Run 2 and the steady state values of Run 1 will be discussed later. In Run 2, steady-state cell numbers decreased with increasing dilution rates in the face of low but constant nutrient levels. As a result, nutrient required per cell at steady state \( q \) increased dramatically at short cell residence times (Fig. 13b). The dramatic increase in \( q \) was associated with very high growth rates. However, at long residence times, steady-state \( q \) appeared to approach some minimum value. This minimum value of \( q \) defines some minimal nutrient cell size \( (q_{\text{min}}) \) suggested in Fig. 13b by the dashed line). Assuming for the moment that \( q_{\text{min}} \) is constant, then the area bounded by the steady-state \( q \) curve and the \( q_{\text{min}} \) dashed line represents the additional nitrogen per cell required to maintain some growth rate; i.e., the area represents a nutrient reservoir. It is important to note that as residence time decreases, some minimum generation time for the population \( (R_{\text{min}}) \) must be
Figure 13. Steady-state and final-state cell numbers and nutrient cell size as functions of dilution rate and residence time, respectively, replotted from Caperon (1968). Symbols as follows: steady-state Run 1 ( △ ); Run 2 ( □ ); final state Run 2 (○ ); last low-dilution-rate experiment in Run 2 noted by star ( ★ ) in appropriate symbol; horizontal dashed line (----) represents a constant steady-state or final-state cell number (13a) or q (13b); and the vertical dashed line (— — ) represents the minimum generation time for the population (R_{min}).
approached. As residence time approaches $R_{\text{min}}$ the maximum growth rate is also approached and an ever greater increase in the size of the nitrogen reservoir is required.

The size of the nitrogen reservoir can be determined in a second fashion, using Caperon's data. When the dilution rate is stopped after steady-state is reached, the cells will continue to divide at an ever decreasing rate until the limiting nitrogen is partitioned into as many cells as possible. Once some minimal nitrogen cell size is reached, cell division ceases, but the cells remain viable at some final-state (analogous to stationary phase in batch culture). Because most of the nitrogen is located within the cells at steady state, the difference between steady-state and final-state cell numbers must be equated with the complete utilization of an intracellular nutrient reservoir. If the minimal nitrogen cell size remained unchanged throughout Run 2, the final-state cell numbers should have been the same in each chemostat and equal to the dashed line in Fig. 13a at $339 \times 10^3$ cells ml$^{-1}$. However, cell numbers exceeded this value in a non-linear fashion (Fig. 13a). As a result, when final-state values of $q$ ($q_{fs}$) are plotted as a function of cell residence time (Fig. 13b), they are not equal to $q_{\text{min}}$, but rather decrease linearly with decreasing residence time. It appears then that minimal nitrogen cell size did not remain unchanged through the course of Caperon's experiments, perhaps because of cell selection as dilution rates were
systematically increased.

Using the $q$ curves, it now seems possible to separate selection effects from dilution rate effects in Caperon's data. As already described, dilution rate increases the intracellular nitrogen reservoir through an increase in nitrogen cell size. Therefore, the difference between steady state $q$ ($q_{ss}$) and $q_{min}$ is a quantitative measure of dilution-rate-induced increases in the nitrogen reservoir (Fig. 13b). In contrast, selection increases reservoir size by decreasing the minimal cell size; i.e., by decreasing $q_{min}$ to $q_{fs}$. A measure of selection effect is thus the difference between $q_{min}$ and final state $q$ ($q_{fs}$) (Fig. 13b). Finally, the summation of these two reservoir fractions, i.e., the difference between steady state and final state $q$ ($q_{ss} - q_{fs}$), is the total nitrogen reservoir per cell ($q_{r}$). This total nitrogen reservoir is reflected in the cell number data (Fig. 13a) as the difference between final-state and steady-state cell numbers.

