COMPETITION BETWEEN TWO AQUATIC MICROORGANISMS FOR OSCILLATING CONCENTRATIONS OF PHOSPHORUS

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COMPETITION BETWEEN TWO AQUATIC MICROORGANISMS FOR OSCILLATING CONCENTRATIONS OF PHOSPHORUS

A

THESIS

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ABSTRACT

The availability of limiting nutrients is a critical factor regulating growth of aquatic microorganisms. In at least some aquatic systems the frequency of addition rather than the absolute concentration of nutrients controls community structure. Gnotobiotic continuous cultures were used to examine the growth characteristics of a green alga (*Selenastrum capricornutum*) and a heterotrophic yeast (*Rhodotorula rubra*) when phosphorus-limited steady-state populations were subjected to varying concentrations of pulsed phosphorus. The responses of these organisms to phosphorus additions were measured both in single and dual species continuous cultures. Both organisms exceeded the maximum transport rates for phosphorus predicted from batch and steady-state continuous cultures. Carbon limitation did not cause a decline in phosphorus accumulation in *R. rubra.* Carbon-limited yeast cultures perturbed with phosphorus attained the highest phosphorus per cell values seen in these studies. The phosphorus pool was not significantly diminished in these cultures only

because the total yeast biomass was limited by carbon. These results suggest that carbon-limitation of heterotrophic populations may be essential to the existence of phytoplankton in low-nutrient aquatic environments.

TABLE OF CONTENTS

Page

ABSTRACT iii

TABLE OF CONTENTS v

LIST OF FIGURES vii

LIST OF TABLES xiii

LIST OF APPENDICES xv

LIST OF SYMBOLS xvi

ACKNOWLEDGMENTS xviii

INTRODUCTION 1

LITERATURE REVIEW 6

Phosphorus in Aquatic Systems 6

Phosphorus Metabolism by Microorganisms 11

Growth and Uptake Kinetics 13

Interactions Between Heterotrophs and Autotrophs ... 27

Competition Models 31

Transient Aquatic Systems 35

METHODS AND MATERIALS 49

Microorganisms 49

Growth Media 49

Radioisotope Labeling 53

Page

Culture Methods 54

Sampling 56

Perturbations 57

Enumeration 58

Carbon 59

RESULTS 61

Overview 61

Phosphate Uptake 64

Transport Rates 83

Biomass Responses 105

Summary 108

DISCUSSION 111

Introduction 111

Phosphorus Uptake and Internal Phosphorus Concentrations 116

Cell Size and Biomass 124

Availability of Carbon 127

Other Population-Controlling Factors 128

Summary 130

APPENDIX 1 133

APPENDIX 2 143

APPENDIX 3 147

LITERATURE CITED 153

LIST OF FIGURES

Page

Figure 1. Schematic diagram of single-stage, single-phase continuous culture apparatus. 15

Figure 2. Dimensionless hyperbolic plots of three basic models for nutrient uptake and growth of nutrient-limited microorganisms. 17

Figure 3. Dimensionless plot of two models for substrate-limited growth of microorganisms. 21

Figure 4. Continuous culture dry weight of *Selenastrum capricornutum* and *Rhodotorula rubra* and both species as a function of available C and P_i (C_o/P_o) ratio and P_i in solution versus C_o/P_o. 30

Figure 5. Percent phosphorus in solution after phosphate perturbation (1087 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 65

Figure 6. Percent phosphorus in solution after phosphate perturbation (2911 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 66

Figure 7. Percent phosphorus in solution after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 67

Figure 8. Percent phosphorus in solution after phosphate perturbation (2911 nM) of a phosphorus-limited steady-state culture of *R. rubra*. 68

Figure 9. Percent phosphorus in solution after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state culture of *R. rubra*. 69

Figure 10. Percent phosphorus in solution after phosphate perturbation (7762 nM) of a carbon-limited steady-state culture of *R. rubra*. 70

Figure 11. Percent phosphorus in solution after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state dual-species culture. 71

Figure 12. Percent phosphorus in solution after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state dual-species culture. 72

Figure 13. Internal phosphorus concentration (Q) after phosphate perturbation (1087 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 75

Figure 14. Internal phosphorus concentration (Q) after phosphate perturbation (2911 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 76

Figure 15. Internal phosphorus concentration (Q) after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 77

Figure 16. Internal phosphorus concentration (Q) after phosphate perturbation (2911 nM) of a phosphorus-limited steady-state culture of *R. rubra*. 78

Figure 17. Internal phosphorus concentration (Q) after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state culture of *R. rubra*. 79

Figure 18. Internal phosphorus concentration (Q) after phosphate perturbation (7762 nM) of a carbon-limited steady-state culture of *R. rubra*. 80

Figure 19. Cell numbers after phosphate perturbation (1087 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 88

Figure 20. Culture absorbance at 686 nm after a phosphate perturbation (1087 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 89

Figure 21. Cell numbers after phosphate perturbation (2911 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 90

Figure 22. Culture absorbance at 686 nm after a phosphate perturbation (2911 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 91

Figure 23. Cell numbers after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 92

Figure 24. Culture absorbance at 686 nm after a phosphate perturbation (7762 nM) of a phosphorus-limited steady-state culture of *S. capricornutum*. 93

Figure 25. Cell numbers after phosphate perturbation (2911 nM) of a phosphorus-limited steady-state culture of *R. rubra*. 94

Figure 26. Culture absorbance at 686 nm after a phosphate perturbation (2911 nM) of a phosphorus-limited steady-state culture of *R. rubra*. 95

Figure 27. Cell numbers after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state culture of *R. rubra*. 96

Figure 28. Culture absorbance at 686 nm after a phosphate perturbation (7762 nM) of a phosphorus-limited steady-state culture of *R. rubra*. 97

Figure 29. Cell numbers after phosphate perturbation (7762 nM) of a carbon-limited steady-state culture of *R. rubra*. 98

Figure 30. Culture absorbance at 686 nm after a phosphate perturbation (7762 nM) of a carbon-limited steady-state culture of *R. rubra*. 99

Figure 31. Cellular carbon after phosphate perturbation (7762 nM) of a carbon-limited steady-state culture of *R. rubra*. 100

Figure 32. Cell numbers after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state dual-species culture. 101

Figure 33. Cellular carbon after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state dual-species culture. 102

Figure 34. Cell numbers after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state dual-species culture. 103

Figure 35. Cellular carbon after phosphate perturbation (7762 nM) of a phosphorus-limited steady-state dual-species culture. 104

LIST OF TABLES

Page

Table 1. Constituents of Culture Media Stock. 50

Table 2. Composition of Culture Media. 52

Table 3. Summary of Perturbation Conditions for Single and Dual Species Continuous Cultures. 62

Table 4. Maximum Internal Phosphorus Concentrations and Maximum Cell Numbers After Perturbations. 82

Table 5. Average Transport Rates for Measured Time Intervals. *Selenastrum capricornutum* Cultures. 84

Table 6. Average Transport Rates for Measured Time Intervals. *Rhodotorula rubra* Cultures. 85

Table 7. Average Transport Rates for Measured Time Intervals. Dual Species Cultures. 86

Table 8. Steady-State Kinetic Constants for *Rhodotorua rubra* and *Selenastrum capricornutum*. 113

Table 9. Maximum Intracellular Phosphorus Concentrations for Phosphorus-Limited Steady-State Cultures Perturbed with Phosphate. 119

Table 10. Highest Observed Transport Rates for Phosphorus-Limited Steady-State Cultures Perturbed with Phosphate. 122

LIST OF APPENDICES

Page

Appendix 1. Data from *Selenastrum capricornutum* Continuous Cultures. 133

Appendix 2. Data from *Rhodotorula rubra* Continuous Cultures. 143

Appendix 3. Data from Dual Species Continuous Cultures. 147

LIST OF SYMBOLS

Symbol	Meaning
a	Specific affinity (l·mg cell-day^{-1})
C	Carbon (concentration)
C/P	Carbon to phosphorus ratio (unitless)
D	Dilution rate (F·V^{-1})
F	Flow rate (ml·hr^{-1})
K_{μ}	Half saturation constant for growth (time^{-1})
K_s	Half-saturation constant for uptake (time^{-1})
P	Phosphorus (concentration)
P_{back}	Phosphorus concentration in media (nM)
$P_{perturb}$	Phosphorus concentration of perturbation (nM)
P_{sol}	Phosphorus concentation in reactor (nM)
PAAP	Provisional algal assay procedure
Q	Cell Quota (nmol·mg cell^{-1})
Q_o	Minimum (subsistence) cell quota (nmol·mg cell^{-1})
Q_{maxs}	Maximum steady-state cell quota (nmol·mg cell^{-1})
Q_{maxt}	Maximum transient (batch) cell quota (nmol·mg cell^{-1})
Q_{maxP}	Maximum transient (perturb) cell quota (nmol·mg cell^{-1})
R	Coefficient of luxury consumption (unitless)
S_t	Threshold concentration of substrate (nM)

Symbol	Meaning
V	Volume
V_{max}	Maximum transport rate
X	Cell numbers ($cells \cdot l^{-1}$)
X_{max}	Maximum cell numbers ($cells \cdot l^{-1}$)
μ	Specific growth rate ($time^{-1}$)
μ_{max}	Apparent maximum specific growth rate ($time^{-1}$)

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INTRODUCTION

Many factors contribute to limit the growth of phytoplankton populations in natural environments and determine the species that exist in those environments. Extremes in light, temperature, pH, oxygen concentration, antimicrobial metabolites, predators, and parasites contribute to the control of populations. However, a major factor which often controls the biomass and community structure of aquatic microorganisms natural ecosystems is the availability of essential nutrients (Tempest and Neijssel, 1978; Morel, 1987).

The nutrient most often considered to be limiting in freshwater systems is phosphorus (Schindler, 1977), while nitrogen is generally attributed to controlling phytoplankton populations in marine environments (Dugdale and Goering, 1967; McCarthy et al., 1977; Eppley et al., 1979). Several experiments have shown heterotrophic microorganisms to be better competitors for these nutrients than algae (Brown et al., 1981; Bratbak and Thingstad, 1985). Therefore, some factor (probably organic carbon concentration) must limit heterotrophic populations in aquatic ecosystems. Bratbak and Thingstad

(1985) described the interaction between heterotrophs (bacteria) and phytoplankton in aquatic systems as an apparent paradox, and pointed out that there is "scant experimental evidence on the subject of bacterial/algal competition."

Numerous models have been presented over the last several decades which have attempted to predict how certain species compete for limiting supplies of nutrients and explain the observed populations in natural aquatic systems. Steady-state models have been used widely in the literature to explain the interactions among phytoplankton species and essential nutrients. Steady-state is defined as balanced growth where the change in biomass and substrate with respect to time is zero (Barford et al., 1982). Uptake and growth constants calculated in steady-state laboratory experiments are often used to attempt to predict which organisms will predominate in low-nutrient environments.

If competition for a single limiting nutrient is the only factor determining the success of a species in natural systems, then one would expect a relatively low diversity of species (competitive exclusion). On the contrary, natural waters generally support many species of phytoplankton simultaneously. This apparent

discrepancy was termed "the paradox of the plankton" by Hutchinson (1961).

Many aquatic systems are subject to pulses of essential nutrients and, therefore, cannot be adequately described by steady-state models. Brown et al. (1978) showed that transport rates may exceed observed steady-state maximum transport rates when phosphorus-starved cells are perturbed with excess phosphorus. Fuhs et al. (1972), Rhee (1973) and Lang (1980) have shown that internal cell phosphorus concentrations under transient conditions reach a maximum cell quota at a higher concentration than observed at the maximum growth rate under steady-state conditions. A similar observation has been made by McCarthy and Goldman(1979) for uptake of nitrogen by phytoplankton. These data suggest that different metabolic mechanisms may control cell nutrient levels, uptake rates and growth rates when transient, rather than steady-state conditions, exist (Lang, 1980). There is evidence that transient uptake rates of nutrients are regulated by cellular nutrient levels, which are in turn controlled by cellular storage capacity (Rhee, 1974; Brown and Button, 1979) Further, transient uptake rates appear to be positively correlated with the previous degree of

nutrient stress (McCarthy and Goldman, 1979; Morel, 1987).

Despite the fact that transient pulses of nutrients on both spatial and temporal scales probably occur routinely in nature, the responses of microorganisms to pulses of nutrients have not been sufficiently studied (Rhee, 1980; Cunningham and Maas, 1982). The lack of study is partly attributable to the difficulties of designing and implementing good transient experiments (Cunningham and Maas, 1982).

I describe herein a study on the growth and nutrient uptake kinetics of two aquatic microorganisms when steady-state phosphorus-limited continuous cultures are subject to perturbations of varying concentrations of phosphorus. The responses of these organisms, a heterotroph and an autotroph, to pulses of phosphorus were measured both in single and dual species continuous cultures. This research is relevant to understanding the mechanisms that control microbial species composition in aquatic environments that have been subject to nutrient perturbations as a result of increased usage or intentional fertilization. Microbial kinetic models are essential in understanding nutrient fluxes in these systems and are extremely useful in predicting the

potential impact on aquatic systems when nutrient concentrations are increased.

LITERATURE REVIEW

Phosphorus in Aquatic Systems

The essential limiting nutrient in many freshwater systems is generally accepted to be phosphorus (Schindler, 1977). Nitrogen availability is often attributed to controlling phytoplankton populations in marine environments (Dugdale and Goering, 1967; McCarthy et al., 1977; Eppley et al., 1979). It has been argued though, that phosphorus must be the ultimate limiting nutrient in nearly all aquatic environments, because, unlike nitrogen, there is no potential for significant phosphorus inputs to aquatic systems from the atmosphere (Broecker and Peng, 1982). Phosphorus has been extensively studied in lakes because of its role in the eutrophication process and in control of primary and secondary production in lakes (Tarapchak and Nalewajko, 1986a).

The phosphorus cycle in the aquatic environment is much less complex than that of nitrogen. Several characteristics of phosphorus account for its simplified cycle. First, phosphorus occurs in the +5 oxidation

state in nature and generally does not change valence during chemical or biological conversions (Fenchel and Blackburn, 1979). Second, there are no significant atmospheric fluxes of phosphorus.

The study of phosphorus chemistry in the environment is complicated by the problem of distinguishing inert from bioreactive phosphorus (Froelich, 1988). Phosphorus fractions are often described in relation to the manner in which phosphorus is chemically measured.

Particulate versus soluble fractions are generally defined by filtration through a 0.45μm membrane filter. Phosphate concentration is commonly measured colorimetrically by the molybdenum blue technique. This method can overestimate orthophosphate concentrations by 10 to 100 times since organic phosphorus can also be measured. In addition, overestimations can be due to interference by arsenate and silicate (silicate interference is minimized by addition of sulfuric acid to the reaction mixture; Nalewajko and Lean, 1980). Therefore, when phosphate is measured by this method it is preferably reported as soluble reactive phosphorus (SRP; Nalewajko and Lean, 1980). The SRP in lakes commonly ranges from a detection limit of about 0.05 μM to 2.0 μM or greater. At the sea surface a typical value

is 0.1 μM or less with the deep water values increasing to 0.5-1.5 μM. The values are more variable in lakes and coastal areas which are subject to allochthonous inputs, and in areas of upwelling. In the open ocean the values are more constant and the system is presumably more dependent on regenerative sources of phosphorus rather than new inputs (Nalewajko and Lean, 1980). In aquatic systems dissolved phosphorus exists in both inorganic and organic forms.

Dissolved inorganic phosphate (DIP), the principal form of dissolved phosphorus in most aquatic systems, generally exists as orthophosphate and is found as HPO_4^{-2} or $H_2PO_4^-$ depending on the hydrogen ion concentration. In seawater, DIP is normally found as HPO_4^{-2} (Raymont, 1980). Dissolved organic phosphorus (DOP) is sometimes found in high concentrations in aquatic systems, particularly those in which productivity is high, and can be a important source of phosphate to aquatic microorganisms in these systems. DOP is not well characterized but includes phospholipids, sugar esters and polynucleotide chains of nucleic acid, and polyphosphate groupings as in pyridine nucleotides (Raymont, 1980).

The ultimate source of phosphorus to aquatic systems

is from the weathering of continental rocks (Froelich, 1988), although local sources may also include solubilization of precipitated metallic phosphates and adsorbed phosphate, excretion by bacteria and other organisms, inputs from soil fertilizers, and industrial and domestic waste (Fenchel and Blackburn, 1979). Approximately 5-10% of the phosphorus is carried to oceans as dissolved phosphorus with the remaining 90-95% associated with particles (Froelich, 1988). Fluvial particles have been estimated by Froelich (1988) to be capable of transporting 1.4-14 x 10^{10} mol/year of reactive phosphate to the sea. Seasonally, phosphorus is tied up in particulate and dissolved organic forms in the summer and remineralized to inorganic phosphorus in the winter (Parsons and Harrison, 1983).

Phosphorus has a strong tendency to be adsorbed by clays to form insoluble salts, particularly in association with Ca^{+2}, Fe^{+3}, and Al^{+3}. Froelich (1988) describes the interaction between dissolved phosphorus and fluvial inorganic suspended particles as a two-step process. The first is the desorption/adsorption on particle surfaces, a kinetically fast reaction (minutes to hours). The second step is the solid-state diffusion of adsorbed phosphorus from the surface to the interior

of particles, a kinetically slow process (days to months). The interaction between dissolved phosphorus and inorganic suspended particles, termed the "phosphate buffer system," is important in the regulation of the dissolved phosphorus concentration in aquatic environments. It is the phosphate buffer system that is responsible for maintaining the dissolved phosphorus concentration at a relatively constant concentration regardless of biological removal or input (Froelich, 1988). Phosphorus associated with particles can also fall out of the water column and be trapped in sediments (Fenchel and Blackburn, 1979). The sediment-trapped phosphorus can be released when biologically mediated processes which result in a change in redox potential occur in the sediments. This can be an important source of phosphorus in stratified lakes which undergo seasonal turnovers. Aquatic sediments, especially the ocean, act as net sinks of phosphate with remineralized phosphorus being returned to terrestrial systems only via tectonic processes on geologic time scales (Fenchel and Blackburn, 1979).

The amount of phosphorus in living organisms generally exceeds the level of dissolved phosphorus in nature. Turnover times between these pools can be very

fast (minutes) during algal blooms or instances of other high biological activity, or can be very slow (> 100 hours) when biological activity is very low or when the orthophosphate concentration is very high (Fenchel and Blackburn, 1979).

Phosphorus Metabolism by Microorganisms

Phytoplankton can fulfill their phosphorus requirement by uptake of DIP or from DOP after hydrolysis to DIP via phosphatase enzymes. Arsenate is a competitive inhibitor in the phosphate transport system (Nalewajko and Lean, 1980). Many phosphate esters are probably usable by aquatic microorganisms (Nalewajko and Lean, 1980). Phytoplankton harvest this DOP source via production of a membrane-bound phosphatase. This enzyme is located near the cell surface and in some cases may be considered to be a true extracellular product (Nalewajko and Lean, 1980). Phosphatase acts by cleaving the ester which allows the free phosphate to be transported in a normal manner by the phosphate transport system (Parsons and Harrison, 1983). Phosphatase activity is commonly enhanced when an algal population becomes phosphorus-limited (Aiba, 1982).

Phosphorus in cells occurs in four main metabolic pools;RNA, DNA, lipid phosphate and ester phosphate (Nalewajko and Lean, 1980; Aiba, 1982). The total phosphorus concentration per cell fluctuates depending on the phosphorus supply and the cell size. Under phosphorus starvation, cells reach a minimum phosphorus content below which no further growth occurs. This has been termed the "subsistence quota" (Q_o; Nalewajko and Lean, 1980). In a survey of literature values, Nalewajko and Lean (1980) found that Q_o values ranged from 0.29×10^{-13} µg per cell to 14×10^{-8} µg per cell. At least part of this variability can be attributed to large differences in cell sizes. These data would be a great deal more useful if they were standardized to a parameter such as cell carbon levels.

Two physiological changes have been identified which accompany phosphorus depletion in aquatic microorganisms and attempts have been made to try to assess the phosphorus status of a population by using these two criteria. The changes include (i) the development of cellular alkaline phosphatase activity, presumably to increase utilization of the DOP fraction, and (ii) the increased initial rate of phosphorus uptake on re-exposure to phosphorus (Nalewajko and Lean, 1980).

When phosphorus is in excess, most aquatic microorganisms respond by storing phosphorus. The primary storage product is polyphosphate (condensed phosphates of various lengths; Nalewajko and Lean, 1980). Polyphosphate is found in bacteria as volutin storage granules (Fenchel and Blackburn, 1979). The highest levels of storage of phosphorus typically occur when previously phosphorus-starved bacteria or algae are supplied with phosphorus. This has been termed "polyphosphate overplus." A related term is "luxury consumption," which is the amount of excess phosphorus taken up by cells beyond that concentration which is required by the cells to replicate.

Growth and Uptake Kinetics

Phytoplankton growth and nutrient uptake are generally studied in the laboratory in batch or continuous cultures. Batch cultures have the advantage of simplicity (Rhee, 1980), but continuous cultures often provide much more controlled and realistic growth conditions. In continuous culture systems very low nutrient concentrations (in the range of the ambient nutrient concentrations found in many natural aquatic

systems) can be used and a dynamic system maintained with continuous material and energy inputs and outputs Williams, 1971; Rhee, 1980). Continuous cultures also provide steady-state data which can be readily manipulated mathematically. Temporary transient conditions can be created by subjecting continuous steady-state cultures to perturbations such as changes in flow rates or pulses in nutrient concentrations (Williams, 1971; Cunningham and Maas, 1982).

A schematic drawing of a typical continuous culture system is shown in Figure 1. In a chemostat, the flow of incoming nutrients is controlled so that population growth is dependent on the dilution rate. The dilution rate (D) is equal to $f \cdot V^{-1}$ where f is the flow rate and V is the volume of the reactor vessel. When the growth rate, μ, is equal to D the culture is said to be at "steady-state." At steady-state the concentration of organisms remains constant over time (Harder et al., 1977).

Steady-state models have been used widely in the literature to describe the interactions between phytoplankton communities and essential nutrients. This information is often used to predict the relative ability of individual species or composites of organisms for

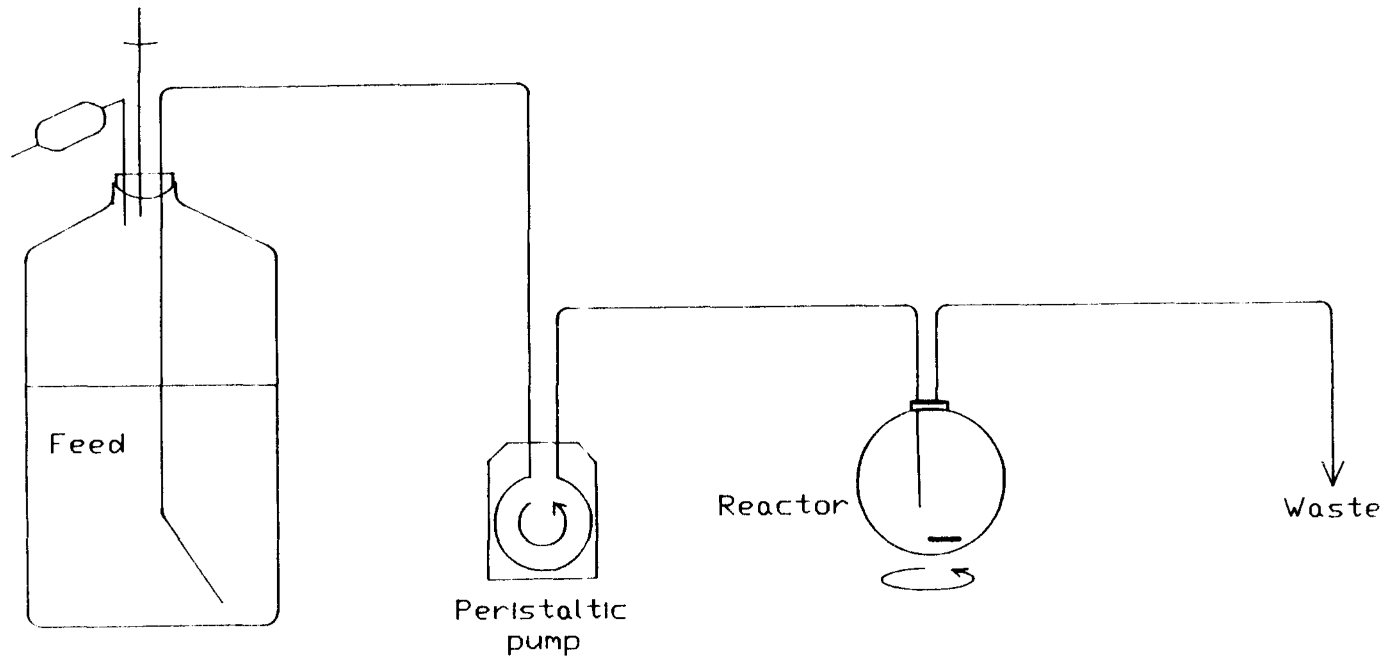

Figure 1. **Schematic diagram of single-stage, single-phase continuous culture culture apparatus.**

growth on, or uptake of, a limiting nutrient. Several steady-state models for both nutrient uptake (Dugdale, 1967) and growth (Monod, 1942) have been adapted from equations based on a basic model of enzyme kinetics.

Caperon (1967) and Dugdale (1967) proposed that phytoplankton nutrient uptake rates and external nutrient concentrations are a hyperbolic function much like the Michaelis-Menten equation from enzyme kinetics (Figure 2). The relationship is expressed as

$$V = V_{max}(S/(K_s + S))$$

where V is the specific uptake rate, V_{max} is the maximum uptake rate, S is the concentration of limiting nutrient, and K_s is the half-saturation constant (the concentration of limiting nutrient where uptake is one-half that of the maximum rate).

Several authors (Paasche, 1973; Law and Button, 1977 and Button, 1978) have found that a threshold substrate concentration exists, S_o, where growth does not occur. The basic uptake equation can then be modified to

$$V = V_{max} ((S-S_o)/ (K_s + (S-S_o)))$$

to take into account this threshold term.

Since uptake is generally assumed to be a function of the number of ion-specific uptake sites on the cell surface, V_{max} can be considered to be a measure of the

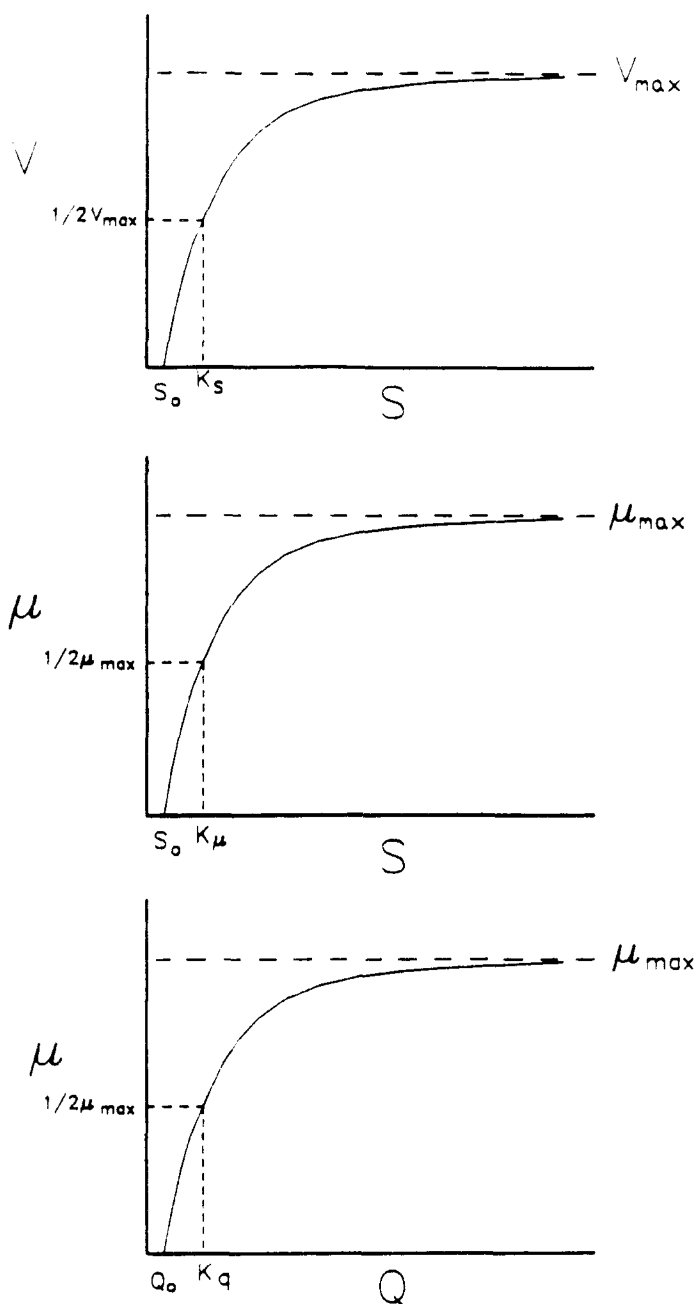

Figure 2. Dimensionless hyperbolic plots of three basic models for nutrient uptake and growth of nutrient-limited microorganisms.

number of sites, while K_s reflects their efficiency at capturing nutrient (Cunningham and Maas, 1982). The uptake process is a combination of absorption and transport (Droop, 1983).

For some nutrients the possibility exists that diffusion kinetics may influence uptake. Unless the rate of diffusion to the cell surface is at least equal to the rate of uptake through the surface, a zone around the cell will occur in which the concentration is less than the bulk medium (Raymont, 1980). This diffusion effect is dependent on the diffusion coefficient of the substrate, the cell shape and the distance from the cell surface to the center of the cell (Pasciak and Gavis, 1974). Uptake studies are also complicated by the fact that the kinetic "constants", K_s and V_{max}, have often been found to be variable.