Assuming that steady-state particle numbers are proportional to cell numbers, then $q$ curves can be applied to my data. Particle-number $q$ curves might be based on total nitrogen available in a chemostat or on particulate nitrogen. If it is assumed that final state of experiment 1B (approximately equal to final-state nitrogen of 2A) is a good estimator of total available nitrogen, then that much nitrogen was required to maintain the number particles at steady state in all chemostats. Therefore, a $q$ based on total available nitrogen per
particle (q') was calculated for each experiment (using experiment 1A as an example):

\[ 1Aq' = \frac{\text{ugN ml}^{-1} \text{ of final-state 1B}}{N_{T} \text{ of steady-state 1A}} \]

The q' values were essentially analogous to the q values of Caperon. If, as discussed earlier, the measured particulate nitrogen values are estimators of organic cellular nitrogen (exclusive of intracellular inorganic pools), then a q might be calculated based only on organic nitrogen per particle (q''), again using experiment 1A as an example:

\[ 1Ag'' = \frac{\text{ugN ml}^{-1} \text{ of steady-state 1A}}{N_{T} \text{ of steady-state 1A}} \]

By definition, q' - q'' is the size of the inorganic nitrogen reservoir \( (a_{r1}) \). Values of q' and q'' have been calculated for each experiment and are plotted in Fig. 14.

The organic fraction of the nitrogen reservoir \( (a_{r2}) \) cannot be estimated without further assumptions in my experiments. In experiments 1A and 1B, I have assumed that no selection effects occur; i.e., \( q_{fs} = q_{min} \) for both experiments. In such a case, differences between q' and q_{min} estimate the size of the complete nitrogen reservoir \( (a_{r}) \), and differences between q'' and q_{min} estimate the size of a_{r2} (Fig. 14). It should be noted that both the a_{r1} and a_{r2} components of a_{r} are larger in experiment 1A than in experiment 1B, the sole result of different dilution rates (if the assumption of no selection in either
Figure 14. Nutrient cell size as a function of residence time. Determination of $q_{r1}$ and $q_{r2}$, and functional relationships is illustrated for Experiment ZA. The lines connecting $q'$ and $q''$ of 1A to $q'$ and $q''$ are hypothetical curves of similar form to those in Figure 13b.
experiment is valid). It is obvious that most of the increase in nitrogen cell size in 1A was in terms of inorganic nitrogen pools ($q_{r1}$).

If significant selection did occur in experiment 1A over the two-day selection period, then the value of $q_{r2}$ would be underestimated, because although $q_{r2}$ should equal $q'' - q_{fs}$, $q_{r2}$ was measured as $q'' - q_{min}$. Also, if part of $q_{r2}$ was lost as labile pools of organics such as free amino acids, $q''$ would be underestimated. As a result, $q_{r1}$ would be overestimated and $q_{r2}$ underestimated.

The increases in synthetic machinery at higher growth rates in experiment 1A (compared to experiment 1B) are in agreement with the higher production per unit biomass (Fig. 10), higher C and N per unit volume (Fig. 8), and more volume per particle (Fig. 8) observed in experiment 1A. Such increases in higher-growth-rate systems are also in agreement with the general concept of nutrient reservoirs.

Effects of selection were dominant in experiments 2A, 2B and 2C, but were confounded with dilution rate. I have suggested a separation of these effects for experiment 2A because I allowed a final state to develop for this experiment (Fig. 14). The decrease in minimal nitrogen cell size of 2A from $q_{min}$ to $q_{fs}$ was sufficiently large so that the steady-state 2A $q'$ and 2A $q''$ values were below the $q_{min}$ line set for the no-selection-phase experiments (1A and 1B). I have assumed that the parent population of 2A (and 2B and 2C) had the same $q_{min}$ as 1A and 1B. As a result of the reduction of minimal
nitrogen cell size in 2A, the total nitrogen per particle is smaller, and the nitrogen reservoirs \(q_{r1}, q_{r2}\), and thus \(q_r\) are also smaller than those in 1A. These smaller nitrogen reservoir sizes are in accord with the smaller physical dimensions of cells in experiment 2A. However, reduction in reservoir size might seem contrary to 1) equally high growth rates observed in 1A and 2A, and 2) the much lower particulate nitrogen levels in 2A. If we examine the series of experiments in order of increasing selection time (1B<2B<2C<2A), a pattern is suggested (Fig. 14). As the dilution rate increases in the series, the difference between \(q'\) and \(q''\) (i.e., \(q_{r1}\)) increases (dilution-rate effect), but at the same time the organic nutrient cell size \(q''\) and by inference the minimal cell size, decreases (a selection effect). By the end of the series, \(q_{r1}\) of Experiment 2A is still smaller than 1A. However, what is probably most significant is that the percent of the total cell nitrogen devoted to synthetic machinery \(q_{r2}\) and inorganic pools \(q_{r1}\) increases sharply in the series along with the decrease in \(q_{\text{min}}\). Because of the decrease in \(q_{\text{min}}\), the cells in Experiment 2A have a greater percentage of the total nitrogen devoted to synthetic functions. The larger inorganic pools on a percent basis, in 2A over 1A, even though less in absolute terms, allow 2A to maintain the same high growth rate as 1A. However, the requirement for a greater percent of the total cell nitrogen as \(q_{r1}\) in 2A results in a smaller percentage particulate nitrogen. As a result,
the observed particulate nitrogen (i.e., the product of $N_T q''$) is
greatly reduced in Experiment 2A versus 1A. It must be noted that,
as I have calculated it, $q_{r1}$ is composed of both ambient and intra-
cellular pools. However, the ambient levels have been shown to be
both cell concentration and dilution rate independent. Therefore, the
produce $q_{r1} N_T$ is most sensitive to changes in cell numbers and intra-
cellular nitrogen reservoirs.