Some of the factors which contribute to the variability for the kinetic constants are temperature and light (Parsons et al., 1984), the nutrient content of the cells, the previous growth history, the growth rate, the geographical location where a given species was isolated, the presence of interfering ions and the physical culturing conditions (Rhee, 1973; Gotham and Rhee, 1981; Cunningham and Maas, 1982). For example, several

continuous culture studies have shown that the maximum uptake rate is dependent on both the growth rate and the cell quota (Gotham and Rhee, 1981; Riegman and Mur, 1984).

These factors limit the use of K_s and V_{max} alone in making inferences about competition among organisms in natural aquatic systems. In addition, it has been shown that phytoplankton uptake of nutrients deviates from simple Michaelis-Menten kinetics, particularly at low substrate concentration (Brown et al, 1978; Tarapchak and Herche, 1986). The implication is that mechanisms controlling uptake of a growth-limiting nutrient when external concentrations are very low may change as the external concentration is increased.

The growth rates of microorganisms are also related to the concentration of growth-limiting substrates. However, a definitive relationship between uptake rates of a growth-limiting nutrient and growth rates has been elusive (Aiba, 1982). In the early 1900's Blackman presented a model for growth as a function of substrate concentration, where growth is linearly-related to substrate concentration until some other factor limits growth (Bader, 1982; Condrey, 1982). The Blackman model

can be expressed as:

$$\mu = \mu_{max}\ (S/2K_{\mu}) \qquad \text{for } S < 2K_{\mu}$$

$$\mu = \mu_{max} \qquad \text{for } S > \text{or} = 2K_{m}$$

In these equations, μ_{max} is the maximum specific growth rate, μ is the observed specific growth rate at a given substrate concentration, S, and K_{μ} is the constant which is equal to the substrate concentration when growth is half-maximal (equations rearranged from Bader, 1982; Figure 3).

The Blackman model fits experimental data quite well. In fact, Condrey (1982) argues that the Blackman model fits the data which Monod used to test his hyperbolic growth model better than the Monod model itself. The primary disadvantage of the Blackman model is that it is a discontinuous function (Bader, 1982; Condrey, 1982) and consequently the Monod model has been more widely used in growth kinetic studies.

Monod (1942) proposed that the specific growth rate of an organism (μ) and the concentration of essential growth rate-limiting nutrient could be described by a hyperbolic equation

$$\mu = \mu_{max}(S/(K_{\mu} + S))$$

where μ is the specific growth rate, μ_{max} is the maximum specific growth rate, S is the concentration of limiting

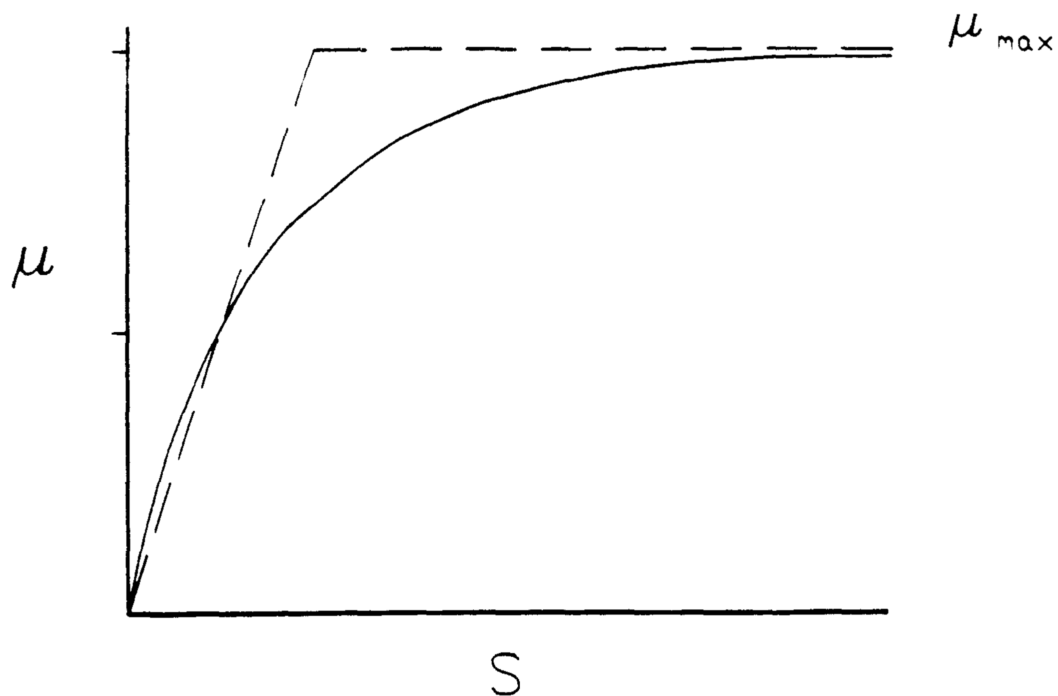

Figure 3. Dimensionless plot of two models for substrate-limited growth of microorganisms. Monod model (——) Blackman model (- - - -).

substrate, and K_{μ} is the half-saturation constant (the concentration of limiting substrate where growth is one half that of the maximum rate; Figure 2).

A major drawback to the use of the Monod type equation in the estimation of growth is that it requires the accurate measurement of external nutrient concentration. This measurement may be difficult, if not impossible, to perform due to analytical problems, particularly in oligotrophic waters where the concentration of limiting nutrient may be extremely low.

Growth can also be described as a function of internal rather than external nutrient concentration (Figure 2). The internal cellular nutrient concentration (cell quota; Q) and specific growth rate may also be represented by a hyperbolic function (Caperon, 1968; Droop, 1968). This empirical model is based on the theory that the apparent nutrient concentration (external concentration) is less important to a given cell than the past history of nutrient availability reflected in its storage of nutrient (internal nutrient concentration). The internal stores model (Droop equation) is expressed as

$$\mu Q = \mu_{max}((Q-Q_o)/(K_q + (Q-Q_o)))$$

where μ is the specific growth rate, μ_{max} is the maximum

specific growth rate, Q is the internal nutrient content of the cell or cell quota, Q_o is the minimum cell nutrient concentration necessary for viability at zero growth rate, and K_q is the subsistence quota or the value of Q at $\mu = 0$. Droop (1968) observed that K_q and Q_o often have similar numerical values and suggested the following empirical simplification of the equation

$$\mu Q = \mu_{max} (1 - (Q_o/Q)).$$

The internal stores model has been used to describe growth limitation in chemostats for a number of nutrients including phosphate (Fuhs, 1969; Rhee, 1973; Brown and Button, 1979; Robertson and Button, 1979) nitrate, (Rhee, 1974, 1978) and ammonia (Thomas and Dodson, 1972). From a management standpoint, the Droop model has the limitation of being dependent on cell physiology, which is only an indirect reflection of the external environment (Auer et al., 1986). Therefore, if limiting nutrient concentrations can be accurately measured in an aquatic environment, the Monod model is significantly easier to use. Another consideration when using the Droop model is that the relationship only holds for the nutrient that is limiting. Elrifi and Turpin (1985) found that the cell quotas of non-limiting nutrients would not fit the Droop hyperbolic curve.

As with the uptake "constants", K_s and V_{max}, the growth "constants", K_μ and μ_{max}, are defined with respect to the environmental conditions in which they were measured. At μ_{max} the population is no longer nutrient-limited and considerations other than the rate of assimilation become important. Therefore only one value should exist for μ_{max} no matter which nutrient initially limited the population (Rhee and Gotham, 1980).

In addition to the accurate measurement of growth constants, predictive models of phytoplankton growth are also dependent on any potential interacting factors. For example, several studies have addressed the question posed by Ahlgren (1980) of whether there is a threshold effect "where growth is controlled by a single nutrient that happens to be in the shortest supply relative to the needs of the cell," or whether a multiplicative effect exists where "growth is dependent on the concentrations of all suboptimal nutrients which might be exerting influence simultaneously." These studies have shown that unialgal cultures are ultimately controlled by the nutrient in shortest supply.

Rhee (1978) and Rhee and Gotham (1980) grew seven different plankton in continuous cultures over a wide range of N:P ratios. They found no evidence of a

multiplicative effect. They also defined an optimum ratio of N:P for each organism equal to a ratio of the subsistence quotas for the two nutrients. Above this ratio phosphorus is the limiting nutrient and below this ratio nitrogen limits growth. There was a wide range of values for the various species but the mean optimum N:P ratio was 17, a value which is very close to 15, the value predicted from the Redfield ratio. Terry (1980) and Terry et al. (1985) define another ratio-- the ratio of nutrients equal to the concentrations at which cells actually require these nutrients. He terms this the critical ratio, R_C. He argues that this is the only point where a cell is simultaneously limited by two nutrients. At all other concentrations the value of the non-limiting nutrient is inconsequential and growth is limited solely on the cell quota of the limiting nutrient.

Data from Elrifi and Turpin (1985) for nitrogen and phosphorus and Turpin (1986)for carbon and phosphorus also support the theory that the optimum ratio is growth rate dependent. Elrifi and Turpin (1985) found that the cellular requirements for phosphorus increased much more rapidly than for nitrogen as the growth rate was increased. This led to a decrease in the N:P ratio with

increasing μ. They additionally found that the capacity for luxury consumption was different for the two nutrients. At a low growth rate the coefficient of luxury consumption was four times greater for phosphorus than for nitrogen. At nutrient ratios other than the critical ratio, growth was controlled by the one nutrient with the lowest cell quota. Examples of a threshold effect exist for a variety of pairs of nutrients other than nitrogen and phosphorus. These include phosphate/vitamin B_{12} (Droop, 1974), and phosphate/carbon (Brown et al., 1981).

Brown et al. (1981) found that, under steady-state conditions, there was no apparent multiplicative effect of carbon limitation on a yeast's (*Rhodotorula rubra*) transport of inorganic phosphate even in the presence of a competing phosphorus-starved phytoplankter (*Selenastrum capricornutum*). Coexistence of these species only occurred when the heterotroph was growth rate-limited by organic carbon (Figure 4). Since the yeast was shown to have a higher affinity for phosphate and since carbon starvation did not affect the yeast's ability to transport phosphate, the concentration of organic carbon directly controlled the biomass of the yeast and at the same time indirectly controlled the biomass of the

phytoplankton. These interactions between heterotrophs and phytoplankton may be very important in aquatic environments.

Interactions Between Heterotrophs and Autotrophs

The relative importance of bacteria and the role they play in the metabolism and regeneration of nutrients in aquatic environments has not been resolved (Harrison, 1980). Some studies have concluded that bacteria are dependent on nutrients supplied from algal exudates (Meffert and Overbeck, 1979). Other studies have concluded that algae may be dependent on nutrients re-mineralized by bacteria (Currie and Kalff, 1984a; De Pinto et al., 1986). Heterotrophs have been shown to be good competitors for nutrients in several studies (Rhee, 1972; Brown et al., 1981; Bratbak and Thingstad, 1985).

In 1972, Rhee studied competition for phosphorus by mixed cultures of the alga, *Scenedesmus* sp., and the bacterium, *Pseudomonas* sp. grown in batch cultures. He found that the growth of the alga was limited by the presence of the bacteria but that the growth of the bacteria was not significantly affected by the presence of the alga. He concluded that carbon must limit

bacterial populations in aquatic environments and that bacteria are much less likely to store phosphorus than algae. These two factors would allow phytoplankton to successfully compete with bacteria in nature.

Several studies have shown that orthophosphate uptake in lakes (Currie and Kalff, 1984a) and California coastal waters (Krempin et al., 1981) is dominated by the bacterial fraction. Currie and Kalff (1984a; Currie et al., 1986) estimated that bacterial uptake accounted for 95-100% of the total community uptake of orthophosphate in 13 lakes with various trophic states. They also found that the uptake of excreted organic phosphorus in these studies was dominated by phytoplankton. However, in 1984 (b) the same authors report that for three bacterial and two phytoplankton species there was no significant difference in alkaline phosphatase activity. This implies that the bacteria should be able to use organic phosphorus as efficiently as the phytoplankton they studied.

Competition between heterotrophs and autotrophs has been examined in continuous culture for a yeast and a green alga (Brown et al., 1981) and for a bacterium and an alga (Currie and Kalff, 1984 a,b,c). In all these studies, the heterotrophic organism was shown to have a

higher affinity for phosphorus than the alga. It was suggested (Currie and Kalff, 1984b) that the uptake superiority of the bacteria may be related to cell size rather than metabolic differences. Cell size may influence uptake. However, Brown et al. (1981) found that a heterotrophic yeast outcompeted the green alga used in their experiments, despite the fact that the two cells are approximately equal in size. Coexistence of the two species only occurred when the heterotrophic population was limited by carbon (Figure 4). It is fairly clear that a tight coupling exists between phytoplankton and heterotrophic populations in aquatic environments.

In a recent study, Vadstein et al. (1988) measured phosphorus and carbon pools in a eutrophic lake. They compared P:C ratios in different fractions and concluded that bacteria have a greater phosphorus requirement than do phytoplankton. Additionally the net consumption by bacteria was four times higher than that of the phytoplankton and it was not clear whether the bacteria were carbon-limited or phosphorus-limited.

Carbon limitation appears to be a major factor controlling the populations of these organisms. Other factors may also be important such as the ability of

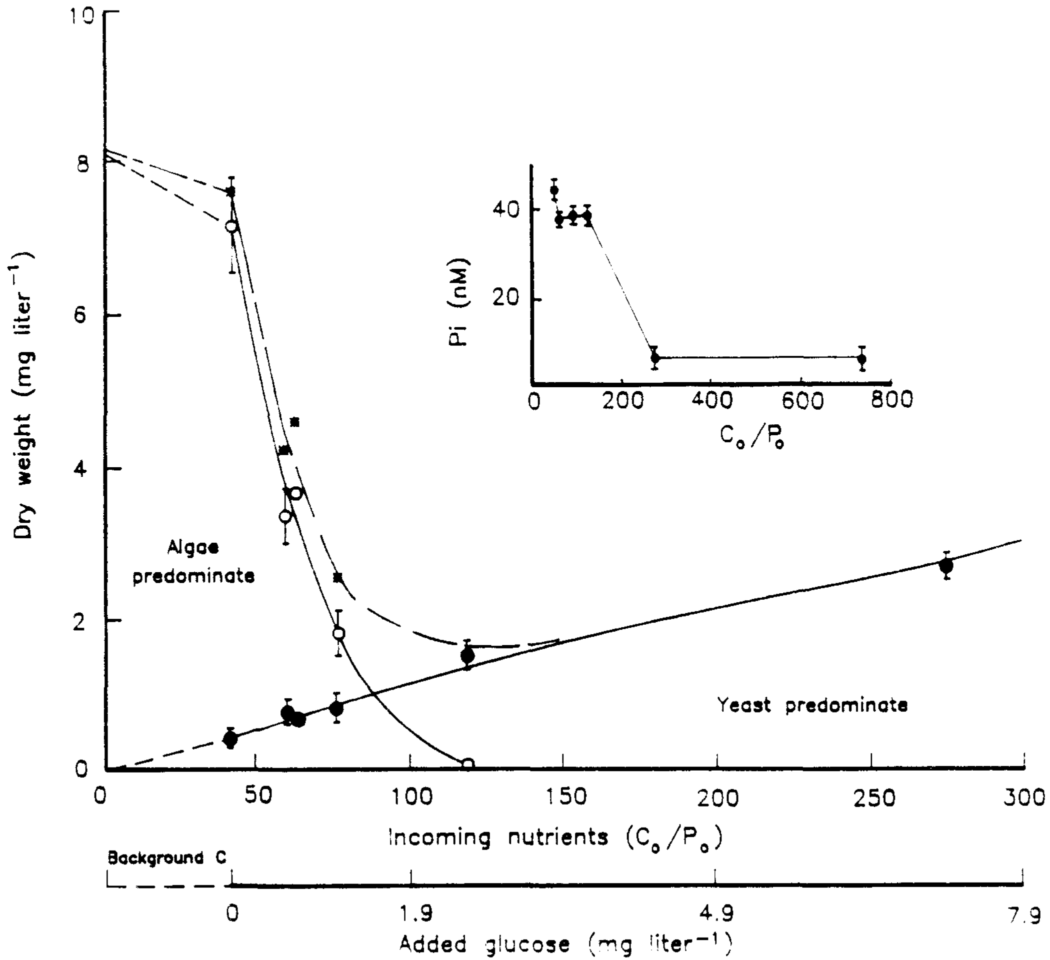

Figure 4. Continuous culture dry weight of *Selenastrum capricornutum* (○—○) and *Rhodotorula rubra* (●—●), and both species (✱- - -✱) as a function of available C and P_i (C_o/P_o) ratio and P_i in solution versus C_o/P_o (inset). Each point with error bars represents the mean value of two steady-state measurements. Those without error bars represent one measurement. Total dry weights are the sum of each species' mean dry weights. Re-drawn from Brown et al. (1981).

these organisms to store phosphorus and the potential differences in phosphorus requirements among aquatic microorganisms. Such factors have been investigated by describing nutrient-limited growth and uptake by pure cultures and by modeling competition for nutrients in microbial consortia under controlled conditions.

Competition Models

There are three major components often incorporated into nutrient-limited algal growth models. These are nutrient uptake (assimilation) from the external pool, nutrient utilization, and cell division. Utilization consists of the processes responsible for conversion of nutrient from the internal nutrient pool to structural cell components and active cell components. Nutrient uptake kinetics have been studied in detail and empirical relationships between uptake rates and nutrient concentrations are relatively well understood. The processes of nutrient utilization and the regulation of cell division have been much more difficult to study (Cunningham and Maas, 1982) thus the universality of kinetic descriptions is much less reliable. The relative abilities of nutrient-limited organisms to grow is

commonly described by comparing half-saturation constants obtained for each species. A better measure for evaluating the competitive ability of an organism in a nutrient-limited system is the maximum slope of a saturation curve (Law et al., 1976; Button, 1978; Healey, 1980; Molot, 1981; Molot and Brown, 1986). This value has been called the "specific affinity" by Button (1978).

The affinity is given by the ratio $\mu_{max} \cdot K_{\mu}^{-1}$ for a Michaelis-Menten type curve. Affinities can also be defined for other saturation curves (Button, 1978; Brown and Button, 1979; Molot and Brown, 1986). The specific affinity (Button 1978, 1985, 1986) is the net specific flux of substrate per unit concentration of that substrate. Since the uptake of substrate at low concentration is dependent on both the concentration of substrate and the biomass of organisms (which further relates to the number of transporter molecules available for transport), Button (1986) argues that a second-order rate equation is necessary to adequately describe these systems. Button (1986) gives a general form for the definition of affinity. The affinity for substrate A is related to uptake rate (v), external substrate concentration (S), and biomass (X) in the following

manner

$$v = a_A S_A X$$

The specific affinity is a useful kinetic constant for assessing the ability of an organism to accumulate substrate and grow when substrate concentrations are low.

One problem in kinetic studies is that the kinetic constants calculated from traditional models such as the Monod equation for growth and the Michaelis-Menten equation for nutrient uptake are sometimes used interchangeably leading to confusion about what was actually measured (Goldman and Glibert, 1983). Growth and uptake can only be equated when Q (the cell quota) is a constant and when V is equal to the product of μ and Q. Since Q is the reciprocal of the cell yield, Y, these criteria also require that the yield be a constant. Although yield is often considered to be a constant, a number of organisms have been shown to have decreasing cell yields as a function of the growth rate, μ (Braddock et al., 1984). There is no particular reason to assume that a cell maintains its cellular components in the same proportions over time. In fact, growth and uptake may normally be loosely coupled and only be directly related when the population is at true steady-state (Droop, 1983).

Another approach to competition models involves the use of kinetic constants derived from relationships such as the Monod model. Some authors have argued that competition for multiple resources determines the community structure in aquatic systems. These models are dependent on a concept presented by Taylor and Williams (1975) postulating that the number of growth-rate limiting nutrients is greater than or equal to the number of species present in a mixed population.

Resource competition models have been supported by extensive batch and continuous culture experiments and by observation of populations in freshwater aquatic systems (Titman, 1976; Tilman, 1980; Tilman and Kilham, 1976; Tilman et al., 1982; Sommer, 1985a). For example, Titman (1976) and Tilman and Kilham (1976) applied this approach to the study of two species of diatoms limited by phosphate and silicate. The results of these studies indicated that the two species of diatoms could coexist only when the growth rate of each species was limited by a different nutrient. The nutrient limiting a given species under a given nutrient regime is that nutrient which leads to the lower growth rate. These authors argue that steady-state single species continuous culture data can be used to predict the outcome of competition

involving two species and two limiting nutrients (Kilham and Hecky, 1988).

A limitation of these models may be the reliance on steady-state data. If some aquatic systems are subject to significant nutrient perturbations, steady-state models may not adequately assess the outcome of competition among microorganisms living in those systems.

Transient Aquatic Systems

Nutrient concentrations in aquatic systems may not be uniform. If this is true, the question is whether phytoplankton respond differently to pulsed nutrient supplies than to constant supplies (Tarapchak and Nalewajko, 1986b). The question of nutrient patchiness historically relates back to Hutchinson (1961) and the "paradox of the plankton." Hutchinson posed the paradox in the following manner: If phytoplankton populations in aquatic systems are limited by one nutrient, one would expect that the organism with the highest affinity for that limiting nutrient would outcompete other species. This would lead to environments with very low species diversity (competitive exclusion). On the contrary, natural waters generally simultaneously support many

species of phytoplankton. Although there have been many attempts to explain the paradox of the plankton, a conclusive explanation of phytoplankton diversity has remained elusive. Several models have been presented in an attempt to explain this paradox.

Hutchinson (1961) proposed that the nutrient regime of aquatic habitats changes too rapidly for equilibrium (steady-state) to be established. Therefore, species composition at any given time is not stable- no organism has the advantage long enough to cause the extinction of the others. Hutchinson points out that this theory may only be applicable to species that have intermediate reproductive rates so that exclusion cannot occur before a significant environmental change. This theory is essentially based on the idea of disequilibrium on a temporal scale.

In 1970, Richerson et al. presented the idea of "contemporaneous disequilibrium" in which an apparently homogeneous water body is imagined to be composed of many separate microhabitats so that heterogeneity is considered to be contemporaneous, rather than temporal as Hutchinson suggested. Richerson et al. (1970) based their theory on observations of the distribution of phytoplankton in a highly oligotrophic lake in

California. They observed a high degree of patchiness in the distribution of phytoplankton species in this lake. They suggest that the simultaneous existence of algae in many different niches is a result of patchy nutrient supplies. McCarthy and Goldman (1979) relate nutrient patchiness to growth kinetics of marine phytoplankton. They argue that marine phytoplankton can use small-scale temporal and spatial patches in nitrogen to maintain near maximal growth rates at nitrogen concentrations which were not measurable by analytical methods at that time. Turpin et al. (1981) have pointed out that spatial patchiness can be modeled identically to temporal patchiness since from the perspective of the phytoplankton there is a finite time between patch encounters.

Lehman and Scavia (1982) and Scavia et al. (1984) have presented more recent evidence for the effect of microscale patchiness on nutrient availability for phytoplankton. These authors used autoradiography and microvideography to look at nutrient patches produced by zooplankton and potential usage of nutrients from micropatches by phytoplankton. They make a strong case for the fact that patchiness may play an important role in phytoplankton species composition, especially in

oligotrophic waters where nutrients are scarce and where excretion by zooplankton is relatively high (summer). Under these conditions, the maximum rate at which cells can take up nutrient under enrichment conditions may be much more important than their absolute affinity for nutrient under low-nutrient conditions. Sakshaug et al. (1983) provide evidence that phosphorus-limited freshwater communities can be affected by the presence of daphnia and whitefish. Excretion of nutrients by these organisms seemed to dampen the nutrient poverty of the algal community.

Some authors have argued, however, that the positive effect on the phytoplankton population from patchy nutrients provided by zooplankton excretions is offset by herbivory (Sterner, 1986). Although excreted nutrients from zooplankton may not increase algal biomass, Scavia et al. (1984) show evidence that the dominant species which exist in phosphorus-limited semi-continuous cultures is different depending on whether the same amount of phosphorus is supplied in pulses or continually. Holligan et al. (1985) present another mechanism for patch generation in the sea. They present evidence that mixing by large soliton-like internal waves can lead to pulses of nutrients in nutrient-depleted

waters, both on the surface and at depth.

The major opposition to the idea of patchiness has been that mixing and dispersion are too rapid in most aquatic systems for patches to be significant in phytoplankton nutrition (Jackson, 1980). Alldredge and Cohen (1987) used microelectrodes to study the existence of oxygen and pH gradients around flocculated marine particles called "marine snow." They found "...experimental evidence (i) that microscale chemical gradients can persist in the ocean against processes of advection and diffusion on a scale significant to microorganisms and (ii) that these patches may have important implications for nutrient recycling in the sea." Thus, the role of patches has not been fully resolved. If limiting nutrient concentrations are not homogeneous, then it is necessary to determine how aquatic microorganisms respond to pulsed nutrient concentrations. There is fairly substantial evidence that short term uptake is different from steady-state uptake. Short term uptake appears to be much more dependent on the previous nutrient history of the cells (McCarthy and Goldman, 1979; Morel,1987). Several authors have attempted to simulate conditions where nutrients are supplied discontinuously. Attempts have been made to describe

transient populations using batch, semi-continuous and continuous cultures.

Nyholm (1977 and 1978) used continuous cultures to study the response of two algae (*Chlorella pyrenoidosa* and *Selenastrum capricornutum*) when phosphorus was supplied in pulses. In his 1977 study, Nyholm concluded that the "time constants for the uptake processes are much smaller than the time constants for growth. Therefore, the uptake is rarely a rate controlling step..." In the chemostat experiments of Nyholm's 1978 study, the pump was turned off at the same time that a pulse of phosphorus was added. Nyholm found that steady-state models could not describe the growth after large phosphorus shocks. His simplified model relates specific growth to the intracellular concentration of phosphorus. Nyholm extends this model (1978) to what he terms a dynamic model to include cellular responses to phosphate shocks. In the extended model he includes a parameter for an intermediate phosphorus pool. This intermediate pool helps account for the observed lags between phosphorus perturbation and growth. Apparently some organisms choose to build up a phosphorus reservoir under these conditions at the expense of an immediate acceleration of growth. The time lag may represent loose

coupling between growth and division under transient conditions (Williams, 1971).

A lag was also seen in growth response for another alga, *Isochrysis galbana,* when nitrate-limited chemostats were perturbed with nitrate (Caperon, 1968). Caperon and Meyer (1972) extended those experiments by looking at uptake of nitrate and ammonium by several other phytoplankton species. They found that different preconditioning of the cells did not significantly affect the half saturation constants for uptake. In contrast, the maximum uptake rates were found to be different for populations maintained at different growth rates. They related this response to an increase in the number of uptake sites. The highest uptake rates were seen in populations grown at higher dilution rates before perturbation.

Conway et al. (1976) and Conway and Harrison (1977) looked at the effects of perturbations of ammonium and silicate in ammonium- and silicate-limited continuous cultures of several diatom species. They found that the organisms adjusted their uptake rates depending on the growth conditions before a pulse was added. The very high uptake rates that they observed following perturbation by a previously limiting nutrient, they

termed "surge uptake," V_s. During surge uptake, the uptake rates of the non-limiting nutrient were depressed. In addition to surge uptake, they defined two other regions of uptake: internally controlled uptake, V_i and externally controlled uptake, V_e. Other studies have looked at the response of nutrient-starved cells.

Collos (1980, 1982a, 1982b, 1984) studied the growth and uptake kinetics of nitrogen-limited and nitrogen-starved diatoms. He used continuous cultures to prepare his cells, either nitrogen-limited cultures obtained by growing the cells at low nitrogen concentrations or nitrogen-starved cultures obtained by turning off the inflow of nutrients to nutrient-limited cultures. He then subjected these prepared populations to pulses of nitrate.

Collos found that the degree of limitation or starvation could rapidly change the resulting maximum uptake rate, V_{max}. This parameter appears to go through its maximum value as nitrogen deficiency increases. This same relationship may exist for phosphorus. He also found that different diatom species have greater or lesser ability to take up nitrogen after being subjected to various degrees of nitrogen starvation. For example, *Chaetoceros affinis* Lauder which demonstrated a decrease

in V_{max} as soon as starvation was begun, was able to use nitrogen immediately upon re-exposure after up to 48 hours of starvation.

Two other diatom species, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*, were less able to use nitrogen after starvation. In contrast to *C. affinis, T. pseudonana* exhibited an initial increase in V_{max} when subjected to nitrogen starvation. In a previous experiment (Collos, 1982b), *C. affinis* was shown to have less ability to store large amounts of nitrate when nitrate-limited cells were subjected to large pulses of nitrate. These studies indicate that various organisms may utilize a variety of strategies to cope with low nutrient environments when nutrients are supplied discontinuously. The results of these single species perturbation studies led to several perturbation studies using two species of phytoplankton.

A number of dual species continuous culture experiments have been performed with diatoms limited by ammonium and silicate (Turpin and Harrison, 1979; Turpin and Harrison, 1980; Turpin et al., 1981; Quarmby et al., 1982). In these experiments the continuous cultures were supplied the same concentration of limiting nutrient but the frequency of nutrient addition was varied. The

authors found that different species would dominate depending on the frequency of nutrient additions. They suggest that optimization of utilization of patches occurred by an increase in V_{max} and of homogeneous conditions by an increase in K_s. Quarmby et al., 1982 also found that the degree of patchiness can control the uptake rate. They found that the V_{max} for ammonium in ammonium-limited continuous cultures of the diatom, *Skeletonema costatum*, was greater in continuous cultures subjected to patch additions of ammonium rather than continuous additions.