It seems apparent that two different modes of adjustment to high
dilution rates occurred in Experiments 1A and 2A. In 1A, adjust-
ments in terms of nutrient cell size may have been sufficient to allow
for higher growth rates and shorter selection phase. However, in
Experiment 2A, both nutrient size and cell dimension shifts occurred.
The factors that control the length of selection phase, the events
occurring during the period, and the factors releasing the higher
growth rate at the end of the phase should be the objective of future
experiments.

In light of the significance of selection effects in altering popu-
lation responses, it is desirable to determine if the process is rever-
sible, and if so, to what extent. The degree of reversibility is of
interest both from the practical standpoint of designing non-selection-
confounded nutrient kinetics experiments and from the theoretical
standpoint that the degree of reversibility leads to implications con-
cerning both the nature of selective processes (genetic, phenotypic,
or adaptive) and the range of variability existing in the initial populations. Although the complete description of the operation of selection and its coupling to the physical cell size must await further experiments, the experiments of Caperon (1968) allow some preliminary insights into the question. These insights are based on deviations of the results expected if one or more of the following possibilities hold:

1) total reversibility expected if the process is "adaptation"; 2) no reversibility if the process is phenotypic or genetic selection on a monotypic population; and 3) the interaction with each of these alternatives by the range of variability of the population characteristics.

After running his chemostat series to the highest dilution rate, Caperon (1968) tried to duplicate results obtained earlier at a lower rate (indicated by a * in Fig. 13a and b). He was unsuccessful.

Because the major feature of selection is a change in minimal cell size, the position of tf for experiment 7 (called tf_s7) in relation to the final state tf line (Fig. 13b) is very important. If the selection process were completely reversible, tf_s7 should lie directly on the tf line (Fig. 13b). If, on the other hand, selection was not reversible, tf_s7 should have the same value as the highest dilution rate system, tf_s6. Although the tf value of experiment 7 lies below the tf line, it is not the same as tf_s6 but instead lies intermediate. An explanation of this result is offered in Figure 15. With any population characteristic, only the mean of the distribution of that characteristic
Figure 15. Suggested distributions of minimal cell sizes for final states of Experiments 1, 6, and 7 of Run 2 (Caperon, 1968).
can be measured. Suppose a fraction of the population was eliminated by the increasing dilution rate series, selecting for smaller minimal cell sizes and giving a lower mean $a_{fs6}$ (Fig. 15). However, the mean $a_{fs}$ observed is considerably less than the maximum value remaining in the distribution. On returning to a lower dilution rate, cells in lower abundance (with higher $a_{fs}$ values than the mean of $a_{fs6}$) remaining in the $a_{fs}$ population increased in abundance. However, because part of the original population was lost, the original mean $a_{fs}$ curve could not be reconstructed. Therefore, a new intermediate $a_{fs}$ distribution resulted, $a_{fs7}$. If this interpretation is accepted, Caperon's results imply that the selective process is irreversible (i.e., it is not "adaptation"). However, deviations from the expected irreversible response will occur in a magnitude proportional to the amount of variability of the population characteristic that remains after selection.