Turpin and Harrison (1980) argue that cell size may be significant in competition for nutrients supplied in patches. They found, for the species they studied, that the mean cell diameter increased as the frequency of the nutrient additions decreased. Turpin et al. (1981) argue that their data supports a threshold model for species existence. Since growth and uptake can be uncoupled when nutrients are supplied in patches and since the degree of patchiness affects the growth rates of individual species, it is possible for the same nutrient to limit co-existing species of phytoplankton.

Sommer (1985a) compared steady-state and non steady-state competition in natural phytoplankton

continuous cultures when either phosphorus or both phosphorus and silica were provided discontinuously. He found that the number of species at the end of a pulsed experiment was greater than the number found at steady-state. He further classifies algae as "affinity specialists," "velocity specialists," and "storage specialists." From his cultures, Sommer concludes that cell size is not the critical parameter in success under pulsed nutrient conditions, but that certain algae are more successful under different nutrient regimes because of their evolutionary history. Pulsed conditions favored green algae and tended to disfavor diatoms.

The responses of natural phytoplankton assemblages to pulsed nutrient supplies have been studied in other experiments (Suttle and Harrison, 1986; Suttle et al., 1987; Suttle and Harrison, 1988a; Suttle and Harrison, 1988b; Suttle and Harrison, 1988c). These authors used nutrient-limited semi-continuous cultures to examine the responses of phytoplankton assemblages from oligotrophic lake waters. They used phosphorus as the limiting nutrient in one set of experiments (Suttle and Harrison, 1986) and phosphorus, ammonium, nitrate, and silicate in other experiments (Suttle et al., 1987). For phosphorus, they found that the highest uptake rates were seen at

intermediate dilution rates. Suttle and Harrison (1986) argue that uptake rates should be standardized to the initial nutrient concentration. For all nutrients, it was concluded that cell size was important under transient conditions. Suttle et al. (1987) found that the average cell volume increased as the frequency of nutrient additions decreased. They conclude that the cells "... which are able to sustain elevated maximum uptake rates, and which are able to store the largest amount of nutrient relative to their minimum requirements, should be successful under conditions where saturating pulses of limiting nutrients occur."

Very few of the studies that have been done have used highly controlled conditions to compare steady-state data to transient data. One reason is that perturbations of phytoplankton populations growing in continuous culture are time-consuming. Cunningham and Maas (1982) suggest three criteria for successful perturbed chemostat experiments. These are the establishment of steady-state growth before the perturbation, the frequent data collection during the transient condition and the continuation of data collection until steady-state is re-established.

In the past few years a number of authors have studied transient growth in culture and have attempted to model the results of these studies. Nevertheless, many questions remain unanswered. For example, (i) how important is nutrient patchiness to phytoplankton community structure in aquatic systems? (ii) are steady-state data applicable in modeling transient populations? (iii) are some organisms better able to compete when nutrients are discontinuous because of their cell size or because of factors correlated with their taxonomic relationships? and (iv) what is the relationship between heterotrophic organisms and phytoplankton?

Empirical models are limited by their inability to account for the actual mechanisms involved in growth and uptake. However, kinetic relationships have been useful to identify the kinds of physiological studies that might be useful to understand the mechanisms involved with growth and transport of nutrients. Very few studies have described the transient kinetics of coexisting heterotrophs and autotrophs. In this study I have used phosphorus-limited continuous cultures of the green alga, *Selenastrum capricornutum*, and the yeast, *Rhodotorula rubra*, to study the kinetics when steady-state cultures

are perturbed with large pulses of phosphorus.

MATERIALS AND METHODS

Microorganisms

Microorganisms used in these experiments were the green alga *Selenastrum capricornutum* Printz and *Rhodotorula rubra*, a pink heterotrophic yeast isolated from seawater (Button, 1969). Both species were maintained free of bacteria at $4^{o}C$ in modified liquid Provisional Algal Assay Procedure (PAAP) medium (Bartsch, 1971) described in Table 1. Stock cultures were routinely checked for bacterial or fungal contaminants by microscopic examination and by plating on plate count agar.

Growth Media

The PAAP medium used for batch cultures is described in Table 1. The yeast medium was modified from the basic PAAP by the addition of $0.5\ g \cdot l^{-1}$ dextrose and $0.2 g \cdot l^{-1}$ $(NH_4)_2SO_4$. Stock solutions were stored at $4^{o}C$ in the dark and were re-made about every six months. All batch strength media contained 20 μM K_2HPO_4. All continuous

Table 1. Constituents of Culture Media Stock

Solution	Compound	Conc. in Stock ($g \cdot l^{-1}$)
1	$FeCl_3 \cdot 6H_2O$	0.053
	$Na_2EDTA \cdot 2H_2O$	0.111
2a	$NaHCO_3$	5.0
2b	$NaHCO_3$	100.0
3	$MgCl_2 \cdot 6H_2O$	4.05
	$MgSO_4 \cdot 7H_2O$	4.99
	$CaCl_2 \cdot 2H_2O$	1.179
4	KCl	0.700
5	$NaNO_3$	8.504
6a	K_2HPO_4	0.1679
6b	K_2HPO_4	0.4429
7	$MnCl_2 \cdot 4H_2O$	1.385
	$ZnCl_2$	0.109
	$CoSO_4 \cdot 7H_2O$	0.0065
	$CuCl_2 \cdot 2H_2O$	0.00005
8	$Na_2B_2O_4 \cdot 8H_2O$	1.045
	$Na_2MoO_4 \cdot 2H_2O$	0.024
	$Na_2EDTA \cdot 2H_2O$	0.00744
9	Vitamin B_{12} in 0.12N HCl	0.020
10	d-Biotin in 0.12N HCl	0.0032
11	Thiamine-HCl in 0.12N HCl	0.0032
12	$(NH_4)_2SO_4$	
13	glucose	

culture media contained 20% strength phosphate-free PAAP medium prepared in a pre-conditioned (Brown et al., 1978) glass carboy. The medium was buffered with 12.1 g Tris (hydroxymethyl)-aminomethane dissolved in 100 ml of water and 6.5 ml of concentrated hydrochloric acid (HCl) per 20 liters of medium. The pH of the medium was 7.8±0.2 after autoclaving.

Prior to autoclaving, phosphorus (as K_2HPO_4) and/or dextrose were added as described for individual experiments. The medium was allowed to cool after autoclaving and a sterile saturated solution of $NaHCO_3$ was added for a final concentration of 0.1 $g \cdot l^{-1}$. Water lost in autoclaving was replaced by bringing the carboy up to volume with sterile water. Lastly, sterile radioisotopes of phosphorus and/or carbon were added to the medium. The specific medium constituents varied for single species yeast, single species algal or dual species experiments (see Table 2). All continuous culture media were prepared with distilled and deionized water (American Society for Testing Standards type 1 quality) which was filtered through a 0.45 μm membrane filter.

Table 2. Composition of Culture Media

Soln. #	Amount of Solution				
	Batch Culture (ml stock/l)		Continuous Culture (ml stock/20 l)		
	algae	yeast	algae	yeast	dual
1	10	10	50	50	50
2a	10	10	--	--	--
2b	--	--	10	10	10
3	10	10	40	40	40
4	--	--	40	40	40
5	10	--	40	40	40
6a	10	10	--	--	--
6b	--	--	10	variable	10
7	1	1	5	5	5
8	1	1	5	5	5
9	--	0.1	0.2	0.2	0.2
10	--	0.1	0.2	0.2	0.2
11	--	0.1	0.2	0.2	0.2
12	--	$0.2\ g \cdot l^{-1}$	--	$1.1\ g \cdot l^{-1}$	$1.1 g \cdot l^{-1}$
13	--	$0.5\ g \cdot l^{-1}$	--	variable	variable

Radioisotope Labeling

The phosphorus in the culture media was labeled by adding carrier-free radio-orthophosphate (^{32}P). The ^{32}P was received and diluted in 0.02N HCl. It was reported by the manufacturer to have radionuclidic and radiochemical purity of 99.00%. In some experiments glucose was also labeled by adding D-[^{14}C(U)]-glucose to the culture medium. The ^{14}C-glucose was diluted into water, autoclaved and stored in the dark. The specific activity was reported by the manufacturer to be 358.6 $mCi \cdot mmol^{-1}$ with a radiochemical and chemical purity of greater than 99%. Radioactivity was measured by placing samples into a 2:1 toluene/Triton X-100 cocktail (v/v) and counting in a Beckman model LS3801 liquid scintillation counter. When both ^{32}P and ^{14}C-glucose were used in an experiment, the ^{32}P was counted immediately after sampling. The samples were then stored in the dark at 4° C for six months before counting the ^{14}C. This allowed decay of the ^{32}P which had an initial activity approximately 1,000 times greater than the ^{14}C.

Culture Methods

The continuous culture apparatus used in these experiments is shown in Figure 1 (see Literature Review). The system is a single-phase, single-stage system (Brown and Button, 1979). The medium, stored in a 20 l glass carboy, was pumped via a variable speed peristaltic pump (Technicon models Varioperpex and Multiperpex; Technicon Instruments Corporation, Tarrytown, NY) through silicone tubing into the reactor vessel. The reactor vessel was a 500 ml round-bottom glass boiling flask which had been modified to remove most of the stem. The reactor vessel contained a Teflon-coated magnetic stir bar and was sealed with a rubber stopper containing a glass tubing inlet port and outlet port. Waste cells and medium were continually removed from the system as fresh medium was added with the peristaltic pump. The reactor flask was banked with cool white fluorescent lights to provide incident radiation of 300-400 $\mu E \cdot m^{-2} \cdot sec^{-1}$. All experiments were conducted in constant temperature rooms at 25°C.

The medium carboy and reactor flask were autoclaved separately for each experiment and connected to each other using sterile techniques. Each culture was started

by adding approximately 200 ml fresh media to the reactor vessel via the pump. The pump was then stopped and approximately 10 ml of fresh batch culture was inoculated into the reactor vessel. The lights were turned off for approximately 24 hr, after which time the pump was started at a slow rate and the lights were turned on. Single species experiments using either *S. capricornutum* and *R. rubra* were conducted under the same lighting conditions described above to maintain similar culture conditions in all the single species experiments. When dual species experiments were run, the algal population was established before inoculation with *R. rubra*.

The flow rate (F, in $ml \cdot hr^{-1}$) delivered by the pump was determined by allowing the effluent to drain into a graduated cylinder for a specific amount of time (generally about 8 hours). The flow rate for each culture was measured several times to assure continuous and constant delivery by each pump at a given flow rate. The dilution rate (D) was calculated as the flow rate divided by the volume of the culture vessel. This value, D, is equivalent to the specific growth rate of the organism (μ) at steady-state (Stanier et al., 1976). After at least three residence times (R; where $R = D^{-1}$ or μ^{-1}) with no change in measured variables at a given flow

rate, a steady-state was assumed and samples of the reactor vessel contents were removed for microbiological and chemical analyses.

All experiments were performed using aseptic techniques and axenic cultures. Checks were made periodically for contaminants by microscopic examination, and by plating out reactor vessel material on plate count agar and checking for microbial growth after several days.

Sampling

Samples were removed and additions made to the reactor vessel by clamping off the effluent line and inserting a sterile, 20 gauge syringe needle attached to a 10 cc, or occasionally 20 cc, syringe through the rubber stopper. In some experiments where many samples were removed in a short period of time, a second cotton-stoppered syringe with plunger removed was inserted in the reactor for sterile air exchange and pressure release.

Immediately after removing a sample from the reactor vessel, two ml were filtered through a 25 mm diameter, 0.22 μm pore size membrane filter to separate cells from

the medium. The filter, one ml of filtrate and one ml of total sample were then placed in scintillation vials, cocktail was added and the samples were counted on a liquid scintillation counter. Sample aliquots were also removed for carbon analysis (5-10 ml) and epifluorescent microscopy counts (1-2 ml). The carbon samples were filtered onto glass fiber filters (Gelman type A/E) and immediately frozen for later analysis.

Perturbations

Just prior to a perturbation with phosphate, a small amount of ^{32}P was added to the reactor through the stopper with a sterile syringe to provide approximately 80,000 additional counts per minute per milliliter. The phosphate for the perturbation was then added with another sterile syringe through the stopper. The phosphate was diluted into between one and five ml to yield the appropriate final concentration in the reactor vessel. As soon as the syringe was removed containing the phosphate for the perturbation, a second syringe was inserted in the rubber stopper and a sample removed for the time 0 point. Another syringe was modified by removing the plunger and placing a cotton stopper in the

top. This syringe was placed through the rubber stopper before sampling so that the reactor could draw in sterile air to replace the fluid loss when samples were drawn.

Enumeration

Cell numbers were determined by epifluorescent direct count microscopy from a method adapted by Hobbie et al. (1977). A measured aliquot (0.5-2.0 ml) of reactor vessel contents was placed in a small test tube containing filtered water. Two to three drops of filtered 0.1% (in water) acridine orange solution were then added to each tube to stain the cells. Each tube of stained cell suspension was then filtered using a 3 ml syringe and Millipore Swinnex filter apparatus (Millipore Corporation, Bedford, MA) onto an Irgalan Black stained Nuclepore filter (Nuclepore Corporation, Pleasanton, CA; 0.2 μm pore size, 13 mm diameter; Hobbie et al.,1977). Three 2 ml aliquots of filtered water were additionally filtered to rinse each tube and the filter apparatus. The damp Nuclepore filter was then placed on a microscope slide with a drop of immersion oil and a cover slip and was held at $4^{\circ}C$ in the dark for 30 minutes to one hour, after which 15-20 microscope fields were counted per

slide using a Zeiss Standard microscope with an epifluorescence light source (Carl Zeiss, Inc., Thornwood, NY). Duplicate or triplicate filters were prepared and counted for each sample. From these data, a mean number of cells per ml was calculated plus or minus one standard error for a given time or dilution rate. The acridine-orange direct-count method was selected because it allowed rapid and reproducible estimates of cell numbers.

Carbon

Carbon samples were collected on pre-combusted glass fiber filter papers (Gelman type A/E, 25 mm; Gelman Sciences Inc., Ann Arbor, MI), placed in ten ml ampules and frozen until carbon analyses could be performed. When a number of samples had been collected and the ^{32}P had decayed, the samples were prepared for carbon analysis. Six ml of 1 N H_2SO_4 were added to each ampule, the ampule was sealed, and the contents autoclaved for 20 min. The supernatant was analyzed for carbon by the wet chemical method described for the Technicon Auto Analyzer II (Technicon Industrial Systems, 1978). This method was selected for its sensitivity (0.4-20.0 mg $C \cdot l^{-1}$) and

small sample-volume requirement (2 ml).

RESULTS

Overview

The results of eight phosphate perturbations of phosphorus-limited steady-state cultures are presented here. A brief description of specific growth conditions for each of the perturbed cultures accompanies these results and is summarized in Table 3. A complete tabulation of all continuous culture data collected in this study is found in Appendices 1, 2 and 3.

In all of the graphs the mean steady-state value is shown to the left of time zero. The error around the steady-state value (one standard error) is shown in dotted lines whenever it fell outside the line of the mean value. The steady-state values represent the mean value of at least three measurements over three residence times.

Three single-species continuous cultures of *Selenastrum capricornutum* were perturbed with different concentrations of phosphate. The first of these cultures, S9, was the only culture with a growth rate

Table 3. Summary of Perturbation Conditions for Single and Dual Species Continuous Cultures.

Culture	$P_{perturb}$ (nM)	C/P
S9	1087	
S12	2911	
S13	7762	
R1	2911	687
R2	7762	608
R3	7762	48
D10	7762	162
D11	7762	122

substantially different from the other continuous cultures. This culture was perturbed at a steady-state growth rate of 0.51 d^{-1}. The other single-species *S. capricornutum* cultures, S12 and S13, had steady-state growth rates of 0.72 and 0.74 d^{-1}, respectively. The concentration of phosphate in the medium for all three of these cultures was either 964 nM (S9) or 970 nM (S12 and S13). Three phosphate concentration perturbation values were used in these runs. S9 was perturbed with 1087 nM, S12 with 2911 nM and S13 with 7762 nM phosphate.

Three steady-state single-species cultures of *Rhodotorula rubra* were also subjected to phosphate perturbations (R1, R2 and R3). The growth rate was approximately the same in all three runs and ranged from 0.69 to 0.74 d^{-1}. The first two *R. rubra* cultures were phosphorus-limited at steady-state. The medium phosphate concentration in these cultures was 970 nM. The carbon source provided to these cultures was glucose and was supplied in non-limiting concentrations under steady-state conditions. The medium for R1 contained 667μM carbon for a C/P ratio of 687. The medium for R2 contained 590 μM carbon for a C/P ratio of 608. The third *R. rubra* culture was carbon-limited. The medium

provided to this culture contained 2097 nM phosphate and 100 μM carbon for a C/P ratio of 48.

In addition to the results from the single-species cultures, the results of two dual species cultures (D10 and D11) are also presented. These cultures were provided with approximately the same steady-state conditions. The growth rate was 0.72 d^{-1} for D10 and 0.66 d^{-1} for D11. The phosphate concentration in the medium was 970 nM for both cultures. The carbon concentration provided for the yeast was slightly different in the two cultures. D10 was supplied with 157 μM carbon for a C/P ratio of 162. D11 was supplied with 118 μM carbon for a C/P ratio of 122.

Phosphate Uptake

Figures 5 through 12 show the uptake response of all eight cultures to phosphate perturbations. In all single-species *S. capricornutum* cultures a rapid decline was seen in the percent phosphorus in solution. The decline was so rapid after perturbation that phosphorus had already been removed from solution in all cultures at the first sampling (time 0) which occurred as quickly as

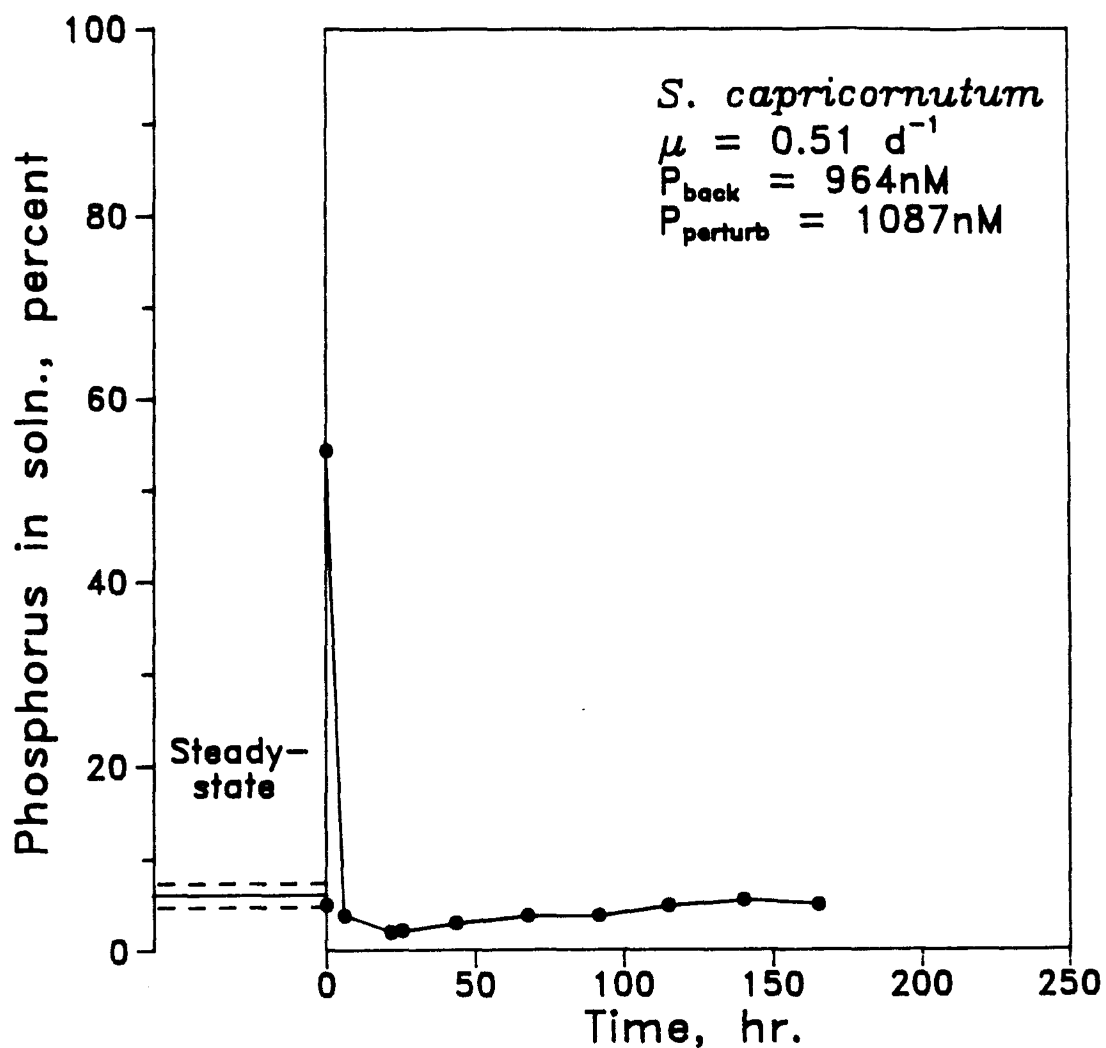

Figure 5. Percent phosphorus in solution after phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

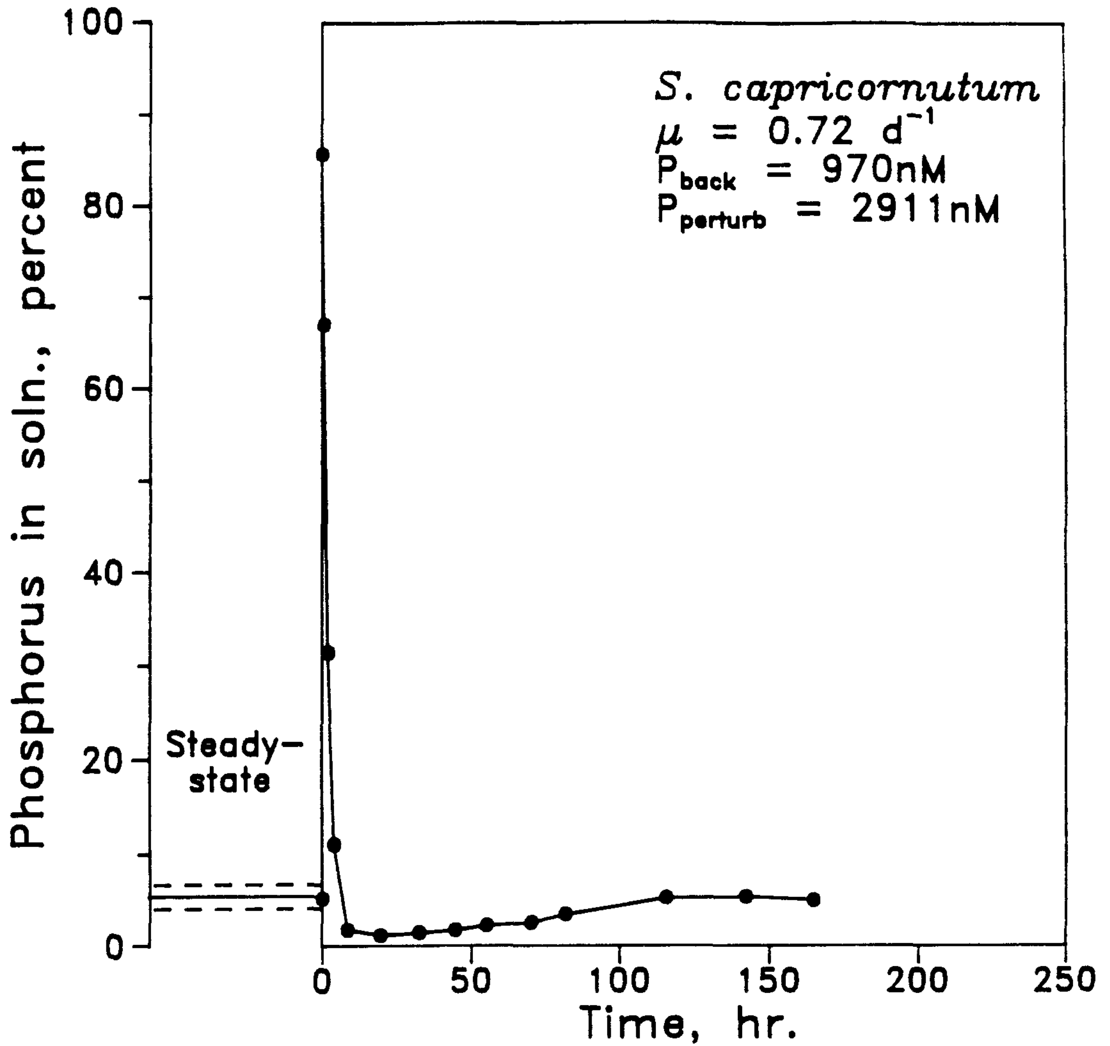

Figure 6. Percent phosphorus in solution after phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

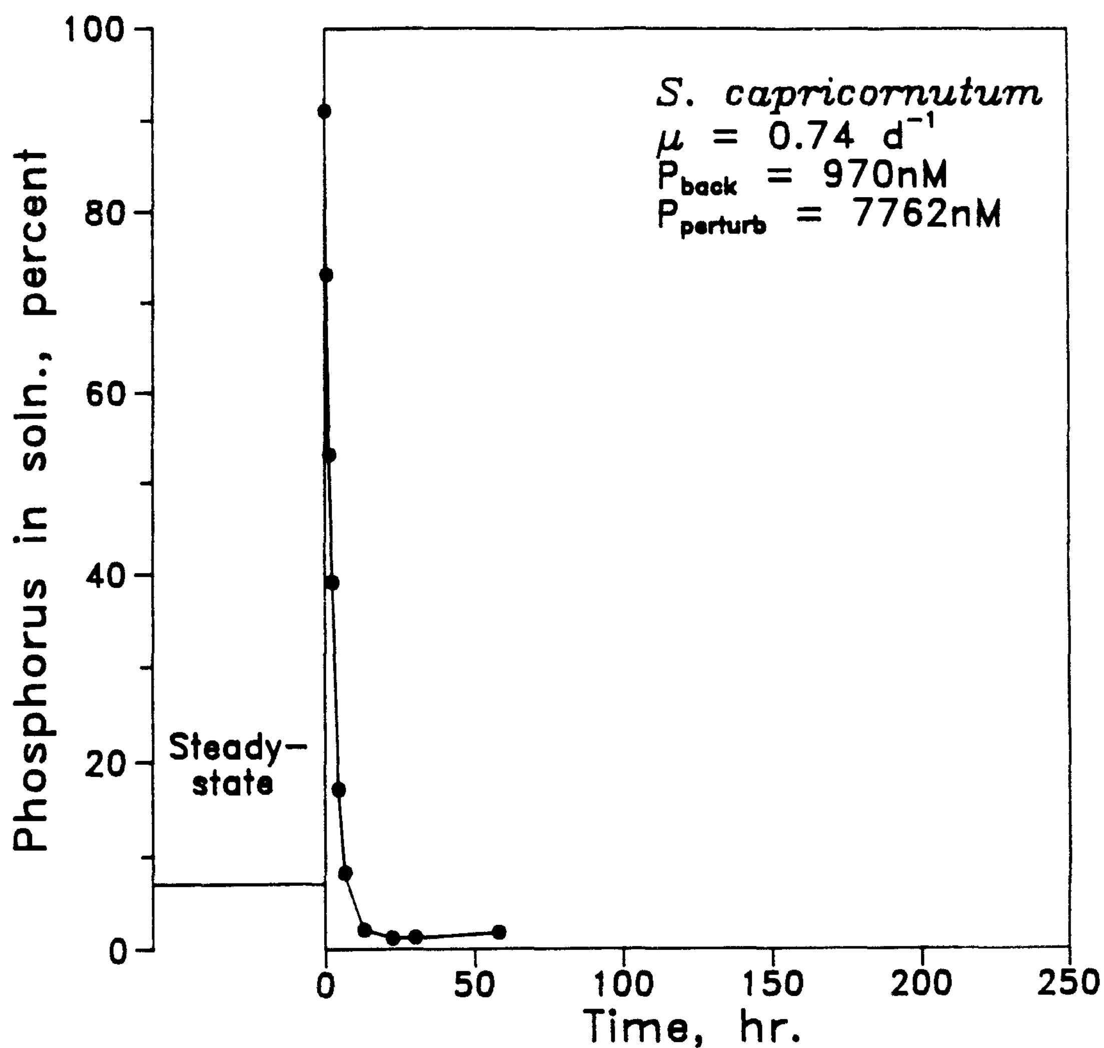

Figure 7. Percent phosphorus in solution after phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

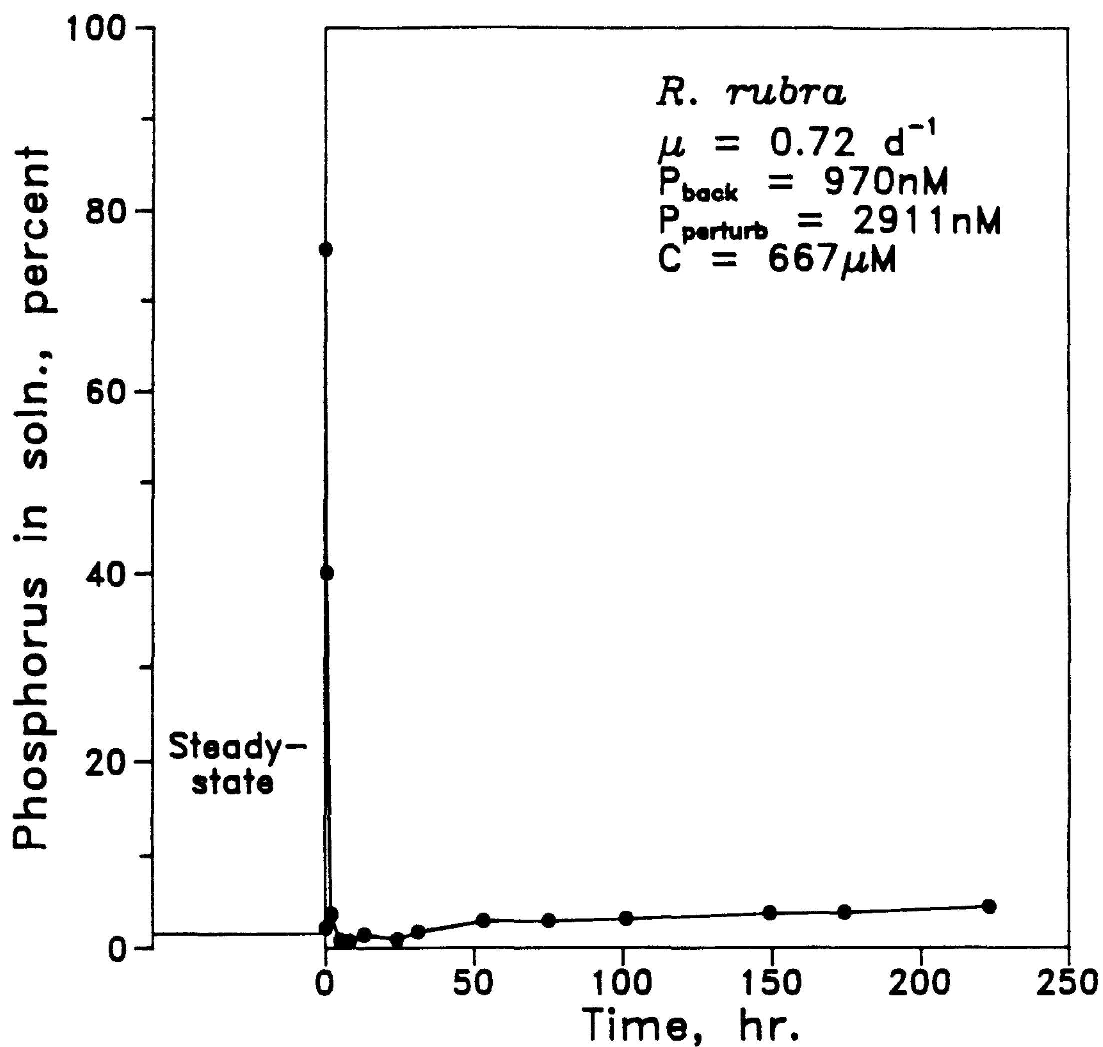

Figure 8. Percent phosphorus in solution after phosphate perturbation of a phosphorus-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

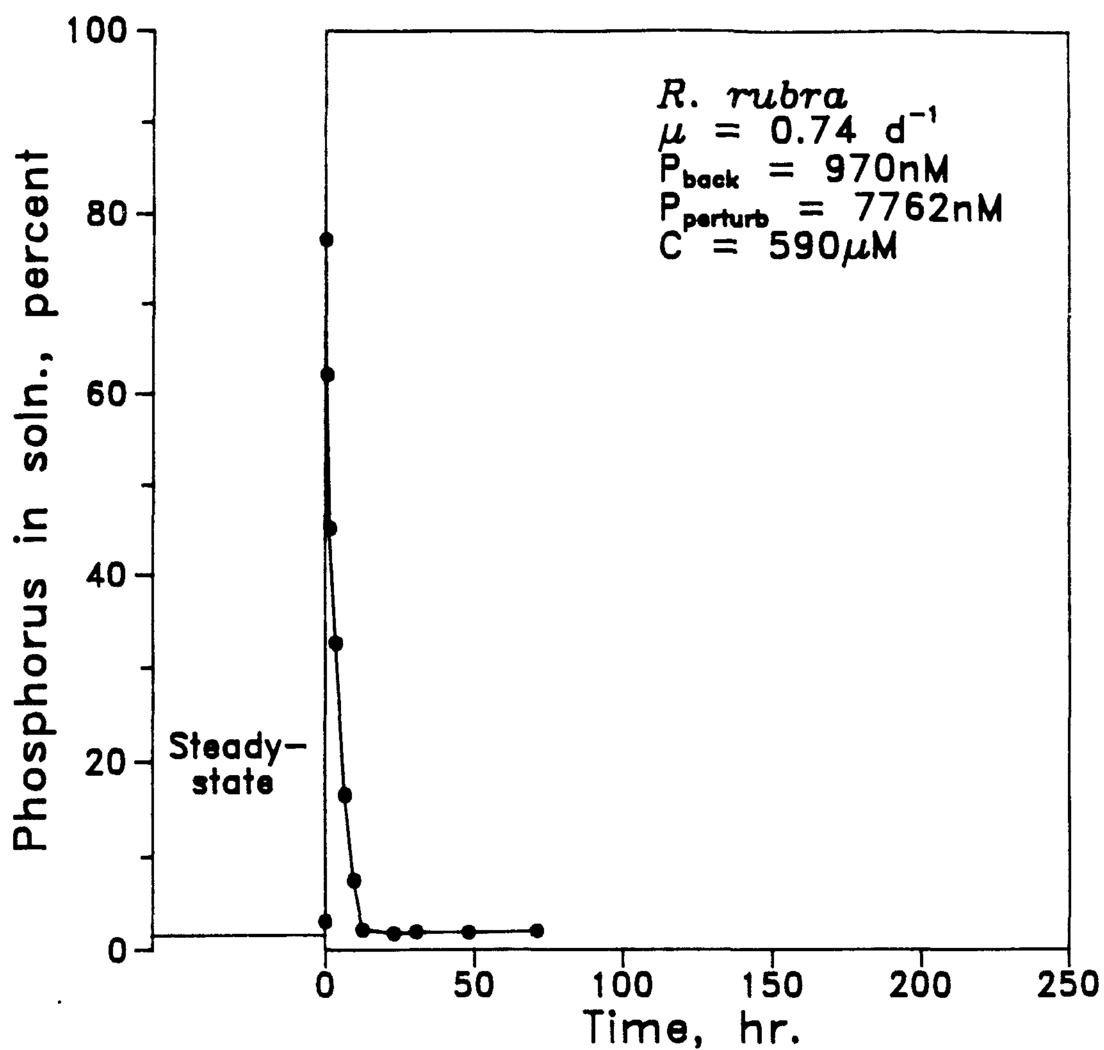

Figure 9. Percent phosphorus in solution after phosphate perturbation of a phosphorus-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

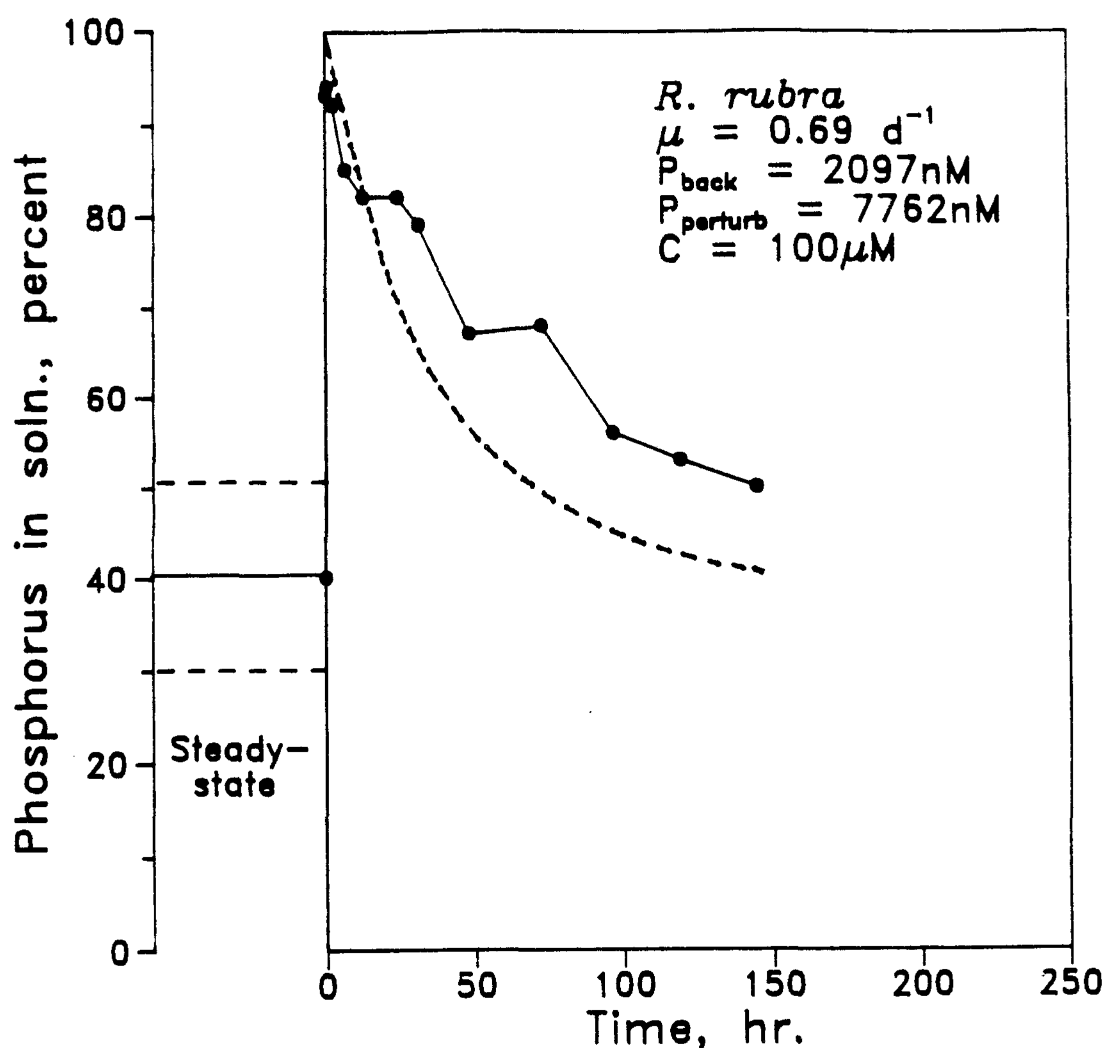

Figure 10. Percent phosphorus in solution after phosphate perturbation of a carbon-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero. The dotted line represents the calculated washout of phosphorus at a dilution rate of 0.69 d^{-1}.

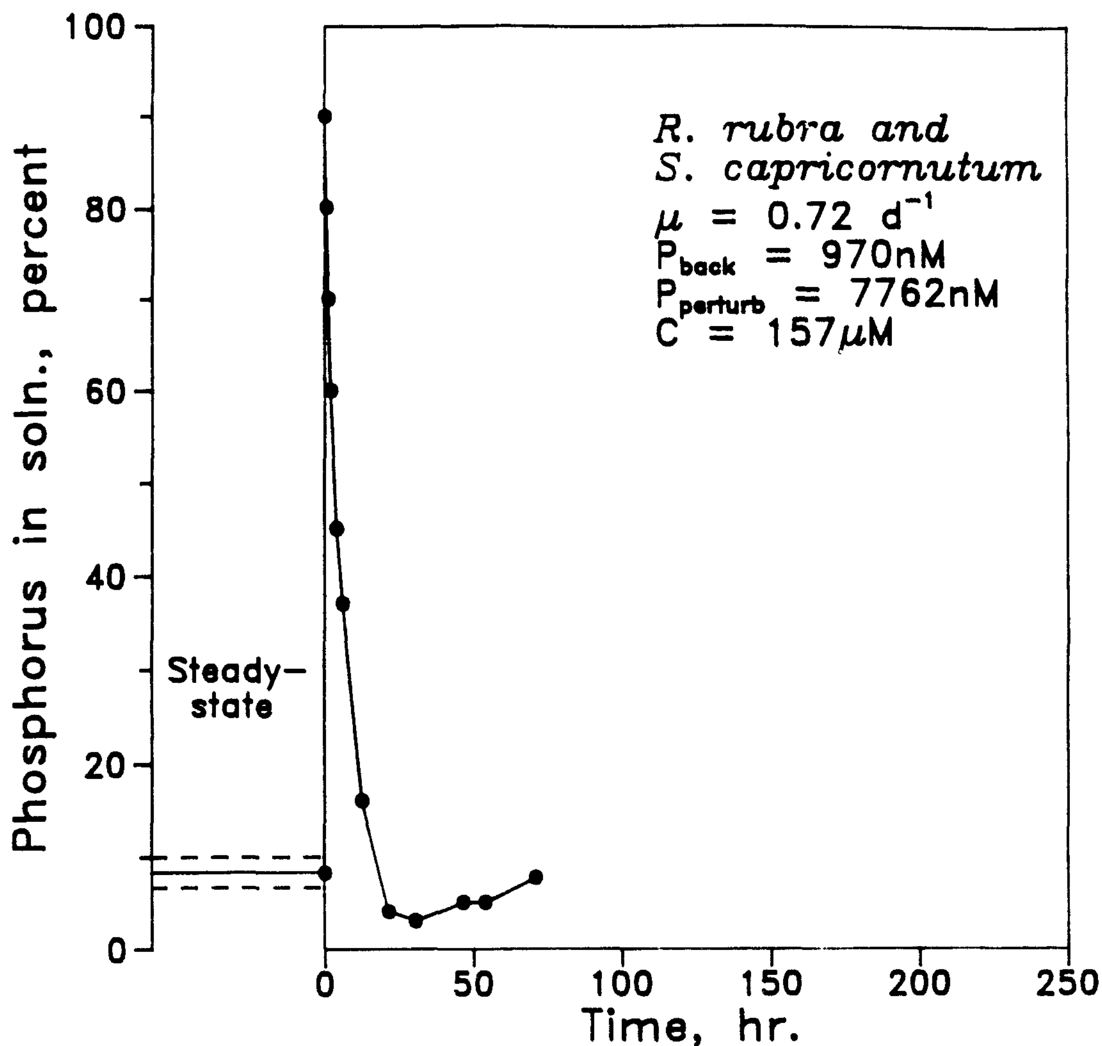

Figure 11. Percent phosphorus in solution after phosphate perturbation of a phosphorus-limited steady-state dual-species culture. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

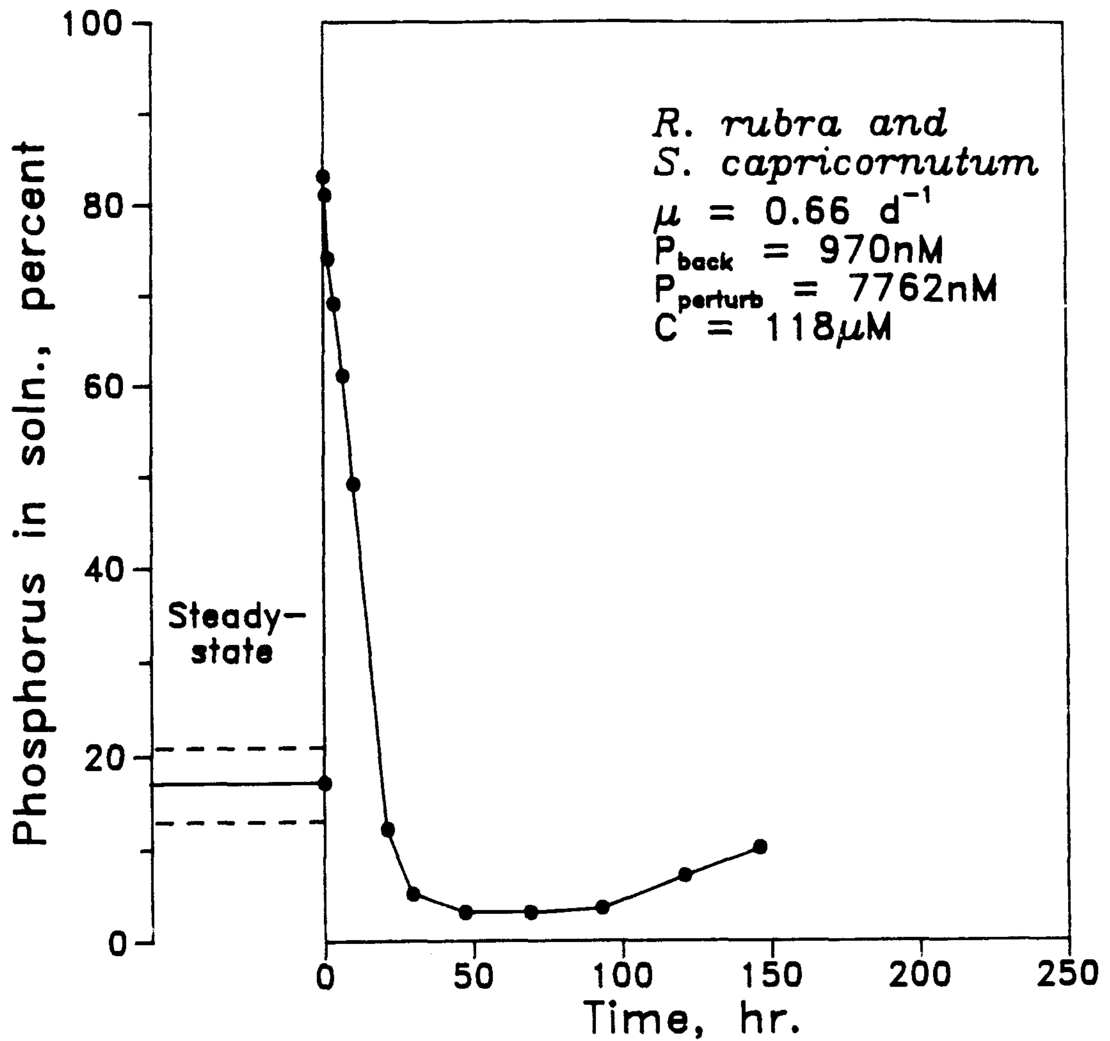

Figure 12. Percent phosphorus in solution after phosphate perturbation of a phosphorus-limited steady-state dual-species culture. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

possible after a phosphorus perturbation. The concentration in solution returned to the steady-state concentration rapidly in all three cultures. In S9 and S12 (Figures 5 and 6), perturbed with less phosphate, this occurred more rapidly. The steady-state values for phosphorus in solution for these cultures were 47 nM for S9, 52 nM for S12 and 67 nM for S13 (see Figures 5, 6, and 7).

Phosphorus-limited single-species cultures of *R. rubra* (R1 and R2) also decreased the percent phosphorus in solution fairly rapidly (Figures 8 and 9). Culture R1 (Figure 8) was subjected to nearly identical conditions to that of S12 (Figure 6). Culture R1 returned to the steady-state background concentration of phosphorus in solution (20 nM) by five hours, while R2 (Figure 9), perturbed with 2.5 times as much phosphate was only approaching the steady-state concentration (29 nM) after 70 hours. The carbon-limited culture (R3, Figure 10) was slower at diminishing the pulsed phosphate from solution because the cell biomass was depressed due to carbon limitation. The culture conditions in R3 were nearly equal to R2 except that R3 was carbon-limited instead of phosphorus-limited. After 120 hours the phosphorus in

solution still had not returned to the pre-perturbation value. Although washout of the perturbed phosphorus was occurring, the decrease in phosphorus in this culture cannot be attributed totally to washout (see dotted line, Figure 10). Even though these cells were not phosphorus-limited, the cells did transport phosphate (see also Figure 17). In fact, the highest internal cellular concentrations of phosphorus in this study were seen in these cells.

The decrease in percent phosphorus in solution for the two dual species cultures after perturbation are found in Figures 11 and 12. The phosphorus in solution again diminished fairly rapidly in these cultures, although generally more slowly than in the single species cultures. In nearly all the phosphorus-limited cultures, the percent phosphorus in solution decreased below the steady-state value as the biomass increased in response to the phosphorus increase.

The internal cellular phosphorus concentration changes with time from perturbation are shown in Figures 13 through 18 for the single-species runs. To convert the measured cell number data to cell dry weight for these figures the following values were used: for *S.*

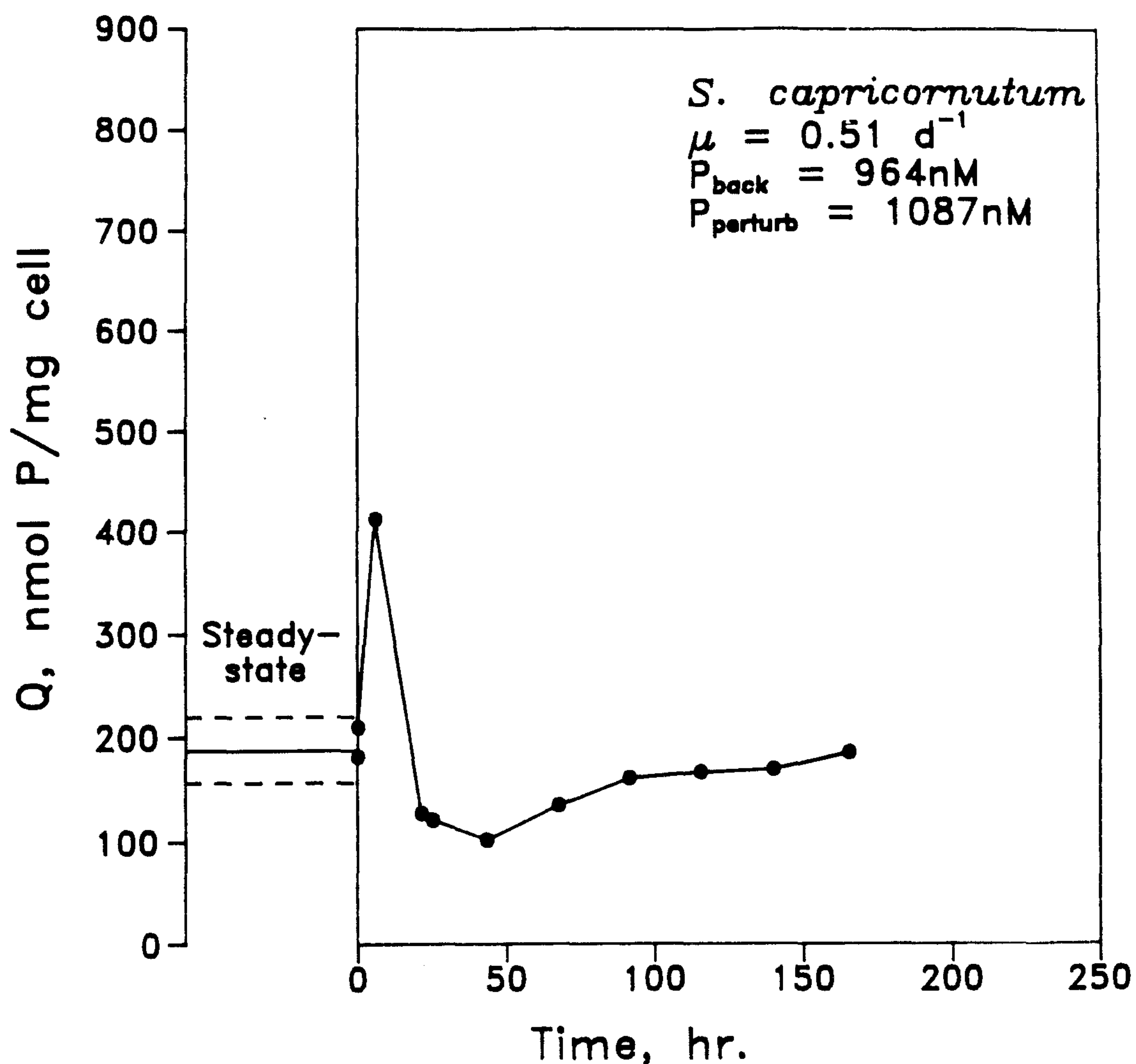

Figure 13. Internal phosphorus concentration (Q) after phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

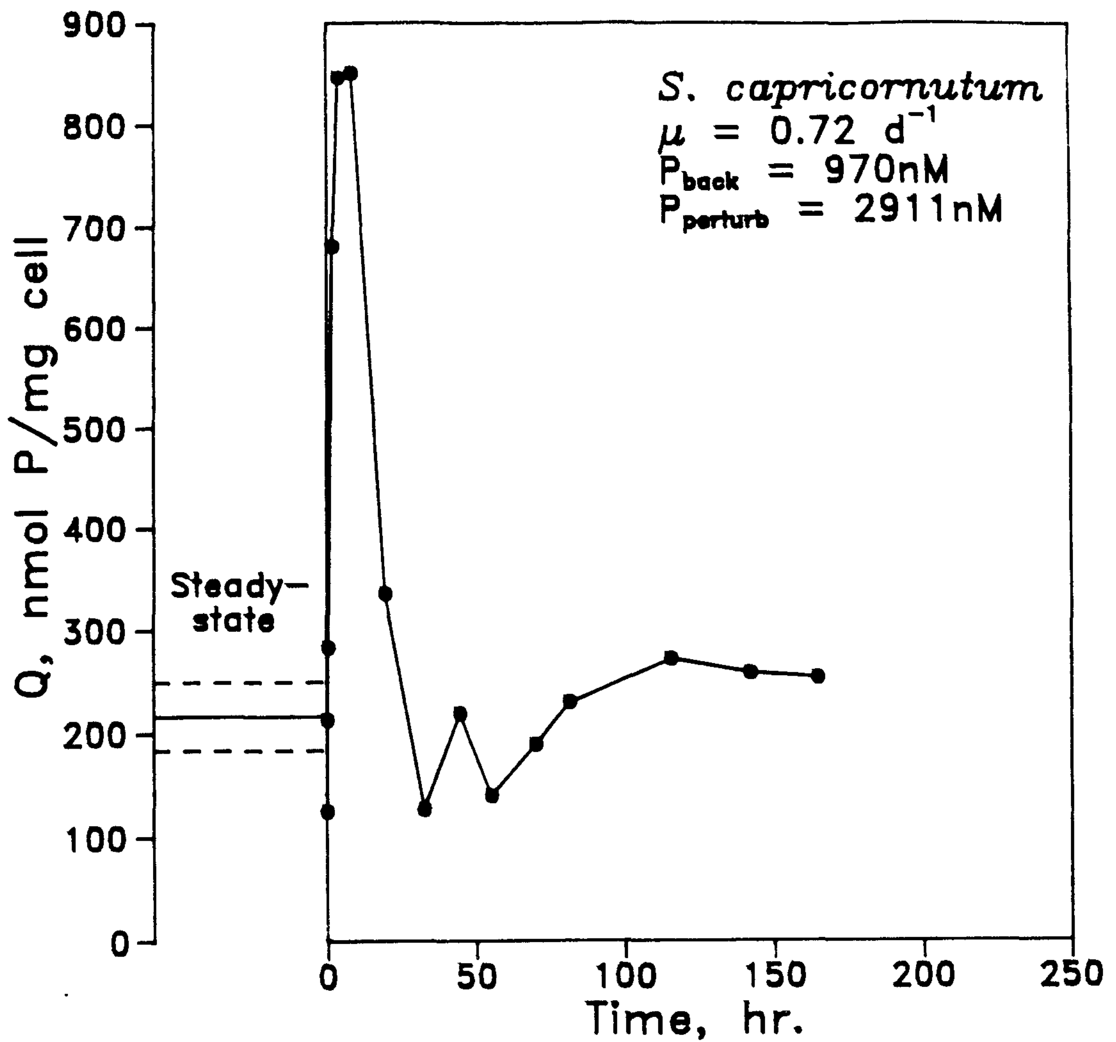

Figure 14. Internal phosphorus concentration (Q) after phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

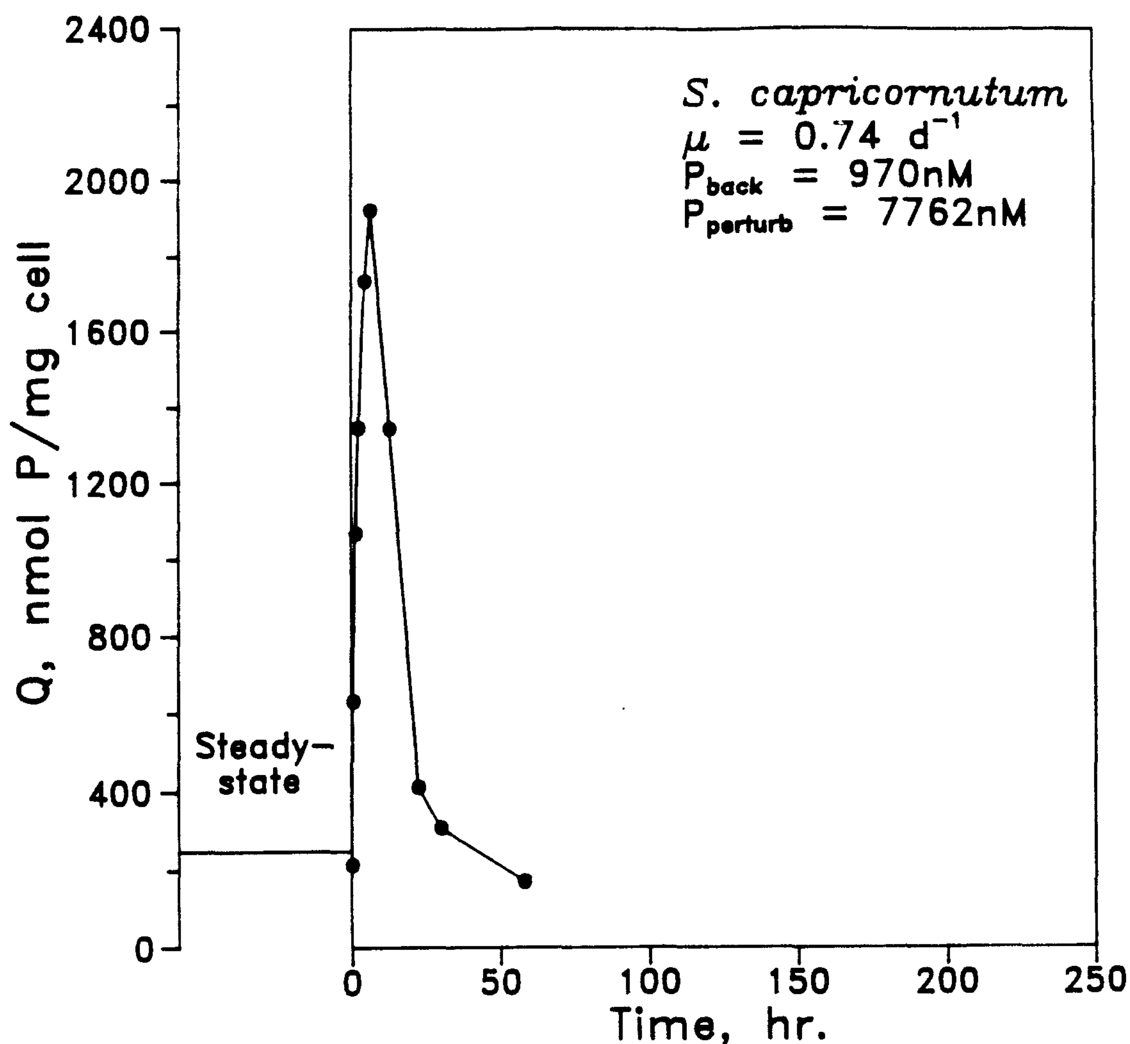

Figure 15. Internal phosphorus concentration (Q) after phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