Implied in Figure 15 is the idea that the occurrence of final states (change to lower growth rate conditions) and steady states (change to higher growth rate) result in selective forces in opposite directions. If this is in fact the case, then if experiments are run without final states between each steady state, the selective pressure should be unidirectional throughout the complete experimental run and the magnitude of changes observed in the final low-dilution-rate duplication experiment should be greater than the changes occurring from
sequential steady-state and final-state experiments. This hypothesis can be examined by comparing Caperon's (1968) Run 2 (final states between steady states) with Run 1 (no final states). Because no final states were done in Run 1, it is not possible to examine the effect of selection using reversibility and deviation from the $q_{fs}$ line as described above with reference to Run 2. However, if the secondary effects of selection on steady-state nutrient requirements are recalled, it becomes possible to use deviations from expected steady state values as a measure of selection. In the discussion of my data in Fig. 14, it was shown that associated with reduction in minimal cell size observed in selected cultures, larger nutrient reservoirs ($q_r$) were required to maintain a particular growth rate ($k$) than in unselected cultures. When the selection-induced steady-state increase in $q_r$ is greater than the reduction of $q_{fs}$ (minimal cell size) arising from selection, then the steady-state value (cell number) always will fall below the unselected steady-state value and the magnitude of such a suppression will be a measure of the combined selection effects on $q_r$ and $q_{fs}$. It should be noted that although this method can lead to an underestimation of the selective effect if changes in $q_{fs}$ are great, it should not lead to overestimations. Using this idea, the deviation below the line of steady-state values of Experiment 6 in Run 1 and Experiment 7 in Run 2 should be quantitative measures of the selection incurred by the populations during the runs. Because these
runs differed principally in that no reverse selection events (final states) existed in Run 1, then the greater depression of the last experiment in Run 1 (Experiment 6, Fig. 13a) implies that the net effect of selection is greater when no final states interrupt an increasing-dilution-rate series. In other words, when the dilution rate is stopped so that a final state may be established in Run 2, individuals with smaller $q_r$, unfavored by the higher dilution rates, could increase in abundance much as described in connection with the terminal lower-dilution-rate duplication experiment at the end of both runs (Fig. 15).

A Nutrient Reservoir Model

From the discussion and interpretation of my results in conjunction with those of Caperon (1968), it seems that a cellular model of nutrient dynamics in phytoplankton systems can be proposed (Fig. 16). The model has been designed to include the ideas of nutrient reservoirs, physiological state, and selection effects on minimal cell size. Compartments within the cell have been developed to correspond to (1) the existence of intracellular inorganic nitrogen reservoirs ($\text{NO}_3^-, \text{NO}_2^-$, and $\text{NH}_3$) which act as substrate for enzymatic reduction ($q_{r1}$); (2) increased synthetic machinery (enzymes, RNA, amino acid pools, etc.) necessary to achieve and maintain a specific growth rate ($q_{r2}$); and (3) a minimal nitrogen requirement per cell that is observed at final state when all cell division stops ($q_{f8}$). The terms $q_{r1}$, $q_{r2}$,
Figure 16. A nutrient reservoir model. Terms are defined in the text and Table 6. Solid lines represent processes measured in previous work; dashed lines represent additional processes that must be quantified in future research.
are measured as nitrogen contained within those compartments per cell (ugN cell\(^{-1}\)). Total nitrogen within the cell is thus \(q_{r1} + q_{r2} + q_{fs} = q\) and total nitrogen within the population is \(q(N_T) = q_{r1}(N_T) + q_{r2}(N_T) + q_{fs}(N_T)\). As discussed above, and summarized in Table 6, each of these compartments may be estimated in terms of \(q\) curves (Figs. 13 and 14) and selection and dilution rate effects thereon observed. An important separation is made in the model between ambient nutrient (\(S_e\)) and intracellular inorganic pools, \(q_{r1}\) \((N_T)\). This distinction was not possible in the data described with the \(q\) curves, but must be made in all future work. In addition to the \(S_e\) and cellular compartments, it is necessary to consider in a dynamic model the concentration of input nutrients \((S_i)\), the rate at which these nutrients enter the system \((k_{di})\), and the rate at which nutrients and cellular fractions leave the system \((k_{de}, k_{dr1}, k_{dr2}, \text{and } k_{dfs})\). In the restricted case of the chemostat, the specific rates of input and loss are equal to the dilution rate. If the model is extrapolated to more natural systems, processes of sinking \((k_s)\), grazing \((k_g)\), and nutrient regeneration \((k_{zi})\) must be added. However, these additional processes will not be considered here. The term \(S_o\) refers to nitrogen that has been taken up and assimilated and then released as organic excretion. Specific rates of transfer relate to net uptake of nitrogen into the cell \((k_1)\), net assimilation of nitrogen into synthetic machinery and organic intermediates \((k_2)\), net excretion of nitrogenous
### Table 6. Definition and Comparison of Model Terms