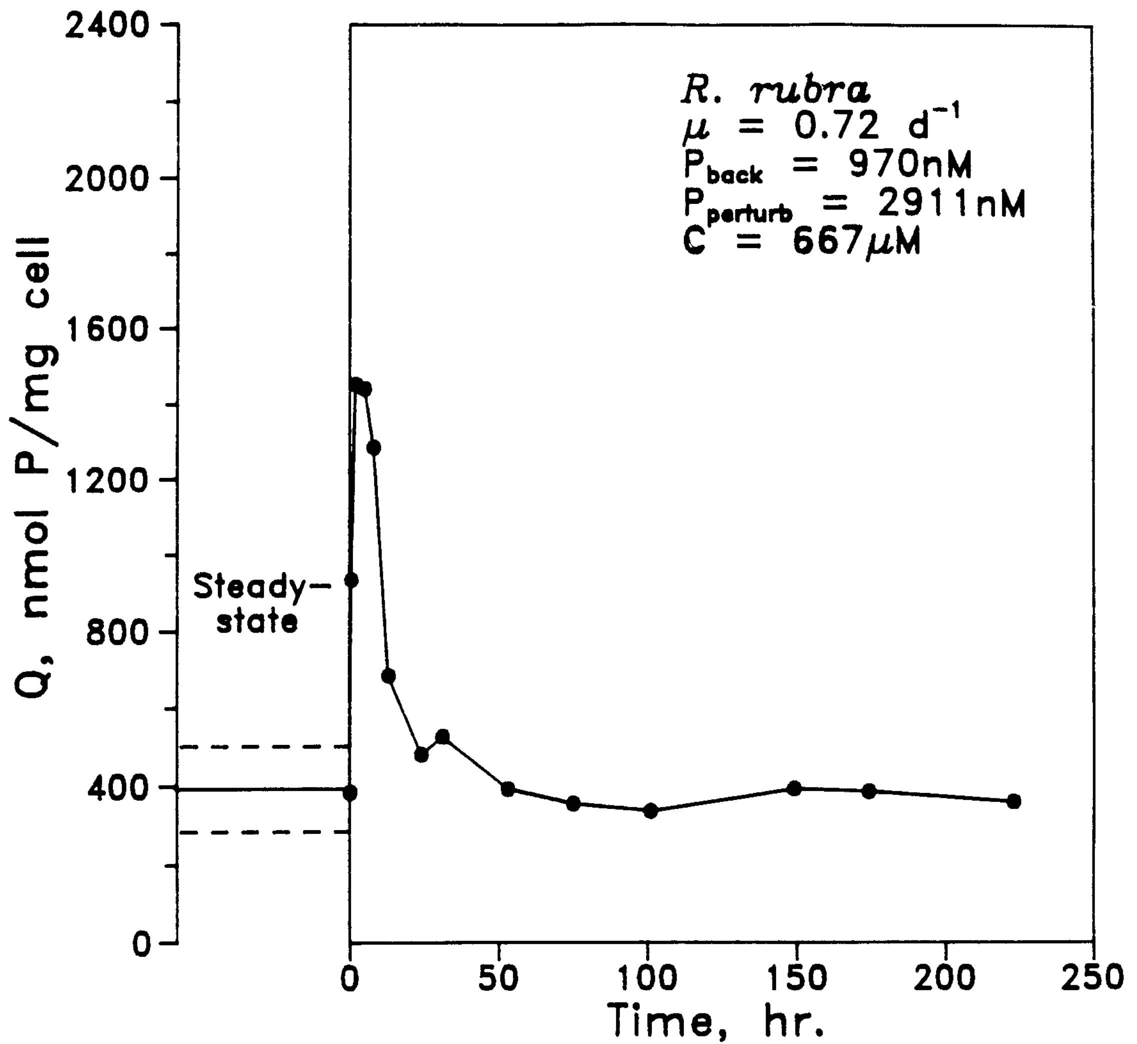

Figure 16. Internal phosphorus concentration (Q) after phosphate perturbation of a phosphorus-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

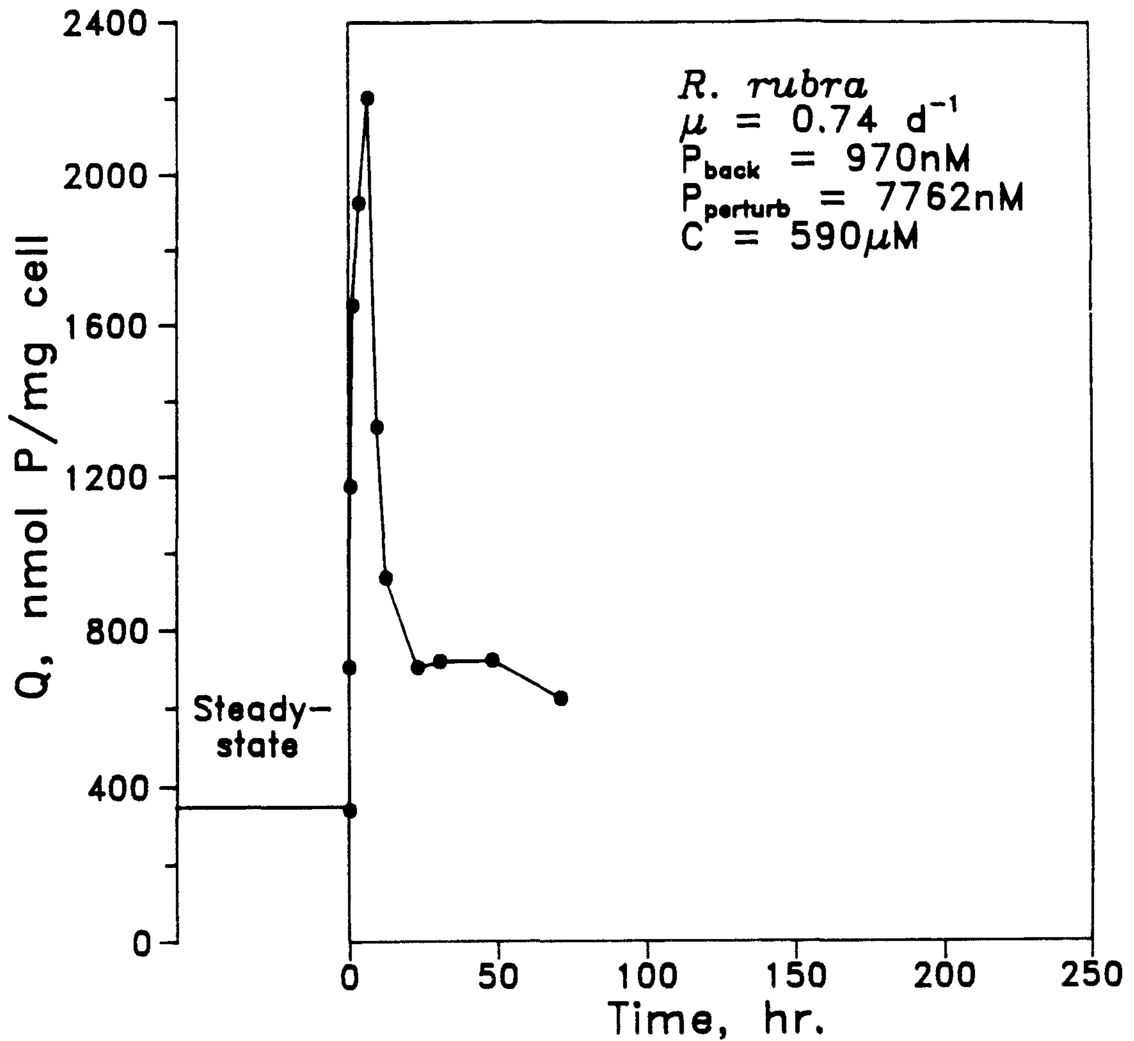

Figure 17. Internal phosphorus concentration (Q) after phosphate perturbation of a phosphorus-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

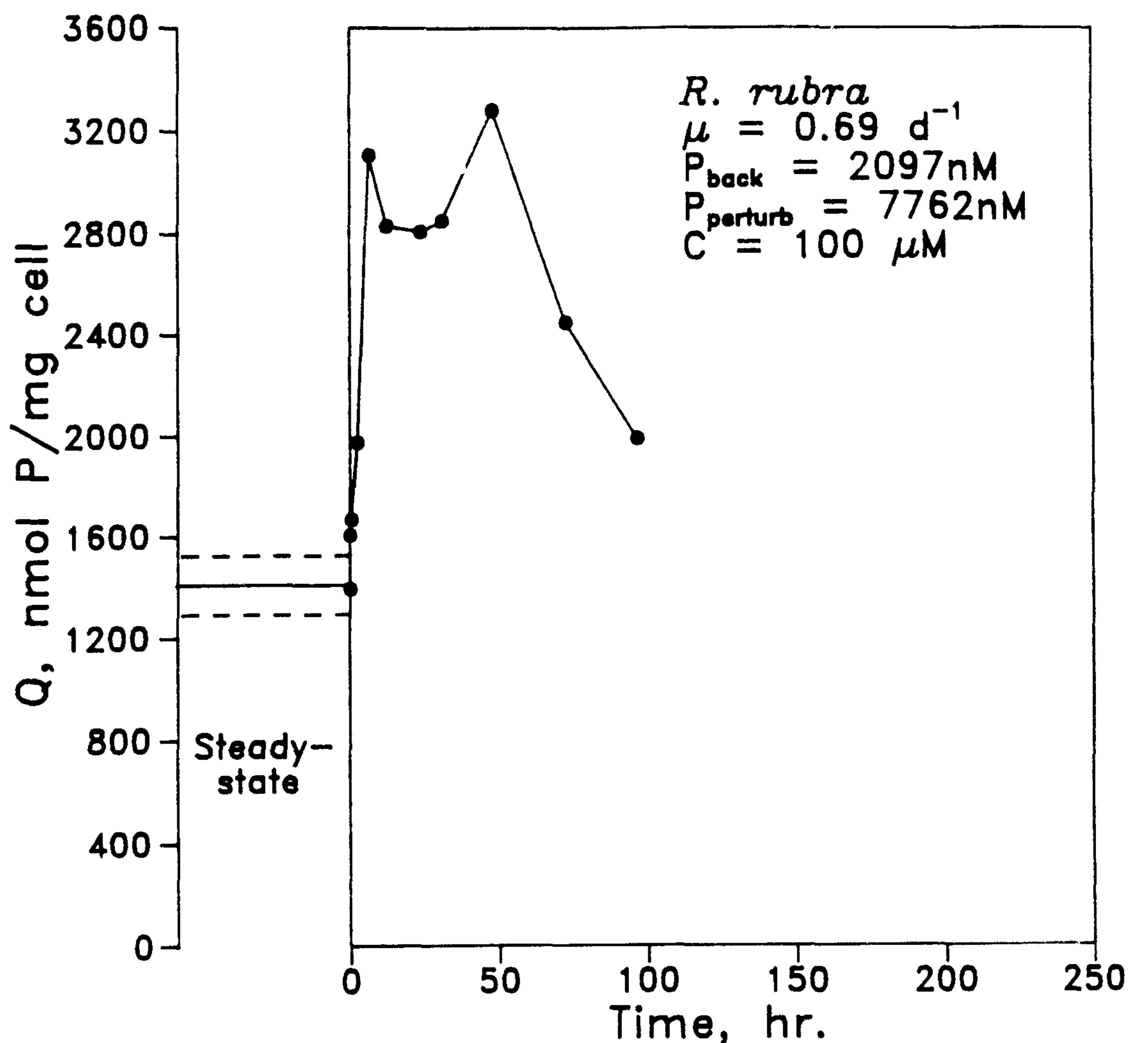

Figure 18. Internal phosphorus concentration (Q) after phosphate perturbation of a carbon-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

capricornutum, 2.5 x 10^{-8} mg dry weight per cell (Brown and Button, 1979) and for *R. rubra* 0.65 x 10^{-8} mg dry weight per cell (Robertson and Button, 1979).

The internal cellular phosphorus concentrations increased in all perturbations, including R3 which was not phosphorus-limited at any time. A summary of the highest internal phosphorus values measured under perturbation conditions (Q_{maxP}) for each culture is found in Table 4. The number of hours after perturbation that this value was attained is found in parentheses after each Q_{maxP} value.

The highest internal cellular phosphorus concentration for *S. capricornutum* was seen in culture S13 (Figure 15), the culture perturbed with the highest concentration of phosphate. These cells attained an internal phosphorus concentration of greater than 1900 nM per mg cells. For all algal cultures, the highest internal phosphorus concentration was seen at between six and eight hours. The phosphorus-limited yeast culture, R2, which had nearly identical steady-state and perturbation conditions as culture S13 attained a slightly higher internal phosphorus concentration maximum of about 2200 nM per mg cell (see Figures 15 and 17). The highest internal phosphorus concentration seen in

Table 4. Maximum Internal Phosphorus Concentrations and Maximum Cell Numbers After Perturbations.

Culture # ($P_{perturb}$)	Q_{maxP}, nmol P/mg cell (time after perturb., hr.)	X_{max}, cells/l (time after perturb., hr.)
S9 (1087 nM)	411 (6)	4.9×10^8 (43)
S12 (2911 nM)	851 (8)	6.6×10^8 (32)
S13 (7762 nM)	1921 (6)	5.3×10^8 (58)
R1 (2911 nM)	1450 (2)	7.7×10^8 (24)
R2 (7762 nM)	2200 (6)	10.5×10^8 (23)
*R3 (7762 nM)	3279 (48)	---

* Carbon-limited

these experiments was in the carbon-limited yeast culture (R3, Figure 18). These cells attained a maximum internal phosphorus concentration of about 3300 nM per mg cell. This peak value was measured at 48 hours after perturbation. These cells kept accumulating phosphate slowly even though cell division was apparently limited by available carbon. These results suggest that both the yeast and the alga are capable of luxury consumption of phosphate. This luxury consumption is seen in phosphorus-limited algal cultures (Figures 13, 14 and 15) and in phosphorus-limited yeast (Figures 16 and 17) and carbon-limited yeast (Figure 18). In all cases the transport system for phosphate did not appear to be saturated as uptake was always more significant than washout. This phosphorus data can be used to estimate average maximum transport rates for each culture.

Transport Rates

Average transport rates were integrated over the time intervals between sampling for each culture (Tables 5, 6 and 7). Generally, the highest transport rates were seen in the cultures receiving the highest perturbation

Table 5. Average Transport Rates For Measured Time Intervals. *Selenastrum capricornutum* cultures.

Culture # ($P_{perturb}$)	Time Interval, hr.	Transport Rate, nmol P/ mg cell-day
S9 (1087 nM)	0.0-6.0	925
S12 (2911 nM)	0.0-0.5	8,032
	0.5-2.0	4,999
	2.0-4.0	2,067
	4.0-8.5	392
S13 (7762 nM)	0.0-0.5	19,908
	0.5-1.5	11,076
	1.5-2.5	7,566
	2.5-4.5	5,604
	4.5-6.5	2,160
	6.5-13.0	434

Table 6. Average Transport Rates For Measured Time Intervals. *Rhodotorula rubra* Cultures.

Culture # ($P_{perturb}$)	Time Interval, hr.	Transport Rate, nmol P/ mg cell-day
RA (D2; 855 nM)	0.0-3.0	2,973
R1 (2911 nM)	0.0-0.5	25,809
	0.5-2.0	8,628
	2.0-5.0	326
R2 (7762 nM)	0.0-0.5	23,177
	0.5-1.5	13,447
	1.5-3.5	5,109
	3.5-6.5	3,950
	6.5-9.5	2,009
	9.5-12.5	627
R3 (7762 nM)	0.0-0.5	685
	0.5-2.5	8,110
	2.5-6.5	8,954
	6.5-12.5	4,980
	12.5-24.0	2,981
	24.0-31.0	2,769
	31.0-48.0	2,194
	48.0-72.5	658
	72.5-96.5	654

Table 7. Average Transport Rates For Measured Time Intervals. Dual Species Cultures.

Culture # (C/P ratio)	Time Interval, hr.	Transport Rate, nmol P/ mg cell-day
D10 (162)	0.0-0.5	12,041
	0.5-1.3	8,408
	1.3-2.0	9,180
	2.0-4.0	4,917
	4.0-6.0	2,664
	6.0-12.5	1,848
	12.5-21.5	622
	21.5-30.5	61
D11 (122)	0.0-0.5	5,952
	0.5-1.5	8,267
	1.5-3.5	3,861
	3.5-6.5	3,591
	6.5-10.0	3,618
	10.0-21.0	1,380
	21.0-29.5	218

concentrations of phosphate. The estimates are the least accurate for S9 because the culture was not sampled for the first time until well after the maximum transport periods seen in the other algal cultures. Both S12 and S13 had initial transport rates which exceeded the maximum value estimated from batch and steady-state continuous cultures. This value is calculated from data in Table 8: the product of μ_{max} and Q_{maxt} and has a value of 2712 nmol P/ mg cell-day for *S. capricornutum* (Brown and Button, 1979). All of the yeast cultures transiently exceeded the value of 8103 nmol P/ mg cell-day calculated for yeast from steady-state and batch culture data (Robertson and Button, 1979). Even the carbon-limited culture was able to transport phosphate at very rapid rates (Table 6). The yeast appears to have higher transport rates than the alga. Cultures S12 and R1 had approximately the same culture conditions as did cultures S13 and R2. In both cases, the yeast transported phosphate more rapidly than did the algae. The dual species cultures also displayed very high transport rates. D10 appeared to exceed D11 perhaps because the very efficient yeast population was less carbon-limited in the D10 culture and transported more phosphate as a population. The

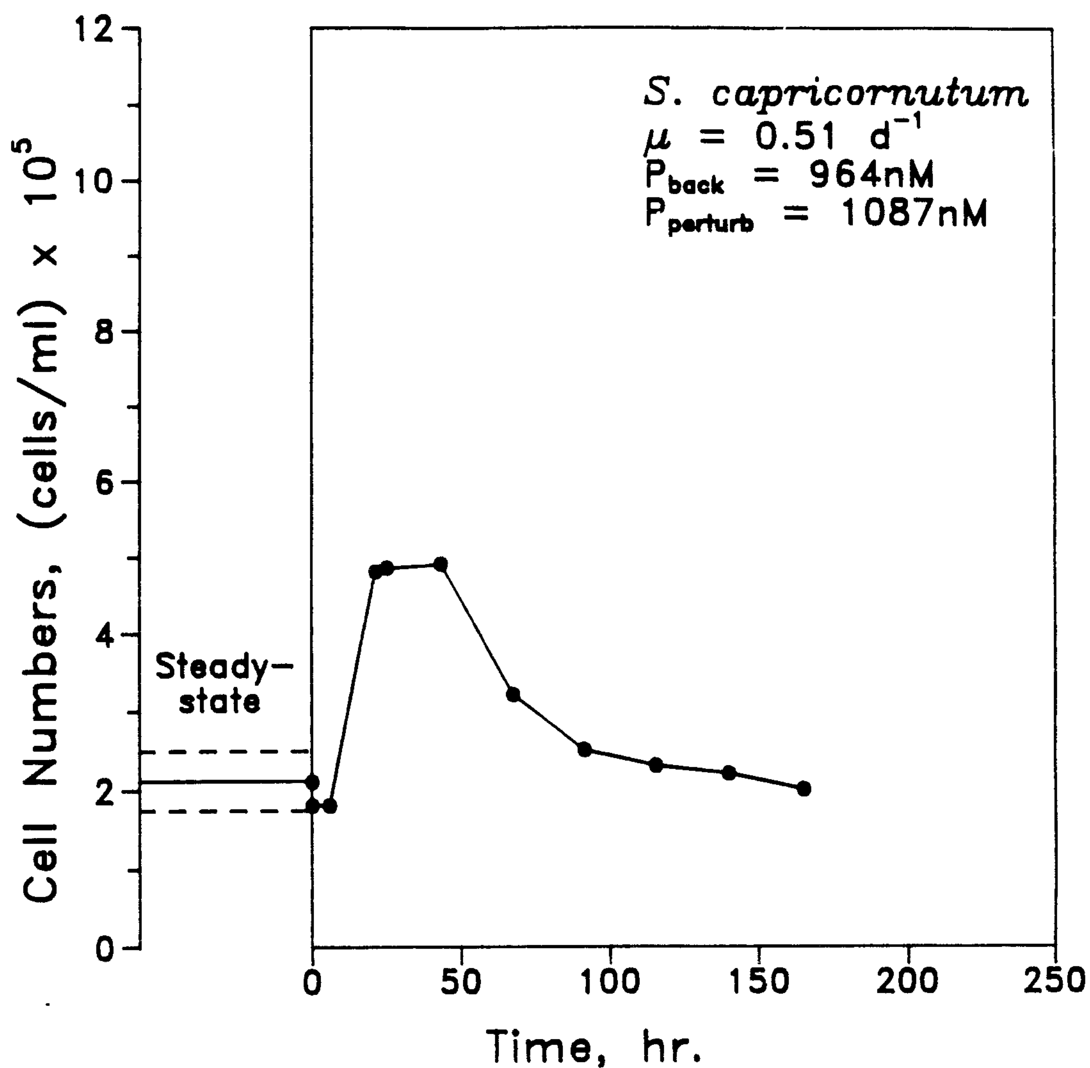

Figure 19. Cell numbers after phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

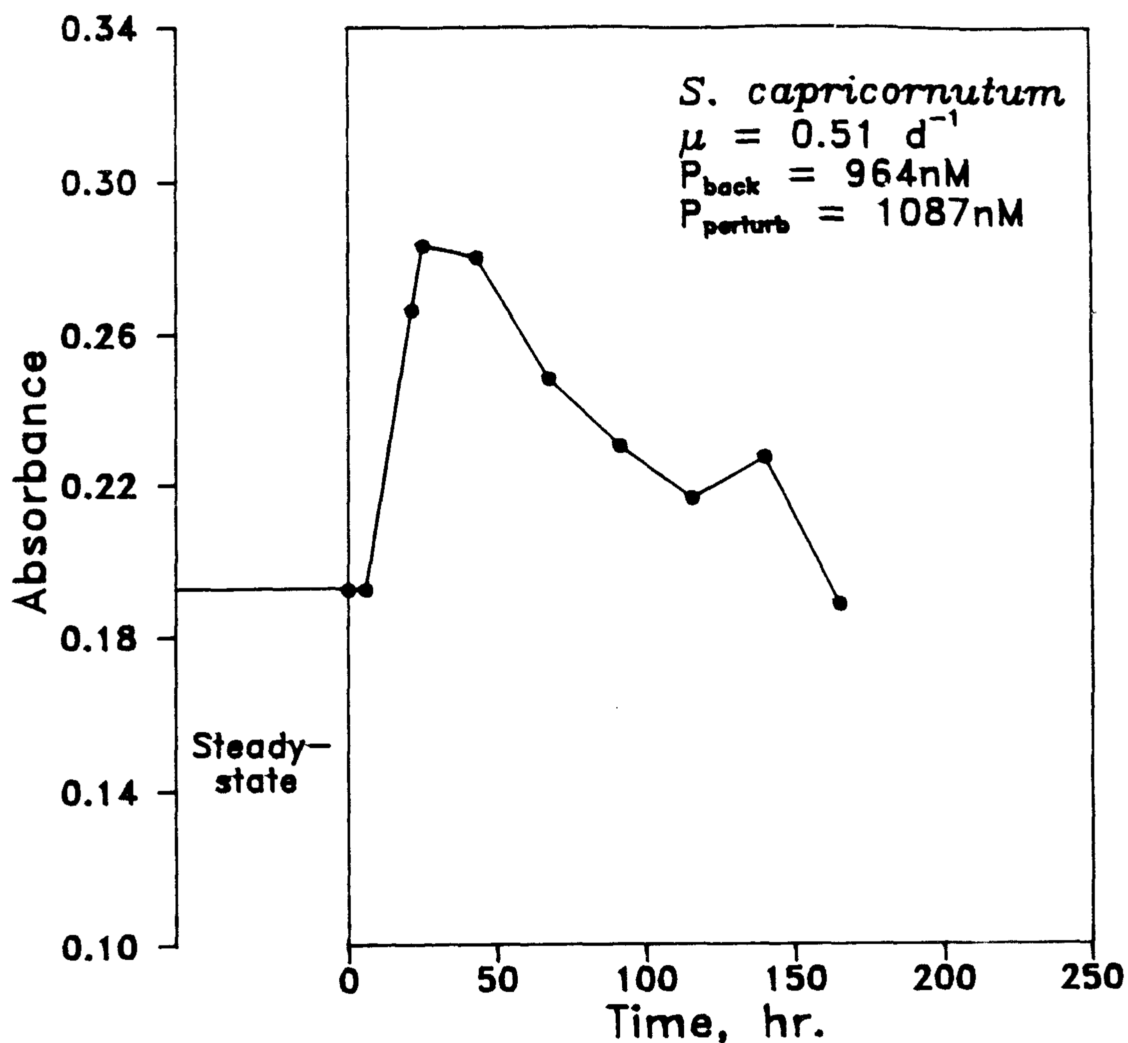

Figure 20. Culture absorbance at 686 nm after a phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

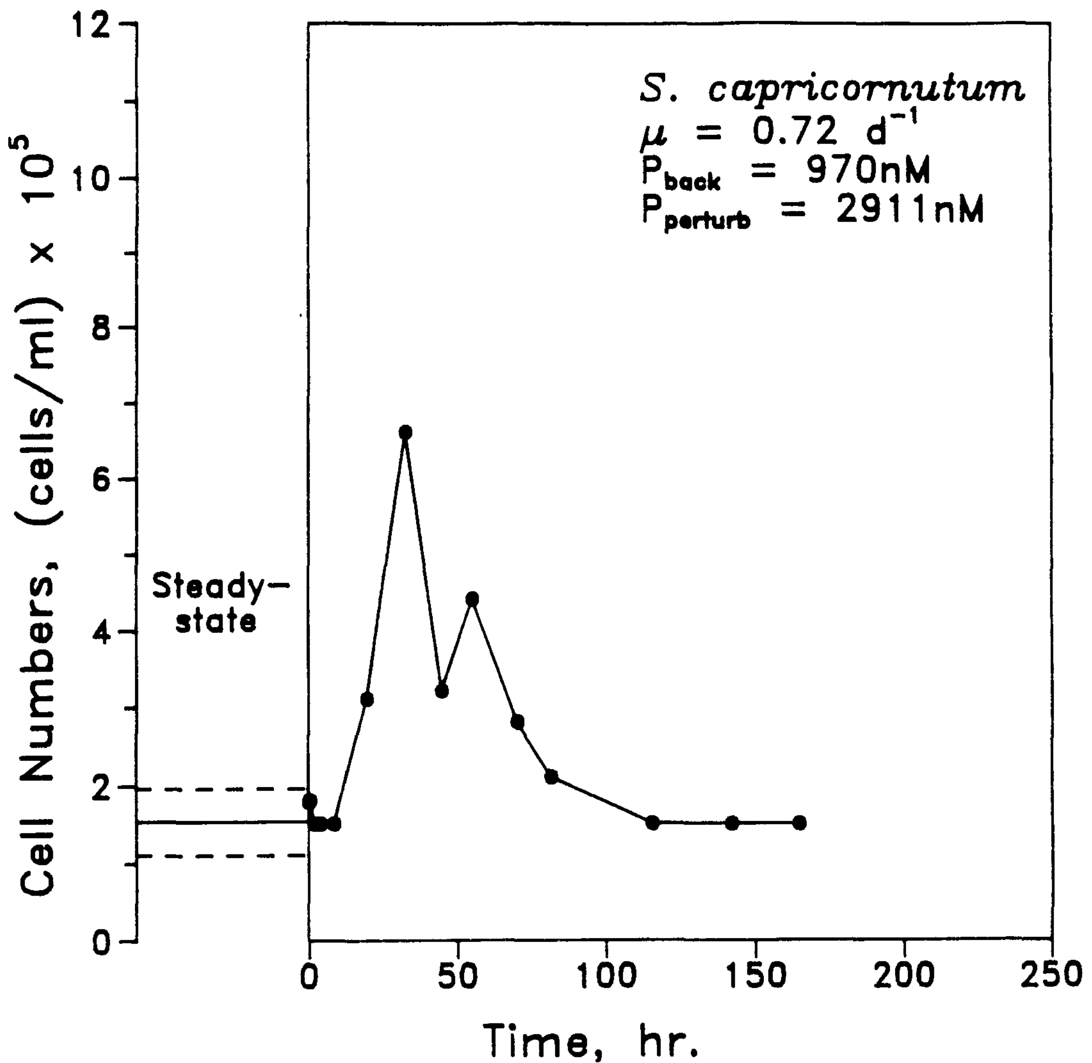

Figure 21. Cell numbers after phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

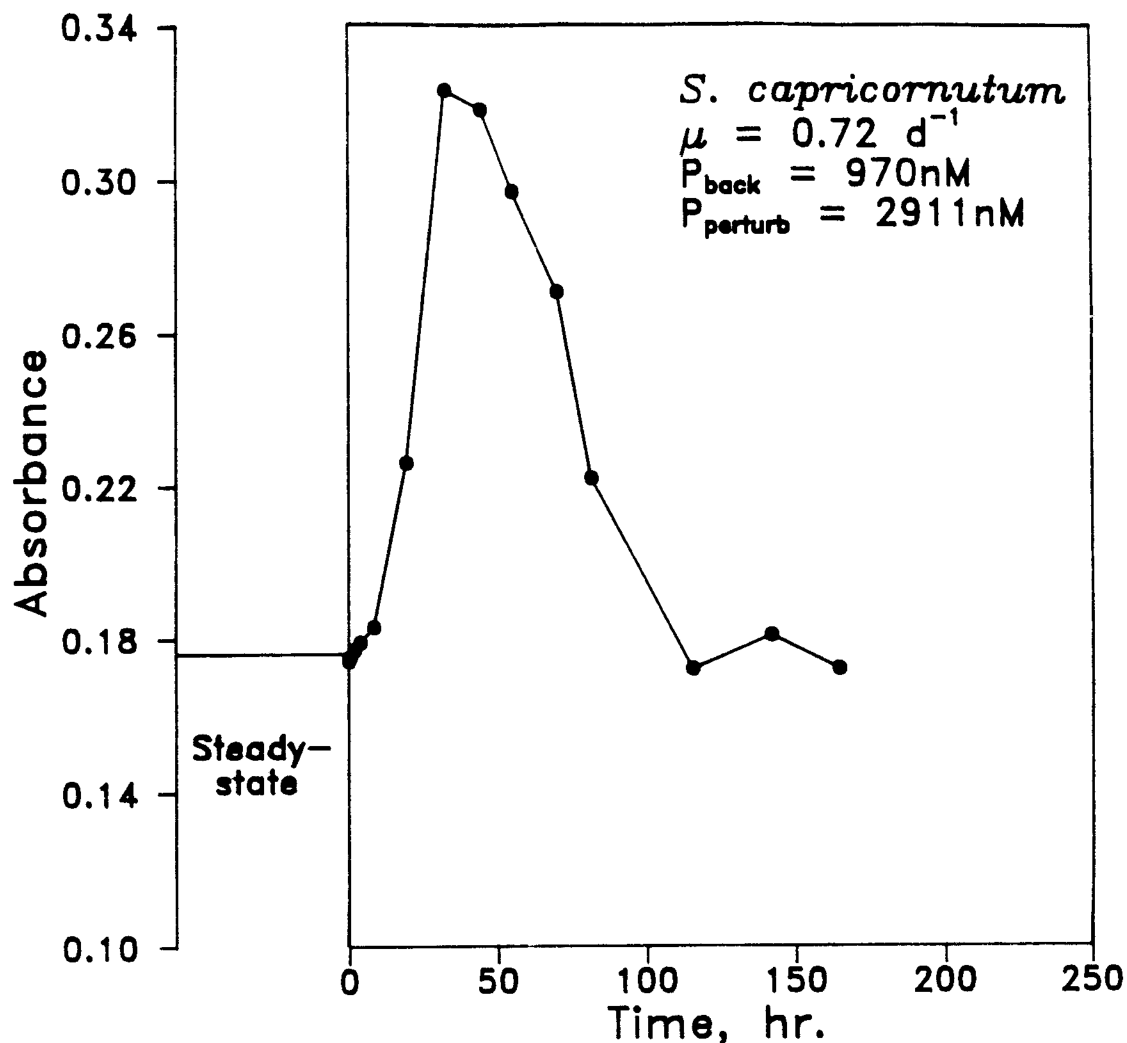

Figure 22. Culture absorbance at 686 nm after a phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

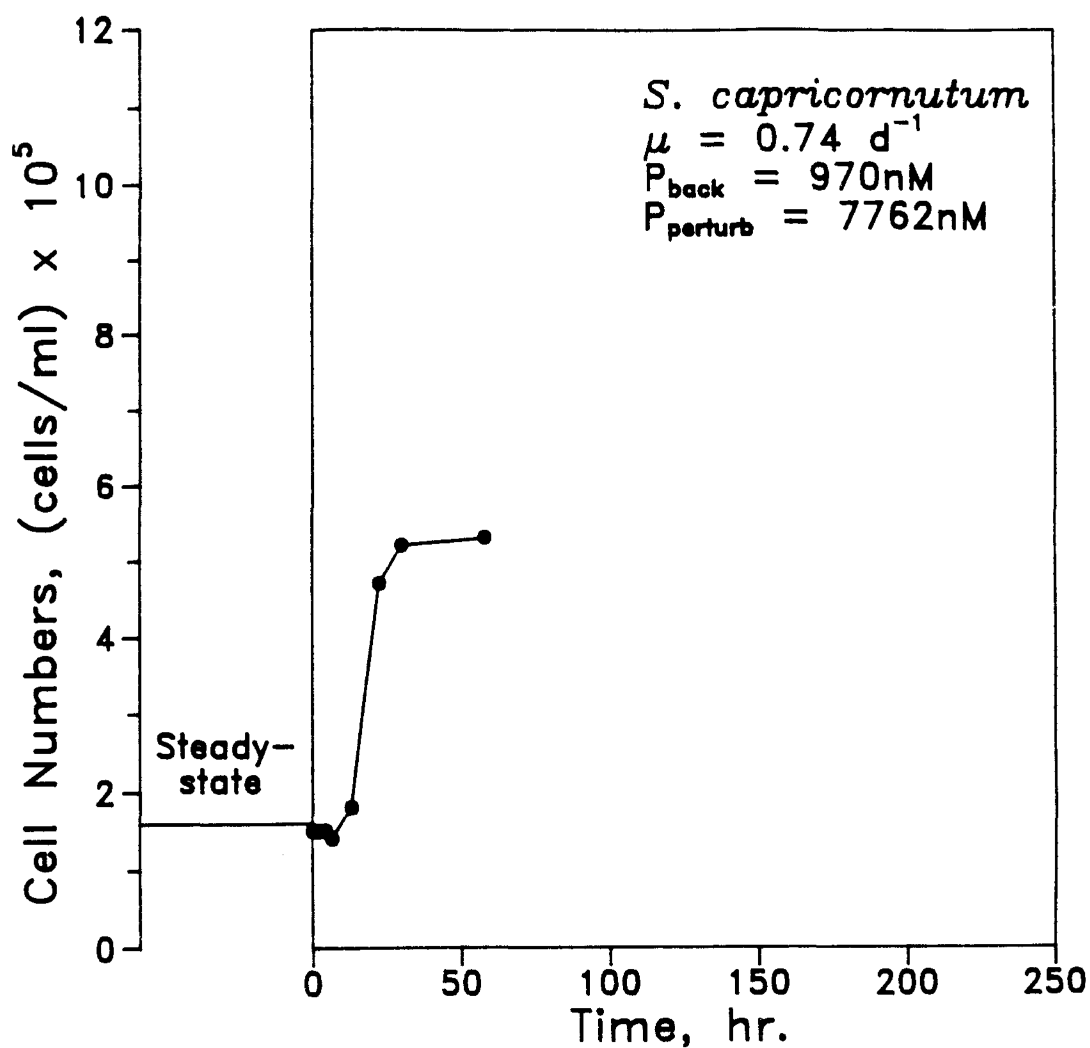

Figure 23. Cell numbers after phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

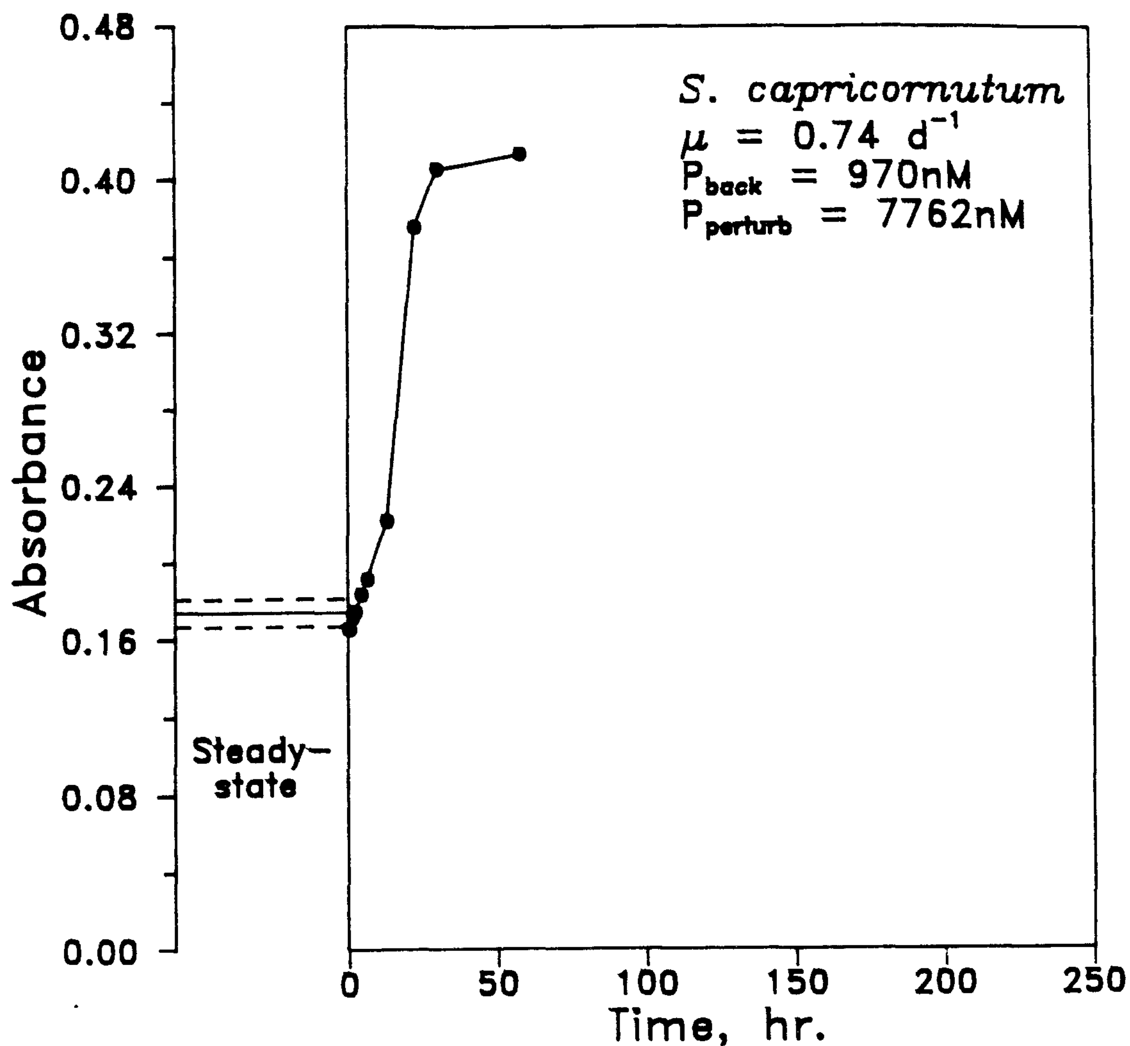

Figure 24. Culture absorbance at 686 nm after a phosphate perturbation of a phosphorus-limited steady-state culture of *S. capricornutum*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

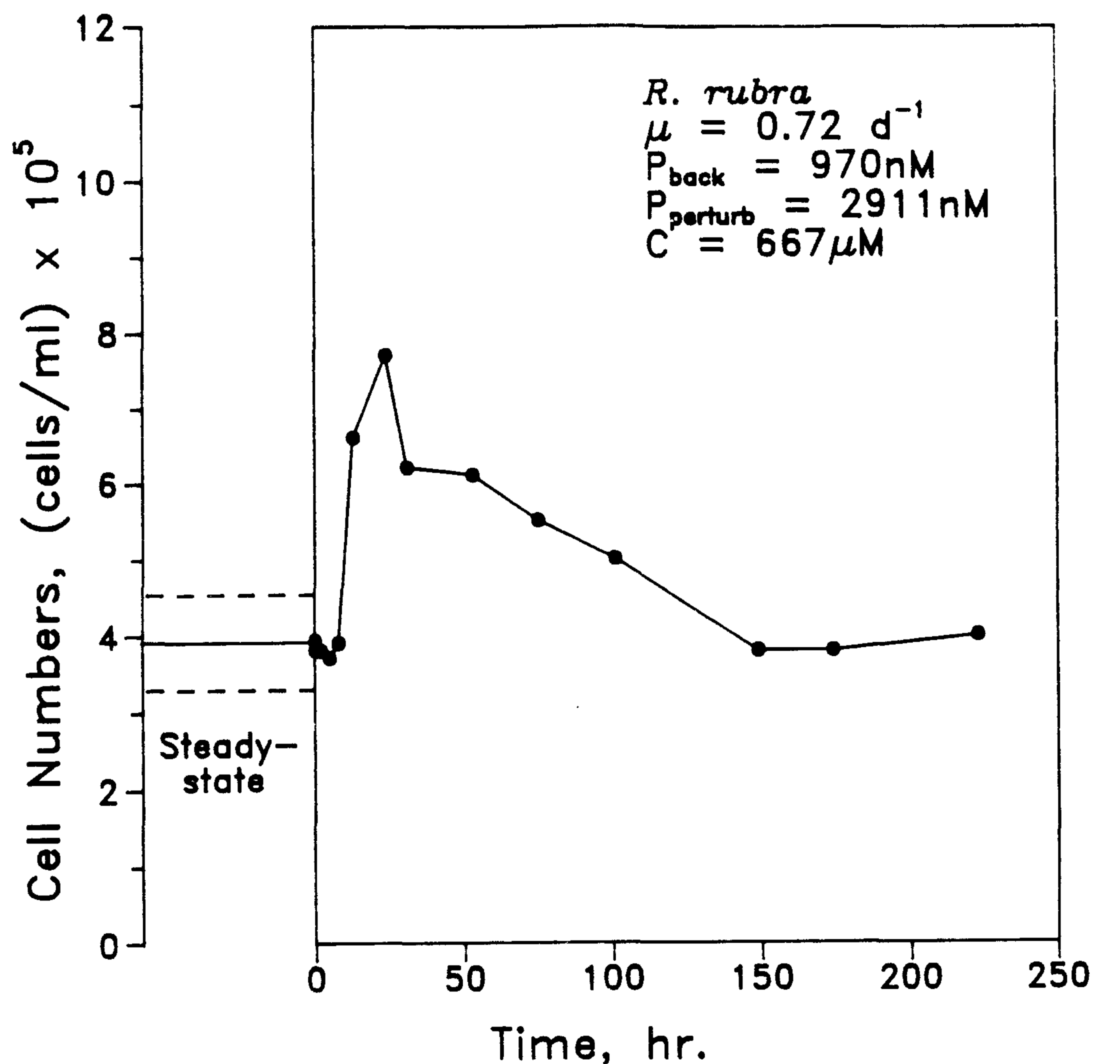

Figure 25. Cell numbers after phosphate perturbation of a phosphorus-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

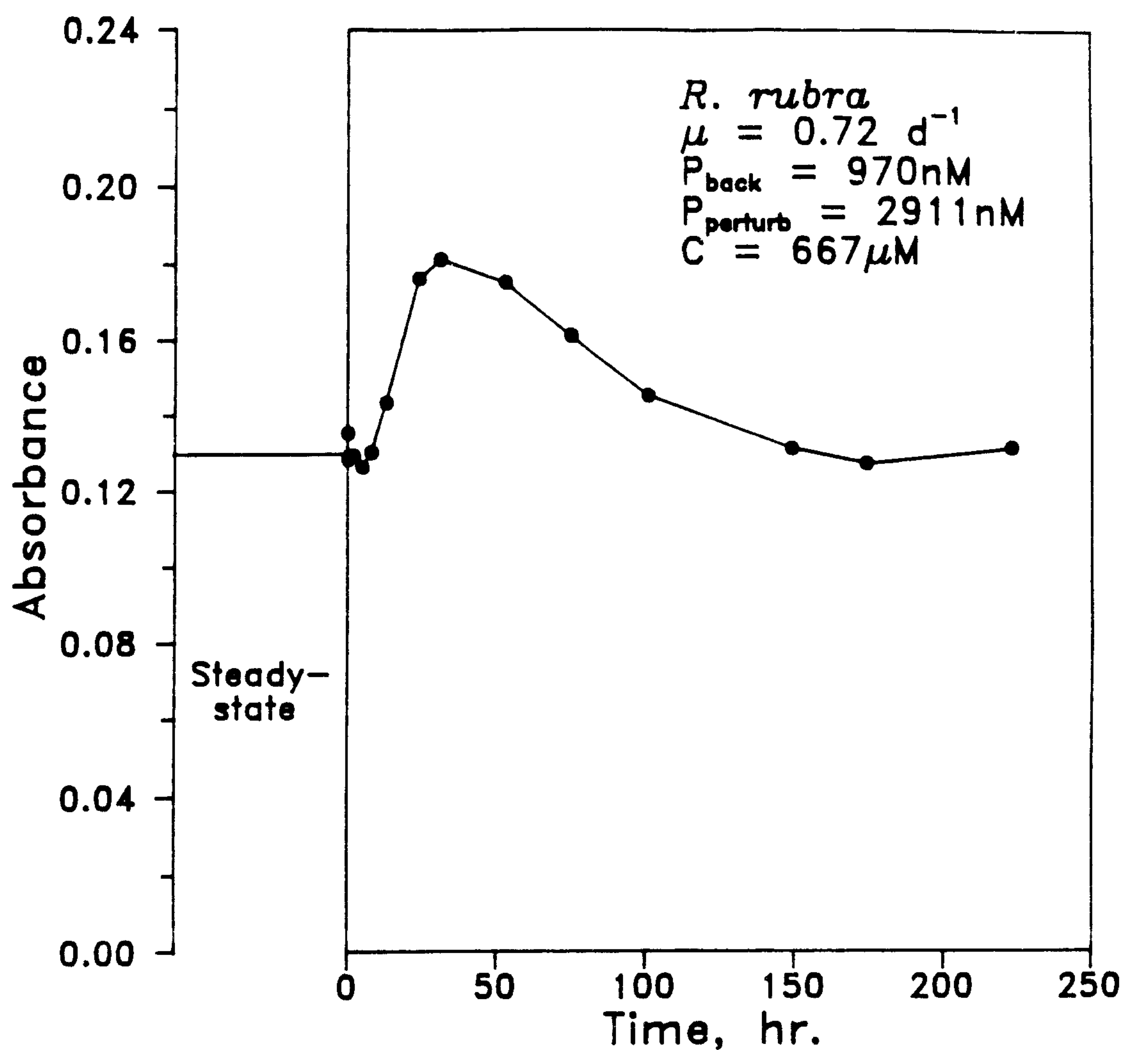

Figure 26. Culture absorbance at 686 nm after a phosphate perturbation of a phosphorus-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

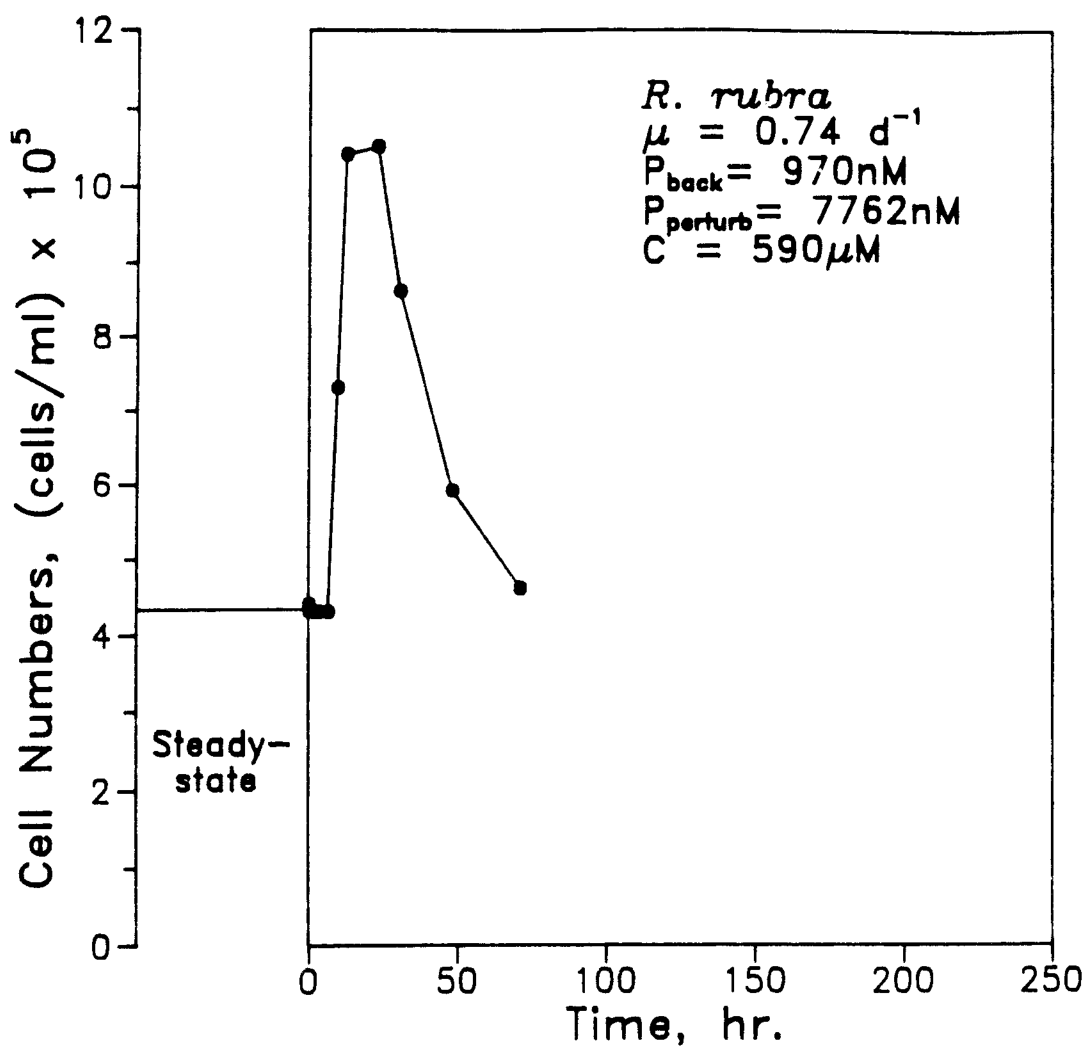

Figure 27. Cell numbers after phosphate perturbation of a phosphorus-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

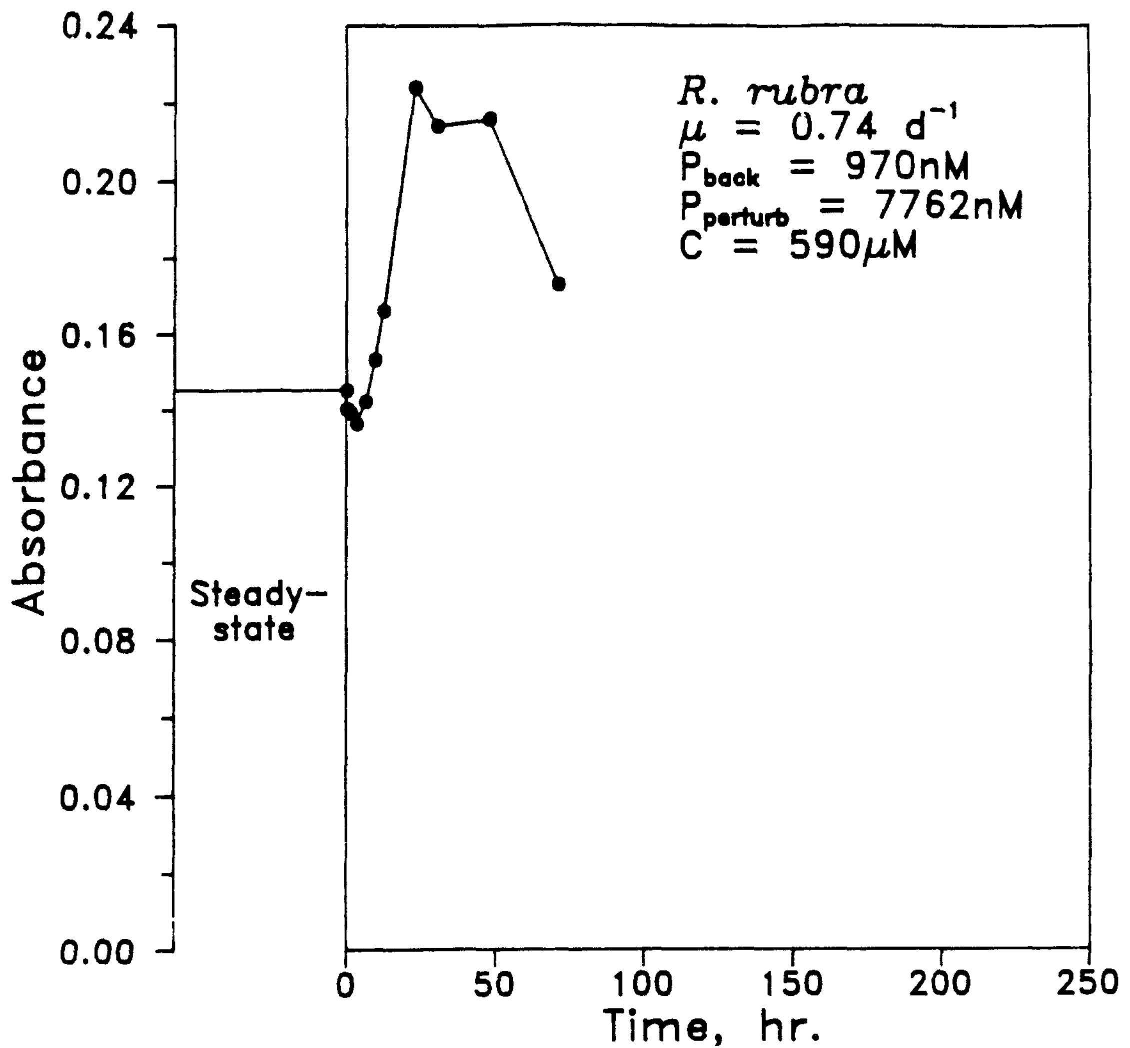

Figure 28. Culture absorbance at 686 nm after a phosphate perturbation of a phosphorus-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

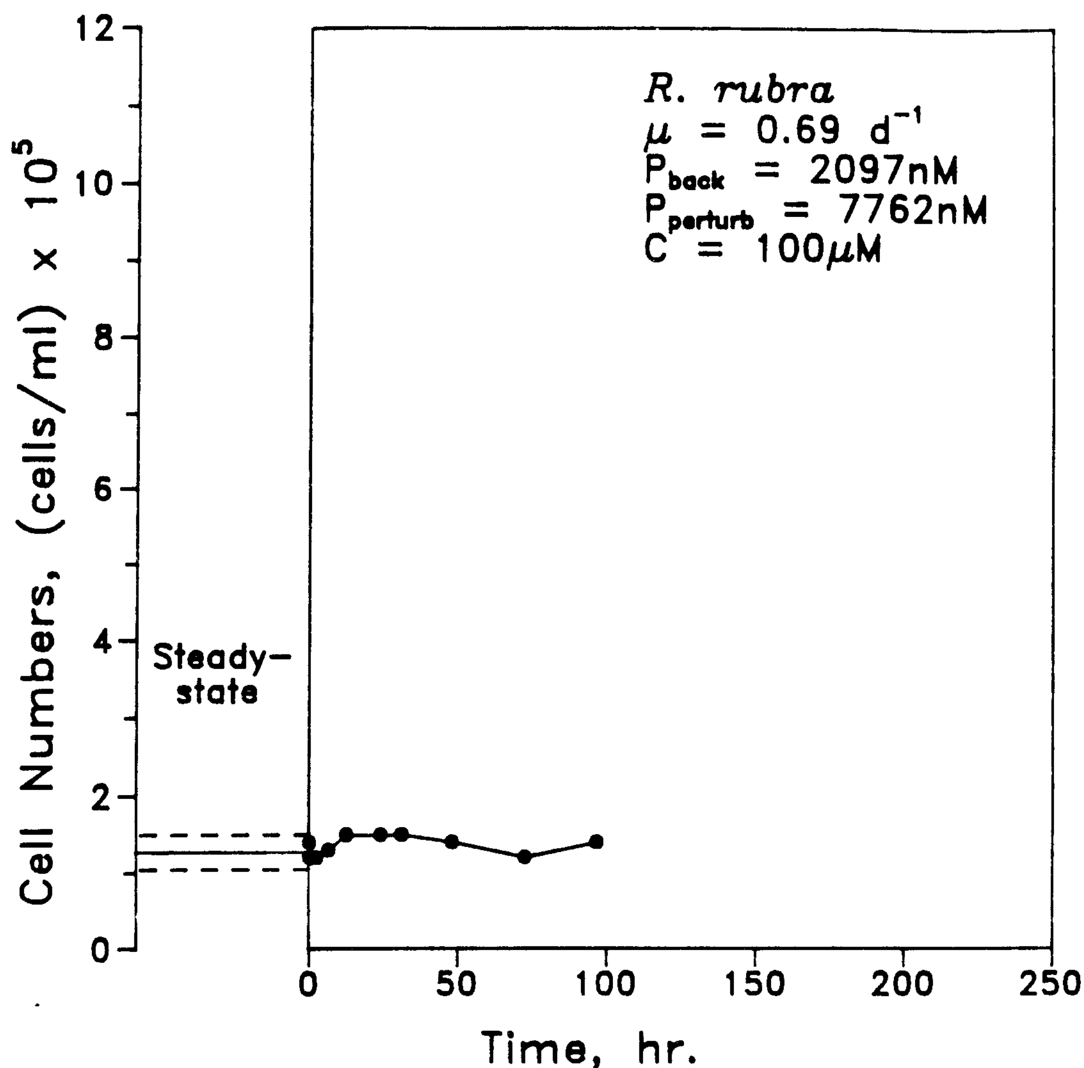

Figure 29. Cell numbers after phosphate perturbation of a carbon-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