<table>
<thead>
<tr>
<th>MODEL TERM</th>
<th>DEFINITION</th>
<th>TERM QUANTIFICATION</th>
<th>Assumptions/Comments</th>
<th>ANALOGOUS TERMS IN PREVIOUS MODELS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_i$</td>
<td>Input nitrogen (ug N ml$^{-1}$)</td>
<td>$q_{i0}$ counterpart</td>
<td>measurement methods available</td>
<td>Caperon (1968) = $N_i^{-1}+$N$^{-1}$+N$^{-1}$+N$^{-1}$</td>
</tr>
<tr>
<td>$S_o$</td>
<td>Ambient N (ug N ml$^{-1}$)</td>
<td>$q_{o0}$</td>
<td>direct: nitex technique (Lonard, 1971); indirect: none</td>
<td>S$^{-1}$</td>
</tr>
<tr>
<td>$S_{is}$</td>
<td>Intracellular inorganic N reservoir (ug N ml$^{-1}$)</td>
<td>$q_{is}$</td>
<td>direct: (NO$<em>3$ after sonic disrupting) (dissolved) indirect: $q</em>{is0}+q_{is}k_2$</td>
<td>S$^{-1}$</td>
</tr>
<tr>
<td>$S_{oe}$</td>
<td>Organic N in minimal N cell size (ug N ml$^{-1}$)</td>
<td>$q_{oe}$</td>
<td>direct: from q$<em>{ie}$ curve, $q</em>{oe0}$</td>
<td>estimated as $N_i^{-1}$ cell numbers + excretion</td>
</tr>
<tr>
<td>$k_{di}$</td>
<td>Net specific nutrient input rate; equals dilution rate, (ml hr$^{-1}$)(ml vessel vol)$^{-1}$</td>
<td>$\omega$</td>
<td>Directly: $f$ Y$^{-1}$</td>
<td>n</td>
</tr>
<tr>
<td>$k_1$</td>
<td>Net cell nitrogen specific uptake rate</td>
<td>$k_1 = k_2 + k_3$ when $\omega = 0$</td>
<td>direct: as cell N specific rates of $S_o$ uptake</td>
<td>$k_2$ (no saturation kinetics assumed)</td>
</tr>
<tr>
<td>$k_2$</td>
<td>Net cell nitrogen specific assimilation rate</td>
<td>$k_2 = k_1 + k_2$</td>
<td>direct: net rate of removal of (norg. N from $q_{oi}$); also $k_2 = k_3$</td>
<td>$k_3$</td>
</tr>
<tr>
<td>$k_3$</td>
<td>Net rate of formation of minimal cells</td>
<td>$k_3 = k_1 + k_2$ when $\omega = 0$</td>
<td>direct: net rate of removal of (norg. N from $q_{oi}$); also $k_2 = k_3$</td>
<td>$k_3$</td>
</tr>
<tr>
<td>$k_4$</td>
<td>Net cell nitrogen specific excretion rate</td>
<td>$k_4$</td>
<td>direct: none available; requires soluble organic N tech., if can directly estimate $k_4$ then $k_4 = k_2 - k_1$</td>
<td>$k_4$</td>
</tr>
<tr>
<td>$k_5$</td>
<td>Net specific cell N rates of loss from system</td>
<td>$k_5$</td>
<td>direct: none available; requires soluble organic N tech., if can directly estimate $k_5$ then $k_4 = k_2 - k_1$</td>
<td>$k_5$</td>
</tr>
</tbody>
</table>

$\omega$ means that the term to the right is equivalent to the sum of the terms to the left.
organic matter \(k_4\), and net formation of minimal cell equivalents \(k_3\). It should be noted that all of the cellular rates \((k_1, k_2, k_3, k_4)\), are specific to total cellular nitrogen (i.e., \(q_r + q_{r2} + q_{fs}\)). For example, \(k_1\) is in units of \(\mu gN\) taken up/\(ug\) cellular \(N/\) unit time. It should be emphasized that the \(k\) values are all net rates and can go to zero. The \(k_1\) and \(k_4\) values can go negative, but it seems unlikely that \(k_2\) and \(k_3\) can do this.

It is my intent here only to establish a biologically based conceptual model of cellular nutrient dynamics that provides a framework for interpreting and summarizing a wide variety of existing data. Although in future work terms of the model should be quantified in terms of \(q\) curves and through direct measurement (Table 6), lack of clear separation of \(S_o\) and \(S_e\) in past work makes definition of mathematical relationships impossible. However, it has been possible to establish functional relationships of the general formulation \(k = f(x)\). These relationships are summarized in Table 6 and are discussed in detail below (see Operation of the Model). I have specifically avoided the use of any kinetic expression (such as the Michaelis-Menten equation) to describe any of the rate processes, because in many cases not enough is known to insure the validity of commonly used equations. The model has been developed in particular for nitrogen; however, the model might be applicable to other nutrients (C, P, Fe, and vitamin \(B_{12}\)) with some modifications.
**Operation of the Model**

Inorganic nitrogen in the environment \( (S_e) \) is taken up at some net rate \( (k_1) \) by the cell for storage in an intracellular inorganic reservoir \( (q_{r1}) \). The maximum uptake rate is assumed to be much faster than the net assimilation rate \( (k_2) \). The uptake rate is a function of the fullness of \( q_{r1} \) down to some very low level of \( S_e \), below which \( k_1 \) is a function of the availability of \( S_e \). At very low \( S_e \), the probability of encounter of a nitrogen molecule by a cell will control availability and thereby uptake. For other nutrients such as iron, process such as chelation as well as concentration may control availability. The rates most often observed in short term nitrate and ammonia uptake experiments involve filling of \( q_{r1} \) in the absence of assimilation (Caperon and Meyer, 1973a; Eppley and Coatesworth, 1968; Eppley et al., 1969).

The size of \( q_{r1} \) might be a species characteristic and a function of temperature, light, and other significant growth rate controlling variables. The fullness of \( q_{r1} \) will depend on the difference between input at the net specific uptake rate \( (k_1) \) and removal by assimilatory processes at rate \( k_2 \). Thus, as \( k_2 \) increases, \( q_{r1} \) might drop below some maximum concentration.

The net specific rate \( (k_2) \) at which \( q_{r1} \) is assimilated into organic fractions is dependent upon the levels of \( q_{r1} \) and \( q_{r2} \). As in
any enzyme reaction the concentrations of substrate and enzyme are both critical. $q_{r2}$ in terms of nitrogen includes the nitrogen concentration in both the enzyme systems and the resultant amino acid pools produced by the multitude of reactions associated with $k_2$. The $q_{r2}$ compartment therefore can be viewed as an operator required to maintain a maximum growth rate in an environment. All of the necessary additional enzyme systems, pools of amino acids, etc. must first be synthesized (i.e., a certain physiological state must be reached) before some specific growth rate can be attained. Because $k_2$ is a function of $q_{r2}$ as well as $q_{r1}$, a cell with little $q_{r2}$ must first synthesize more enzymes, etc. than a cell with much $q_{r2}$ before it can utilize $q_{r1}$. For example, when a cell at final state ($q_{r1} = q_{r2} = 0$, by definition) is resupplied with NO$_3$, $q_{r1}$ may fill rapidly but the low levels of enzymes in $q_{fs}$ must first synthesize the additional enzymes, amino acid pools, etc. at a very slow rate, before high rates of assimilation will occur. This hypothesis implies that substantial lags can occur after $q_{r1}$ is filled, and that maximum $k_2$ will be achieved only after $q_{r2}$ is maximized.

The organic nitrogen assimilated at the rate $k_2$ can either be added to the cell as new protoplasm ($q_{r2}$ and $q_{fs}$) or can be lost as excretion into the environment ($S_o$) at rate $k_4$. The specific growth rate as used throughout this paper is, in terms of nitrogen, the net rate of formation of particulate N, or the net rate of increase in the
compartment \((q_{r2} + q_{fs})\), and is equivalent to \(k_2 - k_4\).