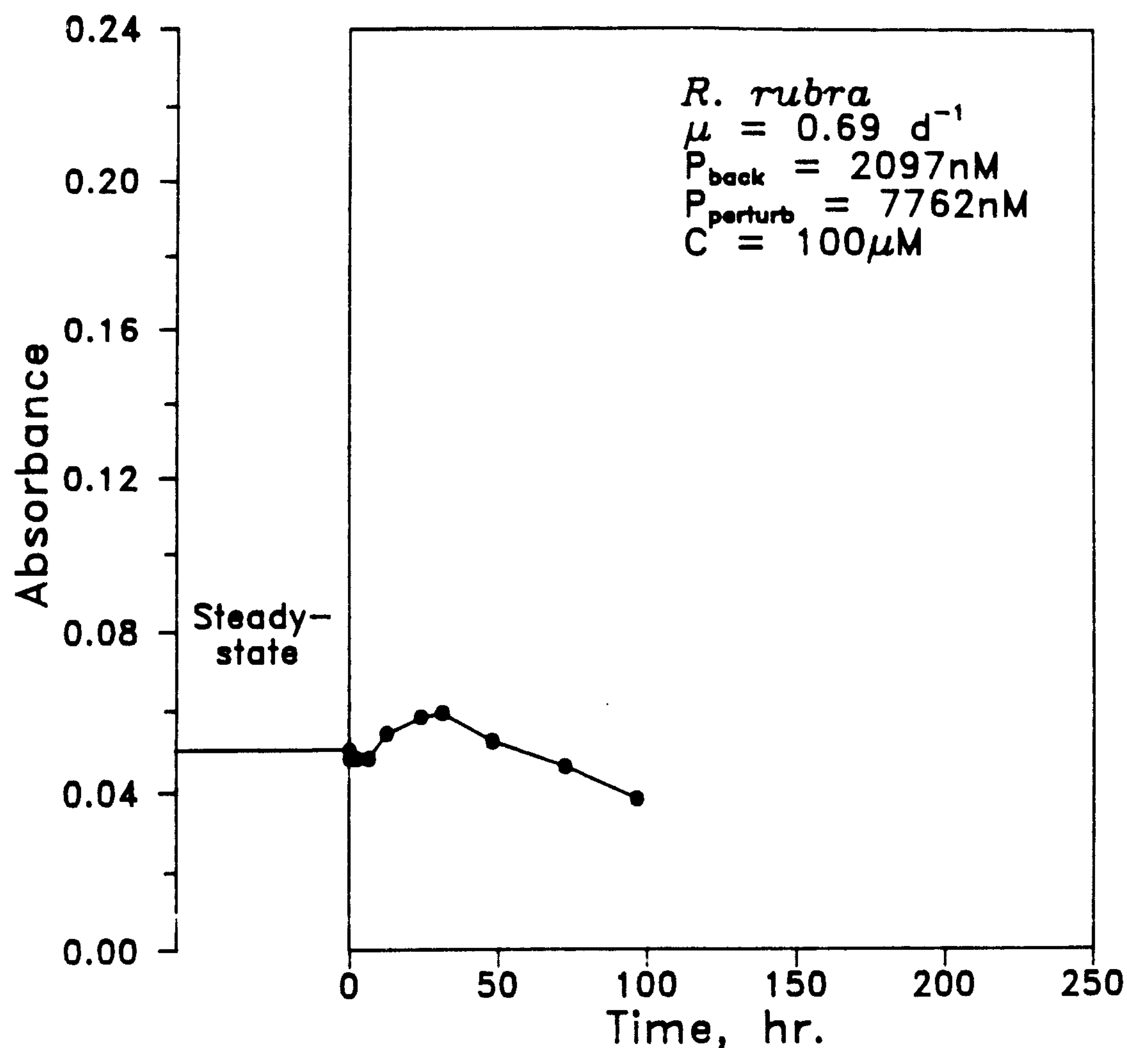

Figure 30. Culture absorbance at 686 nm after a phosphate perturbation of a carbon-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

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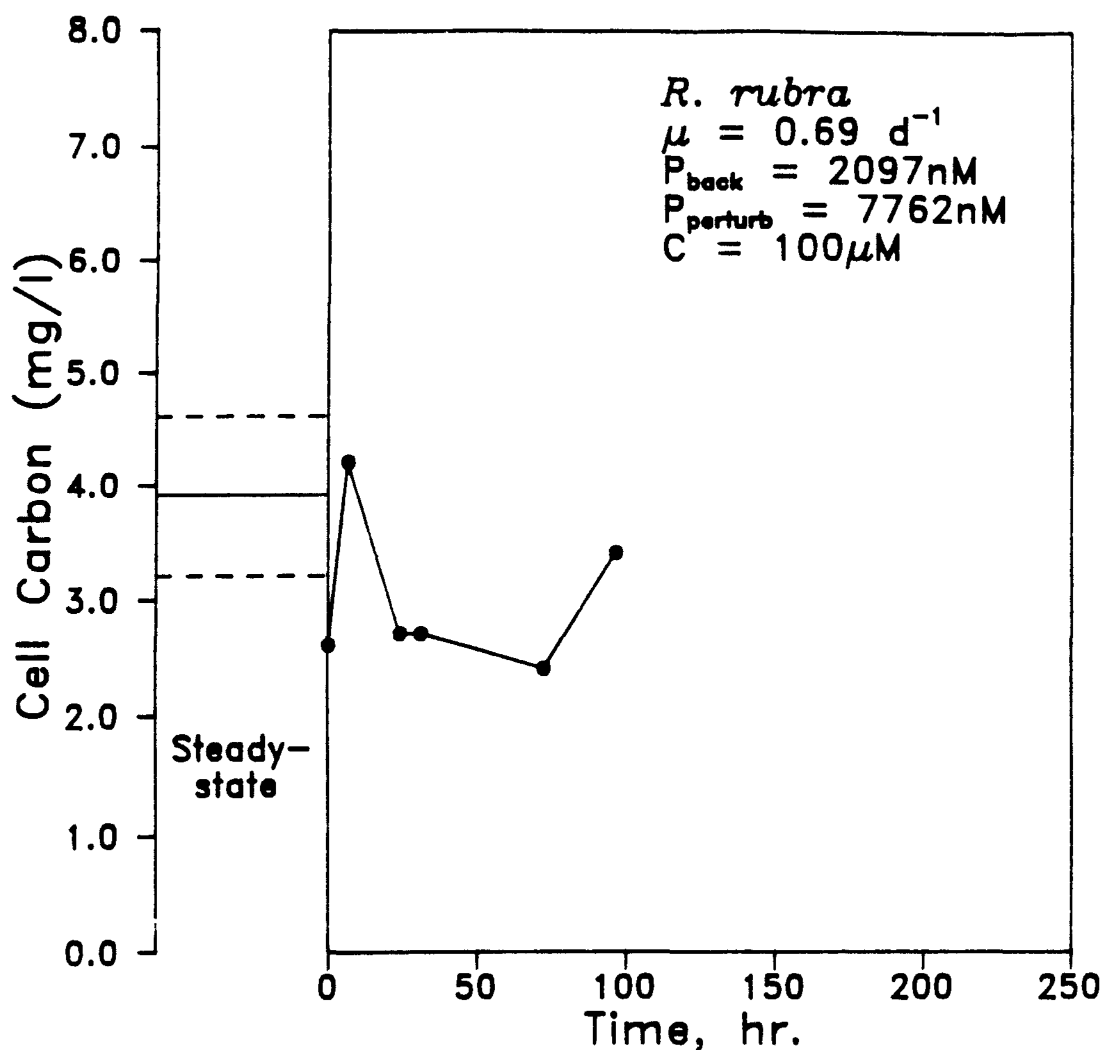

Figure 31. Cellular carbon after phosphate perturbation of a carbon-limited steady-state culture of *R. rubra*. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

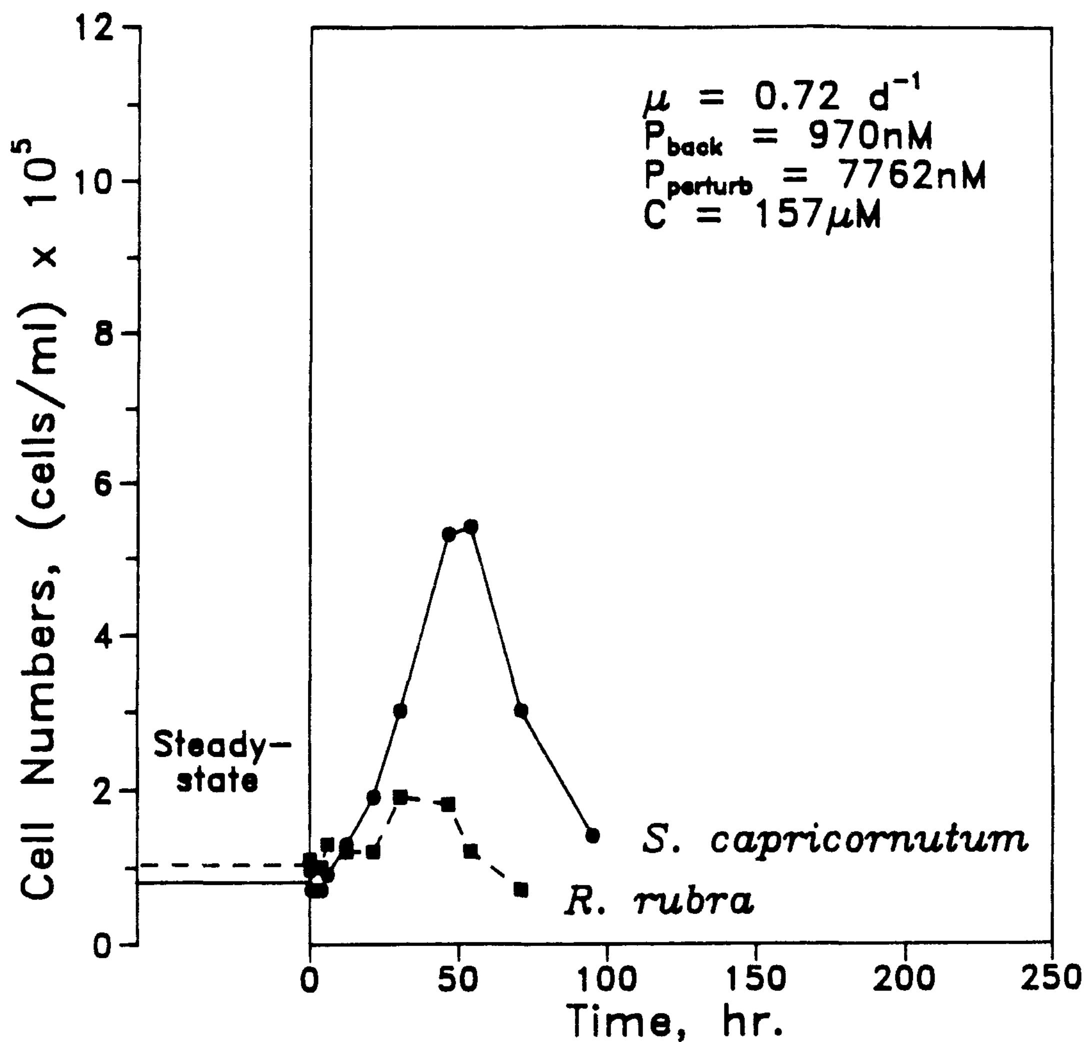

Figure 32. Cell numbers after phosphate perturbation of a phosphorus-limited steady-state dual-species culture. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

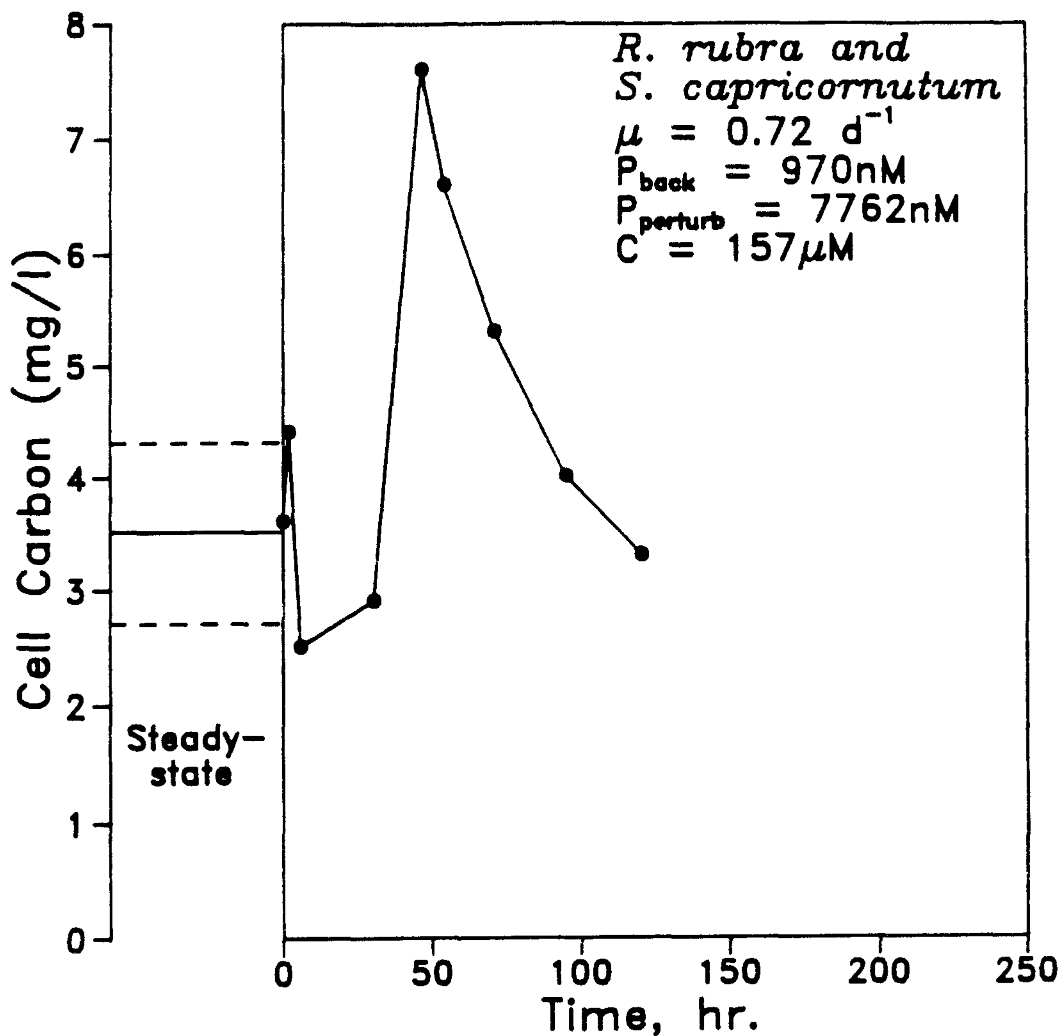

Figure 33. Cellular carbon after phosphate perturbation of a phosphorus-limited steady-state dual-species culture. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

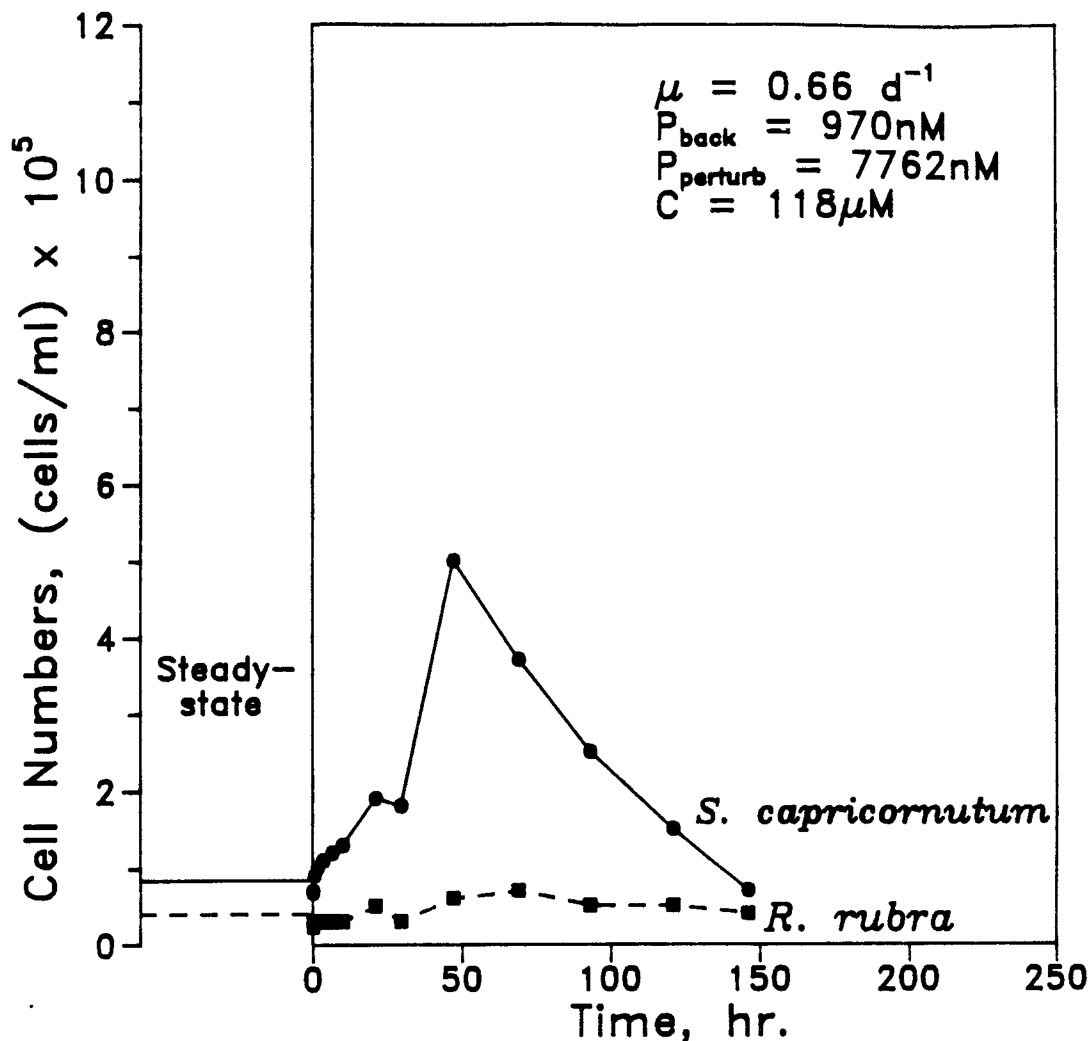

Figure 34. Cell numbers after phosphate perturbation of a phosphorus-limited steady-state dual-species culture. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

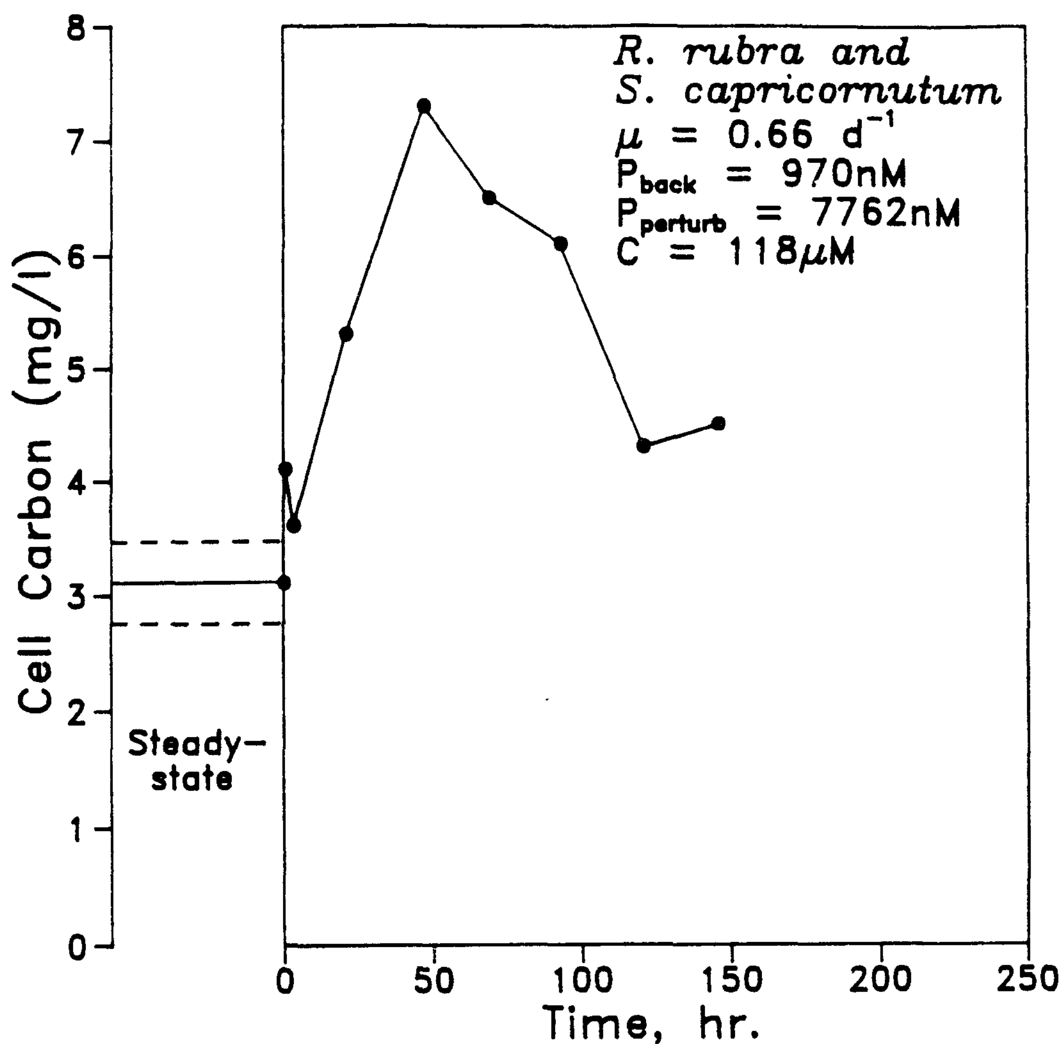

Figure 35. Cellular carbon after phosphate perturbation of a phosphorus-limited steady-state dual-species culture. The mean steady-state value plus or minus one standard error is shown to the left of time zero.

steady-state yeast population in D10 was approximately 1.2×10^8 cells/l and in D11 was approximately 0.4×10^8 cells/l.

Biomass Responses

Biomass was evaluated by measuring the absorbance and cell numbers in all cultures. In addition, cellular carbon was measured for the R3, D10 and D11 cultures. The response in cell numbers with time after perturbation is seen in Figures 19, 21, 23, 25, 27, 29, 32 and 34 for all eight cultures. Absorbance values versus time are shown in Figures 20, 22, 24, 26, 28 and 30. Absorbance values were not plotted for the dual-species cultures because these values would give no information on the proportion of yeast or algae contributing to any increase or decrease measured. The carbon data are found in Figures 31, 33 and 35.

The maximum cell numbers seen in the algal and yeast cultures after perturbation are shown with the maximum internal cellular phosphorus concentrations in Table 4. The value in parentheses in this table is the time after perturbation that the maximum number was observed. For

the *S. capricornutum* cultures the maximum cell numbers were seen between 32 and 58 hours after perturbation. The maximum internal phosphorus concentrations occurred after about six to eight hours in these cultures, indicating a lag of between one and two days between maximum uptake and division in these cultures.

The phosphorus-limited *R. rubra* cultures exhibited maximum biomass about 24 hours after perturbation. The maximum internal cellular phosphorus concentration in these cultures occurred between two and six hours after perturbation. This indicates a lag of less than a day from maximum phosphate uptake to division. In R3, the cell numbers did not change significantly after addition of phosphate. This supports the contention that this culture was carbon-limited rather than phosphorus-limited.

The absorbance values (Figures 20, 22, 24, 26, 28 and 30) yielded information similar to the cell number values about cell biomass in the single-species cultures. Culture absorbance was a very quick and reliable method for obtaining biomass information in these cultures. Absorbance values are not shown for the dual-species cultures.

The cell number data for the dual-species cultures are found in Figures 32 and 34. The primary difference in culture conditions was the addition of more carbon in the medium in culture D10. The C/P ratio for D10 was about 162 and 122 for D11. In both cultures the *S. capricornutum* population responded to the pulse of phosphate. The *R. rubra* population showed some response to pulsed phosphate in the less carbon-limited D10 (Figure 32) culture.

Cellular carbon data are shown for R3, D10 and D11 in Figures 31, 33 and 35. These data show much the same trends as the cell number data. For the R3 (Figure 31) culture, the carbon data imply an average carbon value of 2.5×10^{-8} mg C/ cell. This value is slightly higher than would be expected from dry weight values published for this organism of 0.65×10^{-8} mg dry weight per cell (Robertson and Button, 1979). In the dual species cultures, the carbon cannot be proportioned between the yeast and the algal cells. However, the maximum cellular carbon values occur at the same point after perturbation that is seen for the maximum cell number values.

Summary

The results of eight phosphate perturbations of phosphorus-limited steady-state cultures have been presented. Three single-species steady-state phosphorus-limited algal (*S. capricornutum*) cultures were perturbed with three different concentrations of phosphate. Two single-species steady-state phosphorus-limited yeast cultures (*R. rubra*) were also perturbed with phosphate. The conditions in culture S12 were approximately equal to R1 and the conditions in S13 approximately equal to R2. In addition to these phosphorus-limited cultures, a single-species carbon-limited yeast culture (R3) was perturbed with phosphate. Two dual-species phosphorus-limited steady-state cultures were perturbed with phosphate. These cultures had somewhat different steady-state C/P ratios (162 for D10 and 122 for D11).

In the single species *S. capricornutum* cultures, higher transport rates were seen in the cultures perturbed with increasing concentrations of phosphate. The highest rate observed was 19,908 nmol P/mg cell-day in culture S13, perturbed with 7762 nM phosphate. The maximum algal

internal phosphorus concentration was also seen in this culture. This concentration reached about 1900 nmol P/mg cell at about six hours after perturbation.

In the single species *R. rubra* cultures, the highest average transport rates were seen in the first half hour period after the phosphate perturbation. The transport rates were approximately equal for this time interval in cultures R1 (26,000 nmol P/mg cell) and R2 (23,000 nmol P/mg cell). These two cultures were perturbed with different concentrations of phosphate. Culture R1 was perturbed with 2911 nM phosphate and R2 with 7762 nM phosphate. Culture R3, the carbon-limited culture, had the highest maximum internal phosphorus concentration in the single-species yeast cultures. This culture attained an internal concentration of 3300 nmol P/mg cell at about 48 hours after perturbation.

In the phosphorus-limited dual species cultures, the highest transport rate measured was 12,000 nmol P/mg cell-day. This value was observed in the D10 culture in the first half hour time interval after perturbation. The highest transport rate observed in the D11 culture was about 6,000 nmol P/mg cell-day. For all eight of the continuous cultures, the transport rate data are summarized

in Tables 5, 6 and 7, and the maximum internal phosphorus concentration data in Table 4.

DISCUSSION

Introduction

Phosphorus is often considered to be the nutrient which limits growth of phytoplankton in freshwater systems (Schindler, 1977). If the ability to use phosphorus was the only criterion for success of a species, one would expect that the species most able to use phosphorus when supplied at low concentrations would outcompete the other members of the population. This process would be expected to lead to a very low species diversity-- the "paradox of the plankton" of Hutchinson (1961). A second paradox exists: bacterioplankton appear to greatly outcompete phytoplankton for phosphorus in uptake studies (Rhee, 1972; Faust and Correll, 1976; Currie and Kalff, 1984a,b,c). Bacterioplankton have also been shown to have a much greater phosphorus requirement than phytoplankton (Brown et al., 1981; Vadstein et al., 1988).

It has been suggested and supported by both field (Suttle et al., 1987) and laboratory (Sommer, 1984; Sommer, 1985b; Suttle and Harrison, 1986) studies that

the "paradox of the plankton" may in part be explained by the response of populations to nutrients supplied discontinuously. Measurements made in aquatic environments generally measure only average concentrations of nutrients. However, aquatic microorganisms may live in a microenvironment of heterogeneous rather than homogeneous nutrient supply (Lehman and Scavia, 1982; Scavia et al., 1984; Holligan et al., 1985).

Field studies can be useful in determining phytoplankton community structure under natural or enhanced nutrient conditions. However, because of the complexity of natural systems, little information is gained about the mechanisms that actually control community structure. Laboratory studies, continuous culture studies in particular, can be very useful in pinpointing the potential mechanisms that dictate which organisms will thrive under a given set of conditions. The level of environmental control afforded by continuous cultures is essential in separating out the wide range of factors which affect phytoplankton community structure.

Table 8 includes a summary of steady-state and batch culture data published for the yeast, *R. rubra* (Robertson

Table 8. Steady-State Kinetic Constants for *Rhodotorula rubra* and *Selenastrum capricornutum*.