When \(k_1 < k_2\) (i.e., when the cell can no longer take up \(S_e\) as fast as it can assimilate \(q_{r1}\)) the syntheses of additional enzyme systems (increases in \(q_{r2}\)) ceases and degradation of those systems and/or repartitioning into cells or size \(q_{fs}\) commences at some rate \(k_3\). The rate \(k_3\) is only of significance in describing the decreasing cell division rate during the period from steady state to final state in a chemostat. Likewise, the term \(q_{fs}\) is mainly useful in describing selection effects. However, the repartitioning, like the synthesis of \(q_{r2}\), is much slower than the filling of \(q_{r1}\). Therefore, the level of \(q_{r2}\) along with its associated physiological state might persist for some time after \(k_1\) becomes less than \(k_2\). If new \(S_e\) is made available at this time, \(q_{r2}\) will control the observed short term velocity of uptake by the cell. For example, in experiments such as those of Caperon and Meyer (1973b) uptake rates (for chemostat steady state populations) were initially very high (i.e., filling of partly empty \(q_{r1}\), with \(k_1 = \text{maximum } k_1\)) followed by a fairly constant rate (their \(V_{\text{max}}\) that was a function of the growth rate in the parent chemostat. The \(V_{\text{max}}\) of Caperon and Meyer appeared to be a function of \(q_{r2}\) in the parent chemostat and was a measure of the rate \(k_2\).

Excretion as used in the model is limited to the loss of various fractions of \(q_{r2}\) to the external environment from viable cells by diffusion or active transport processes. The \(S_o\) term does not include
spillage of internal labile fractions by cell disruption either through cell lysing during death or through mechanical breakage. Because filtration techniques have been used in most determinations of $S_o$ in the past, qualification and quantification of $S_o$ and $k_4$ must await the application of the Nite technique (Lundy, 1973) and the development of a method of soluble organic nitrogen analysis.

Based on the operation of the model, it seems possible to speculate on factors controlling excretion. First, $S_o$ might occur normally and consistently at some level because of diffusion or leakage from $q_{r2}$. This "normal" leakage should be proportional to the level of labile fractions in $q_{r2}$. Secondly, when cells enter a new environment the composition of $q_{r2}$ might have to undergo adjustment, with the result that diffusion of "old" materials from $q_{r2}$ might occur. In addition, if the new environment has very low levels of $S_o$, increased diffusion of labile factions might result from the larger gradient across the cell wall. Thirdly, when previously nutrient-deficient cells are filling $q_{r2}$, some initially synthesized compounds (perhaps certain amino acids) might reach high levels because of blockage of further steps in the synthesis process by temporary absence of necessary enzymes that must be produced. Then, such compounds might be actively or passively exuded into the environment. A fourth consideration of loss of $S_o$ might come when $k_1 < k_2$. Under such a condition, $a_{r1}$ begins to fall, $a_{r2}$ is repartitioned into $a_{fs}$, and
k₄ appears to reach a maximum (Fogg, 1968). Maximum excretion (k₄) will occur if fractions of \(q_{r2}\) are not rapidly degraded or resynthesized into the components of \(q_{fs}\). For example, when dilution rate is reduced, or an exponential-phase population reaches steady state, the cells must adjust \(q_{r2}\) to a level appropriate to that lower growth rate. This requires a reduction in synthetic machinery (\(q_{r2}\)) achievable either by excretion or by creation of more non-synthetic cell fractions (\(q_{fs}\)) such as cell walls. It is particularly difficult in examining populations to tell if some cells are lysing, or if all are excreting as described above. In my chemostat system, excretion in transition phase might result from such repartitioning processes. As a fifth possibility, \(S_o\) could be actively excreted by a cell to affect other cells or to enhance the availability of other non-S nutrients to itself. Acting as chelators, some of the compounds in \(S_o\) might insure the availability of trace metals that are necessary for the synthesis of enzyme systems in \(q_{r2}\). Thus, \(S_o\) might be viewed as acting as an operator on \(q_{r2}\) through its effect on the uptake of other elements necessary to the development of \(q_{r2}\). The operator character of \(S_o\) might be most strongly felt when small populations are placed in environments with high dilution rate and low organics such as 1) freshly upwelled deep water (Barber et al., 1971), or 2) high-dilution-rate chemostats using activated-carbon-filtered water (present work, Experiments 1A and 2A). The role of excretion in dynamic
systems should be an objective of future work. It should be noted that, except for the fourth possibility above, excretion can be at very low levels and might support beneficial bacteria (Castellvi, 1972), act as chelators, or act as direct sources of nutrient elements.


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