Parameter	[1]*R. rubra*	[2]*S. capricorn.*
μ_{max} (d^{-1})	5.8	1.2
Q_{maxt} (nmolP/mg cell)	1397	2260
Q_o (nmolP/mg cell)	106	40
a (l/mg cell-day)	30.7	2.8
R (Q_{max}/Q_o)	9.6	56.5
Median Cell Volume (μm^3)	30-80	50-130
Cell Dry Weight (mg/cell)	0.65×10^{-8}	2.5×10^{-8}
$\mu_{max}Q_{max}$ (nmolP/mg cell-day)	8103	2712
S_t (nM)	2.5	10

[1]Robertson and Button (1979), pH 4

[2]Brown and Button (1979)

and Button, 1979), and the green alga, *S. capricornutum* (Brown and Button, 1979), used in these studies. Using these data Brown et al. (1981) tested the hypothesis that the yeast would be a better competitor for phosphorus under phosphorus-limiting conditions in dual-species cultures than the alga. This hypothesis was based on the greater affinity for phosphorus observed in single-species phosphorus-limited steady-state cultures of the two organisms.

Brown et al. (1981) also found that the yeast could outcompete the alga for phosphorus as long as no other nutrients were limiting. Co-existence of the two species only occurred when these researchers limited the supply of carbon to the yeast. A medium C/P ratio of less than 272 was required for co-existence. They also found that carbon-starvation did not affect the yeast's ability to transport phosphorus. Other studies have shown that heterotrophs and cyanobacteria tend to have higher affinities for phosphate than eucaryotic algae (Lang and Brown, 1981). However, most eucaryotic algae appear to have higher Q_{maxt} values than cyanobacteria and yeasts (Brown et al., 1981 and Lang and Brown, 1981). These findings led the authors to suggest that eucaryotic algae

may dominate in environments where nutrient supplies fluctuate. In such environments, storage capacity rather than high affinity might prove to be a more important nutrient utilization strategy.

I hypothesized that the manner in which nutrients are supplied (i.e. constant versus pulsed) might also affect the competition between heterotrophs and autotrophs for limiting nutrients. I sought to determine if steady-state and batch culture data for two well-characterized organisms-- an autotroph (*Selenastrum capricornutum*) and a heterotroph (*Rhodotorula rubra*)--could be used to predict the response of these organisms to perturbed conditions. While whole lake and microcosm studies give results which can lead to predictions of the outcomes of nutrient perturbations, the number of variables involved in these studies makes it difficult to determine the mechanisms by which these outcomes occur. Therefore, to test my hypothesis, I chose to control the number of variables in these studies by using continuous cultures.

Phosphorus Uptake and Internal Phosphorus Concentrations

I found that both single-species steady-state cultures of *S. capricornutum* and *R. rubra* perturbed with phosphate pulses rapidly removed phosphorus from solution (see Figures 5-9). The carbon-limited yeast culture (R3, Figure 10) also was capable of effectively removing phosphate pulses from solution. This finding supported the steady-state data of Brown et al. (1981), that carbon-limitation does not affect the ability of *R. rubra* to transport phosphate. This culture attained very high (9,000 nmolP/mg cell-day) transport rates of phosphate. Notably, this culture attained the highest internal cellular phosphorus concentrations seen in these experiments (3300 nmol P/mg cell; Table 5). Despite these observations, the effect of a severely carbon-limited yeast population on competition for pulsed phosphate supplies would not be expected to be very great. This is attributable to the biomass suppression caused by carbon-limitation. For example, the C-limited culture (R3) attained a steady-state cell number average of approximately 1.5×10^8 cells/l while the steady-state population in the two single species P-limited cultures

was maintained at about 4.0×10^8 cells/l.

The maximum internal phosphorus storage capacity of these and other aquatic microorganisms has been shown previously to vary under different culturing conditions (Brown and Button, 1979; Lang and Brown, 1981). These authors found that cells grown in batch cultures attained higher internal phosphorus concentrations than those grown in continuous culture. Both *S. capricornutum* (Brown and Button, 1979) and *R. rubra* (Button et al., 1973; Robertson and Button, 1979) have been shown to be capable of transporting phosphate at rates many times greater than required for growth at the maximum growth rate. For *R. rubra,* Robertson and Button (1979) estimated that the transport capacity was at least ten times that required to sustain its maximum growth rate. The highest internal cellular phosphorus concentration values seen for *S. capricornutum* were in batch cultures. Lang and Brown (1981) define this maximum value as Q_{maxt}, or the maximum value under transient conditions, to differentiate it from Q_{maxs}, the steady-state value (see Table 8).

I found that for the perturbation concentrations used in these experiments, the Q_{maxt} of 2200 nmol P/mg cell

measured by Brown and Button (1979) for *S. capricornutum* was never exceeded. For *R. rubra* I found that the maximum internal phosphorus concentrations in perturbed cultures exceeded the previous reported value of 1397 nmol P/mg cell calculated by Lang and Brown (1981) from the data of Robertson and Button (1979). If the data from the *R. rubra* culture RA (see Appendix 2) are included here, I have data comprising three perturbation concentrations for phosphorus-limited yeast cultures (Table 9). In addition, I have data from three different perturbation concentrations for *S. capricornutum* (see Table 9). I measured the maximum internal cellular phosphorus for each culture after perturbation and called this Q_{maxP} to distinguish these values from those from steady-state or batch transient cultures. It is apparent from these data that the maximum internal storage capacity for steady-state cells subject to nutrient pulses was not attained for either organism. If the values I obtained for Q_{maxp} for the *S. capricornutum* cultures are plotted versus the total phosphorus concentration, the three values display a linear relationship. There is no indication of saturation. The lack of saturation implies that much higher internal

Table 9. Maximum Intracellular Phosphorus Concentrations for Phosphorus-Limited Steady-State Cultures Perturbed with Phosphate.

Culture # ($P_{perturb}$)	Q_{maxP} (nmol/mg cell)	Perturbation Conc. (nM)
S9 (1087 nM)	411	1087
S12 (2911 nM)	851	2911
S13 (7762 nM)	1921	7762
RA (855 nM)	632	855
R1 (2911 nM)	1450	2911
R2 (7762 nM)	2200	7762
*R3 (7762 nM)	3279	7762

* Carbon-limited

concentrations of phosphorus might be obtained if greater perturbation values were used. If a similar plot is made for the phosphorus-limited *R. rubra* cultures, there is an indication of possible saturation. However, additional data would be required at different perturbation levels to determine the true saturated maximum internal phosphorus concentration for either organism. The perturbation values selected in these experiments reflect values that would be realistic or slightly higher than expected for natural aquatic systems where elevated nutrient concentrations might exist from inputs such as zooplankton excretions or mixing events. While the determination of the saturation values for these two organisms might be physiologically interesting, the saturation values for these two organisms appear to be much larger than the values that would normally be expected to occur in natural environments.

The ratio of the maximum internal substrate concentration (Q_{max}) to the minimum internal substrate concentration (Q_o) has been defined by Droop (1974, 1975) and suggested by several authors (Tilman and Kilham, 1976; Lang and Brown, 1981) to be a good indicator of the nutrient storage capacity of microorganisms under

transient conditions. Since the observed values for Q_{max} seem to vary for the same organism grown under different conditions (Lang and Brown, 1981; this study), caution should be applied to the use of these ratios.

The product of the maximum growth rate times the maximum internal nutrient concentration has been used as an estimate of the maximum transport capacity of a microorganism (Droop, 1974). From previous steady-state and batch culture data, these values were calculated to be 2712 nmol P/mg cell-day for *S. capricornutum* (Brown and Button, 1979) and 8103 nmol P/mg cell-day for *R. rubra* (Lang and Brown, 1981 from data of Robertson and Button, 1979). Transport rates in nearly all the perturbed culture experiments in this study exceeded those predicted from steady-state and batch culture data (Table 10). The maximum transport rate ($\mu_{max}Q_{max}$) estimate is dependent on the internal phosphorus storage capacity of the cell. In the perturbations done in this study, neither *S. capricornutum* or *R. rubra* appeared to be saturated with respect to phosphorus storage capacity. Therefore, even the very high transport rates observed for the organisms in this study are probably not maximal rates for either organism. Several transport mechanisms

Table 10. Highest Observed Transport Rates for Phosphorus-Limited Steady-State Cultures Perturbed with Phosphate.

Culture # ($P_{perturb}$)	Transport Rate (nmol P/mg cell-day)	Time Interval (hr.)
S9 (1087 nM)	925	0.0-6.0
S12 (2911 nM)	8,032	0.0-0.5
S13 (7762 nM)	19,908	0.0-0.5
RA (855 nM)	2,973	0.0-3.0
R1 (2911 nM)	25,809	0.0-0.5
R2 (7762 nM)	23,177	0.0-0.5
R3 (7762 nM)	8,954	2.5-6.5
D10 (7762 nM)	12,041	0.0-0.5
D11 (7762 nM)	8,267	0.5-1.5

for *S. capricornutum*, $\mu_{max} Q_{maxt}$ = 2712 nmol P/mg cell-day (Brown and Button, 1979)

for *R. rubra*, $\mu_{max} Q_{max}$ = 8103 nmol P/mg cell-day (Lang and Brown, 1981 from data of Robertson and Button, 1979)

are known to occur in other microorganisms for phosphate transport (Boos, 1987). The relative importance of different transport systems is dependent on genetic regulation, phosphate concentration, internal pH and surface potential (Borst-Pauwels and Peters, 1987).

In the yeast, *Saccharomyces cerevisiae*, three different phosphate transport pathways have been identified. These include a low affinity transport system, a high affinity system and a sodium ion dependent phosphate co-transport system. The high affinity system and the sodium-dependent system both become derepressed when cells are incubated in the absence of phosphorus (Borst-Pauwels and Peters, 1987). The high affinity is a proton-phosphate co-transfer system and has been shown to be highly dependent on the pH of the medium. Uptake via this system is favored at low pH. The sodium-phosphate co-transfer system appears to contribute more significantly to phosphate uptake when the pH of the medium is relatively high. However, Borst-Pauwels and Peters (1987) have shown that all three systems can occur simultaneously. Other microorganisms also have low and high affinity phosphate transport systems (Boos, 1987). The true maximum transport capacity of a microorganism

might then be expected to occur when the maximum number of transport systems are derepressed. Therefore, phosphorus-limited steady-state populations which are perturbed with pulses of phosphorus might be expected to display maximum capacity for phosphate uptake.

I hypothesized that the alga would have a greater capacity for nutrient storage than the yeast under perturbation conditions but this was not found. It is possible that the alga would have greater storage capabilities if the perturbation values were greater. The ecological significance for algae would not be great under most circumstances, however, since perturbation values in natural environments would rarely be expected to exceed the values used in these experiments.

Cell Size and Biomass

With respect to the competitive ability of microorganisms to take up nutrients at low concentrations, there has been a debate as to the importance of the size of microorganisms. Some authors argue that size is critical in strategy for uptake of nutrients at low concentrations (Smith and Kalff, 1982;

1983;). These authors argue that these cells benefit from a favorable surface to volume ratio. Other authors argue that the competitive advantage occurs across taxonomic groups and is unrelated to cell size (Sommer and Kilham, 1985; Sommer, 1985b).

There is one other aspect of the cell size question which relates to transient rather than steady-state environments. Turpin and Harrison (1980) and Turpin et al. (1981) noted that the mean cell diameter was variable when natural phytoplankton assemblages were grown in cultures supplied with different frequency additions of nutrients. They found that the mean cell diameter increased as the frequency of pulse additions decreased. They concluded that large cells may be favored in environments where nutrients are supplied in pulses--even though the average ambient concentration remains the same. Other studies (Suttle et al., 1987) found that nutrient pulses may account for changes in both mean cell size and community structure of phytoplankton populations. Suttle et al. (1987) found that the dominant algal species was different in semi-continuous cultures when phosphorus was supplied in different addition frequencies. The average cell volume also

increased as the frequency of addition decreased. Other studies have found that nutrient pulses allow the co-existence of species competing for the same nutrient in a phytoplankton assemblage (Sommer, 1984; 1985b).

In this study the two organisms were selected at least in part because they have relatively similar mean volume ranges (see Table 8). This minimized the potential competitive advantage that might be provided by size considerations alone.

Generalizations about nutrient uptake ability based on cell size alone are probably too simplistic. In fact, cell size is not necessarily a constant for a given organism. For both of the organisms used in this study, cell size has been shown to vary with growth rate under phosphorus-limited growth (Brown et al., 1981). Other differences may be more important in determining the competitive ability of a group of organisms to compete under nutrient-limited conditions. These differences might be more likely to be found between heterotrophs versus autotrophs or eucaryotes versus procaryotes.

Availability of Carbon

In the early 1970s several authors observed that heterotrophs were good competitors for phosphorus (Rhee, 1972; Button et al., 1973). These authors further suggested that carbon must limit heterotrophic growth in natural aquatic systems. This hypothesis is supported by the results of one field study. Schindler (1975) reported the results of lake fertilization studies. One lake was fertilized with sucrose and nitrogen, which caused a decrease in the phytoplankton population compared to the lake in which phosphorus was added. In addition the C/P ratio in the lake declined after the addition of carbon.

The results of this study support the hypothesis that many natural aquatic systems must be carbon-limited. Unexpectedly, the yeast had the capacity to outcompete the alga for phosphorus not only under steady-state conditions as was hypothesized but also when phosphorus was supplied in a pulse. Both the transport and storage capacity exceeded those values predicted from steady-state data (Tables 8, 9 and 10). There may be other factors relating to nutrients which contribute to

competition in natural systems. For example, there is some evidence that phytoplankton may be more dependent on organic phosphorus than orthophosphate (Currie, 1986). Heterotrophs may also leak phosphorus at a greater rate than algae.

Under steady-state conditions Brown and Button (1979) found that *S. capricornutum* leaked some phosphorus. Leakage was related to the dilution rate but never exceeded 3% of the internal phosphorus concentration. For *R. rubra* grown in steady-state continuous cultures, the total phosphorus leakage ranged from 10% of the transported phosphorus at low growth rates to 4% at high growth rates (Robertson and Button, 1979). Other heterotrophs may have much greater leakage rates, however, in aerobic environments, those organisms that fix carbon (autotrophs) are more likely to leak metabolites when growth conditions become unbalanced.

Other Population-Controlling Factors

In this study steady-state populations were perturbed with pulses of phosphorus. The cultures were monitored until steady-state was re-established. One general

observation in the dual species cultures was that, for the pulse concentration used in these experiments (7762 nM), the cultures both returned to steady-state with both organisms still in the assemblage (Figures 32 and 34). This co-existence might be altered by pulses provided in series. These types of studies have been done in semi-continuous cultures (Suttle and Harrison, 1986; Suttle et al., 1987) and in continuous cultures (Sommer, 1985b) on natural phytoplankton assemblages. Other factors which have not yet been identified may be important in determining the composition of species that co-exist. I observed that the alga, *S. capricornutum,* could never outcompete the yeast, *R. rubra*, unless the yeast was carbon-limited. In semi-continuous culture experiments at C/P ratios of 100, I was unable to keep the yeast population from washing out at three different "dilution rates" ranging from 0.33 to 0.75 d^{-1}. A similar observation was made by Lang and Brown (1981). They studied the steady-state and batch transient growth kinetics of the cyanobacteria, *Synechococcus* Nageli, and the green alga, *Scenedesmus quadricauda*. They found that the cyanobacteria had both a higher affinity and a greater storage capacity for phosphorus. As a result,

Synechococcus always excluded *S. quadricauda* in phosphorus-limited continuous cultures. However, in dual-species batch cultures *Synechococcus* did not predominate, illustrating the mechanistic complexities that determine the response of an organism to a changing environment. From these data, Lang and Brown (1981) argued that *Synechococcus* Nageli exhibits adaptive traits which would enhance its competitive abilities in oligotrophic environments. In fact, there is evidence that small unicellular autotrophic organisms may comprise a large portion of the microbial biomass in oligotrophic waters. These organisms include cyanobacteria and may also include the newly described chlorophyll *b*-containing procaryote, prochlorophyte (Chisholm et al., 1988).

Summary

This study examined the responses of two aquatic microorganisms when phosphorus-limited steady-state growth in continuous cultures was perturbed by pulses of phosphorus. The kinetic results of this study point out the need for a better understanding of the biochemical processes involved in transport before interpretations of

observations of species successions in aquatic environments will prove useful. For example, I found that the responses of these two microorganisms to pulsed phosphorus were different than predicted by both steady-state and batch culture experiments. In addition, these kinetic studies provide good justification for the importance of studying phosphate transport mechanisms in microorganisms.

Both organisms were able to transport phosphate at rates that exceeded previous measurements and store large amounts of phosphorus. The internal cellular phosphorus concentrations for *S. capricornutum* in these experiments attained values similar to those seen in batch-starved algal cultures (Brown et al., 1978). The yeast, *R. rubra*, attained internal cellular phosphorus concentrations two to three times greater than previously reported (Robertson and Button, 1979).

The competitive responses that the two organisms in these studies exhibited are representative of the types of interactions that do occur in natural systems. The yeast, *R. rubra*, displayed some of the same characteristics also observed in phosphorus-limited growth of heterotrophic bacteria including a very high

affinity for phosphorus while the green alga, *S. capricornutum,* displayed growth characteristics consistent with other eucaryotic algae. The two organisms I studied are within the same size range, which minimizes a transport advantage arising from one organism having a more favorable surface to volume ratio due to size. Thus aquatic heterotrophs, whether eucaryotic or procaryotic, may be favored in phosphorus-limited environments, with the possible exception of procaryotic autotrophs (cyanobacteria).

The results of this study show the kinds of interactions that can occur in environments subject to oscillating nutrient supplies. It is apparent that transient uptake reflects a different metabolic response than that seen in steady-state growth. In these heterogeneous environments, biomass and community structure may be controlled by the type and amount of nutrients supplied to the microbial population. In addition, the phytoplankton community may be indirectly controlled by the supply of carbon to the heterotrophic population.

APPENDIX 1

DATA FROM *SELENASTRUM CAPRICORNUTUM* CONTINUOUS CULTURES

Culture #S1 (pH=7.5, P=964 nM)

Absorbance measured with 5 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
04/14/86		0.40	30	3.1	--	--
04/14/86		0.38	57	5.9	4.5±2.3	--
04/18/86		0.39	87	9.0	--	--
04/19/86		0.39	92	9.6	--	--

Culture #S1b (pH=7.5, P=964 nM)

Absorbance measured with 5 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
05/02/86		0.37	37
05/07/86		0.37	38
05/12/86		0.37	34
05/14/86		0.37	38
05/16/86		0.37	36
05/20/86		0.37	37
05/21/86		0.37	35
- PERTURBED WITH 964 nM P -			
05/21/86	0.0	0.37	60
05/21/86	2.5	0.37	89
05/21/86	5.5	0.37	85
05/21/86	11.0	0.37	69
05/21/86	15.5	0.37	94
05/22/86	24.0	0.37	71
05/23/86	27.0	0.37	64

$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
3.8	--	--
4.0	--	--
3.5	2.8±3.1	--
4.0	--	0.271
3.7	4.0±0.5	0.259
3.8	2.9±1.0	0.289
3.6	3.3±2.0	0.278
3.1	3.4±1.8	0.276
4.6	--	--
4.4	3.5±2.4	0.300
3.6	3.6±2.7	0.299
4.9	3.1±2.4	0.308
3.7	3.0±2.9	0.334
3.3	--	0.376

Culture #S2 (pH=7.5, P=964 nM)

Absorbance measured with 5 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
04/14/86		0.70	51
04/17/86		0.76	89
04/18/86		0.79	97
04/19/86		0.79	91

$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
5.3	--	--
9.2	5.4±2.7	--
10.1	--	--
9.4	--	--

Culture #S2b (pH=7.4, P=964 nM)

Absorbance measured with 5 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
05/02/86		0.74	37
05/07/86		0.74	44
05/12/86		0.73	49
05/14/86		0.73	57
05/16/86		0.73	48
05/20/86		0.73	44
05/21/86		0.73	53
- PERTURBED WITH 964 nM P -			
05/21/86	0.0	0.73	71
05/21/86	2.5	0.73	104
05/21/86	5.5	0.73	105
05/21/86	11.0	0.73	101
05/22/86	15.5	0.73	74
05/22/86	24.0	0.73	62
05/23/86	27.0	0.73	104

$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
3.8	--	--
4.6	--	--
5.1	2.3±4.2	--
5.9	--	0.240
5.0	3.0±1.2	0.249
4.6	2.1±1.0	0.230
5.5	2.4±1.2	0.221
3.7	2.0±1.5	0.264
5.5	--	--
5.7	3.9±1.8	0.311
5.7	3.3±3.2	0.272
4.3	3.3±3.1	0.303
3.8	5.1±5.8	0.341
6.5	--	0.279

Culture #S6 (pH=7.4, P=964 nM)

Absorbance measured with 5 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
07/29/86		0.92	52
08/05/86		0.92	49
08/07/86		0.74	45
08/11/86		0.72	52
08/13/86		0.72	46
08/19/86		0.72	53

%P$_{6OL}$	#CELLS/ml (mean$\pm$SD$\times 10^5$)	ABSORB (686 nm)
5.4	--	--
5.1	--	0.286
4.7	--	0.268
5.4	--	0.223
4.8	--	0.235
5.5	--	0.238

Culture #S7 (pH=7.5, P=964 nM)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
09/15/86		0.48	70
09/18/86		0.48	85
09/19/86		0.48	77
09/26/86		0.47	55
09/29/86		0.47	61
10/01/86		0.47	61
10/07/86		0.47	47
10/08/86		0.45	53

%P_{SOL}	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
7.3	--	--
8.8	--	--
8.0	--	--
5.7	--	--
6.3	--	--
6.3	--	0.147
4.9	--	0.157
5.5	--	0.160

Culture #S9 (pH=7.7, P=964 nM)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$
10/28/86		0.52	50	5.2
10/30/86		0.52	44	4.6
11/14/86		0.52	51	5.3
11/15/86		0.52	45	4.7
- PERTURBED WITH 1087nM P -				
11/15/86	0.0	0.52	1112	54.2
11/15/86	6.0	0.52	71	3.7
11/16/86	21.5	0.52	29	1.9
11/16/86	25.3	0.52	31	2.1
11/17/86	43.3	0.52	37	2.9
11/18/86	67.5	0.52	41	3.7
11/19/86	91.5	0.52	39	3.7
11/20/86	115.5	0.50	48	4.8
11/21/86	140.0	0.50	53	5.4
11/22/86	165.0	0.50	48	4.9
12/11/86	--	0.23	91	9.4
12/19/86	--	0.23	47	4.8
12/24/86	--	0.22	51	5.3

#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
1.9±1.7	--
1.9±1.3	--
2.3±1.1	0.196
2.6±1.4	0.187
1.8±1.9	0.192
--	--
4.8±3.2	0.266
--	0.283
4.9±2.7	0.280
3.2±1.1	0.248
2.5±1.4	0.230
2.3±1.1	0.216
2.2±1.2	0.227
2.0±1.1	0.188
2.2±1.2	0.153
2.9±0.8	0.161
2.5±1.4	0.177

Culture #S10 (pH=7.5, P=964 nM)

Absorbance measured with 4 cm cells

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
01/20/87		0.28	68
01/23/87		0.28	35
01/28/87		0.28	60

%P_{SOL}	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
7.1	--	0.146
3.6	2.1±1.2	0.133
6.2	1.5±0.8	0.166

Culture #S12 (pH=7.2, P=970 nM)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
08/25/87		0.50	55
08/28/87		0.63	59
08/30/87		0.73	53
09/01/87		0.73	57
09/03/87		0.73	48
09/05/87		0.73	43
- PERTURBED WITH 2911 nM P -			
09/05/87	0.0	0.73	3322
09/05/87	0.5	0.73	2569
09/05/87	2.0	0.73	1163
09/05/87	4.0	0.73	388
09/05/87	8.5	0.73	55
09/06/87	19.5	0.73	29
09/06/87	32.5	0.73	29
09/07/87	44.5	0.73	30
09/07/87	55.0	0.73	34
09/08/87	70.0	0.73	34
09/09/87	81.5	0.73	42
09/10/87	115.5	0.73	56
09/11/87	142.0	0.73	54
09/12/87	164.5	0.71	49

$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
5.7	2.5±1.1	0.188
6.1	--	--
5.5	--	--
5.9	2.0±1.5	0.178
4.9	1.5±0.7	0.164
4.4	1.8±1.0	0.180
85.6	1.8±0.6	0.175
66.9	--	0.175
31.3	1.5±0.8	0.177
10.9	--	0.179
1.7	--	0.183
1.1	3.1±2.0	0.226
1.4	6.6±4.9	0.323
1.7	3.2±1.6	0.318
2.2	4.4±1.3	0.297
2.5	2.8±1.0	0.271
3.4	2.1±1.0	0.222
5.2	1.5±0.4	0.172
5.3	--	0.181
4.9	1.5±0.5	0.172

Culture #S13 (pH=7.2, P=970 nM)

Absorbance measured with 4 cm cells

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
01/04/88		0.74	65
01/08/88		0.74	74
01/12/88		0.74	65
01/13/88		0.74	65
- PERTURBED WITH 7762 nM P -			
01/13/88	0.0	0.74	7946
01/13/88	0.5	0.74	6287
01/13/88	1.5	0.74	4441
01/13/88	2.5	0.74	3180
01/13/88	4.5	0.74	1312
01/13/88	6.5	0.74	592
01/13/88	13.0	0.74	123
01/14/88	22.5	0.74	53
01/14/88	30.0	0.74	48
01/15/88	58.0	0.74	38

$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
6.7	--	--
7.6	1.6±1.3	0.164
6.7	1.6±1.6	0.171
6.7	1.6±1.0	0.177
91.0	--	0.165
73.0	--	0.165
53.0	--	0.171
39.0	--	0.174
17.0	--	0.183
8.1	1.4±1.0	0.191
2.0	1.8±1.1	0.221
1.1	4.7±3.7	0.375
1.2	5.2±3.4	0.405
1.7	5.3±3.5	0.413

APPENDIX 2

DATA FROM *RHODOTORULA RUBRA* CONTINUOUS CULTURES

Culture #A(D2) (pH=7.5, P=964 nM, C=38.6 μM, C/P=40)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
01/16/87		0.53	40	4.2	3.6±2.3	0.094
01/20/87		0.49	34	3.5	4.3±1.5	0.104
01/23/87		0.49	31	3.2	2.7±2.0	--
01/28/87		0.49	27	2.8	4.0±2.3	0.104
02/02/87		0.49	33	3.4	4.6±2.5	0.103
- PERTURBED WITH 855 nM P -						
02/02/87	0.0	0.49	1093	60.1	--	--
02/02/87	3.0	0.49	175	9.9	--	0.115
02/03/87	17.0	0.49	35	2.2	--	0.106
02/04/87	43.0	0.49	38	2.9	6.4±5.1	0.112
02/06/87	93.0	0.51	44	4.0	--	0.119
02/09/87	165.0	0.51	25	2.5	2.4±1.1	0.112

Culture #1(R1) (pH=7.8, P=970 nM, C=

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
10/27/87		0.72	22
10/29/87		0.72	20
11/02/87		0.72	18
11/04/87		0.70	18
11/10/87		0.70	22
- PERTURBED WITH 2911 nM P -			
11/10/87	0.0	0.70	2934
11/10/87	0.5	0.70	1536
11/10/87	2.0	0.70	134
11/10/87	5.0	0.70	28
11/10/87	8.0	0.70	23
11/10/87	13.0	0.70	41
11/11/87	24.0	0.70	22
11/11/87	31.0	0.70	37
11/12/87	53.0	0.70	46
11/13/87	75.0	0.70	38
11/14/87	101.0	0.70	35
11/16/87	149.0	0.70	37
11/17/87	174.0	0.70	37
11/19/87	223.0	0.70	42

=667 µM, C/P=687)

$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
2.3	4.8±3.0	0.148
2.1	4.5±2.0	0.140
1.9	4.1±1.4	0.138
1.9	3.5±1.4	0.145
2.3	2.9±1.9	0.135
75.6	--	0.128
40.0	--	0.129
3.6	--	0.129
0.8	--	0.126
0.7	--	0.130
1.4	6.7±4.1	0.143
0.9	7.7±3.3	0.176
1.7	6.2±2.5	0.181
2.9	6.1±2.3	0.175
2.9	5.5±1.9	0.161
3.1	5.0±2.3	0.145
3.7	3.8±2.7	0.131
3.8	--	0.127
4.4	4.0±1.9	0.131

Culture #R2 (pH=7.2, P=970 nM, C=590 μM, C/P=608)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)
12/14/87		0.74	28	2.9	--	0.150
12/15/87		0.74	25	2.6	--	0.148
12/17/87		0.74	33	3.4	--	0.148
12/21/87		0.74	31	3.2	4.2±2.4	--
- PERTURBED WITH 7762 nM P -						
12/21/87	0.0	0.74	6723	77.0	4.4±1.5	0.140
12/21/87	0.5	0.74	5342	62.0	--	--
12/21/87	1.5	0.74	3776	45.0	--	0.139
12/21/87	3.5	0.74	2586	32.5	--	0.136
12/21/87	6.5	0.74	1206	16.4	--	0.142
12/21/87	9.5	0.74	504	7.4	7.3±3.4	0.153
12/21/87	12.5	0.74	132	2.1	10.4±4.8	0.166
12/22/87	23.0	0.74	83	1.7	10.5±4.8	0.224
12/22/87	30.5	0.74	77	1.9	8.6±4.5	0.214
12/23/87	48.0	0.74	50	1.8	5.9±4.9	0.216
12/24/87	71.0	0.74	38	2.0	4.6±2.4	0.173

Culture #R3 (pH=7.3, P=2097 nM, C=100 μM, C/P=48)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5)	ABSORB (686 nm)	CELL CARBON (mg/l)
06/10/88		0.69	671	32.0	1.4±0.6	0.055	4.4
06/15/88		0.69	923	44.0	1.0±0.9	0.048	3.4
06/16/88		0.69	923	44.0	1.5±0.8	0.050	-
- PERTURBED WITH 7762 nM P -							
06/16/88	0.0	0.69	9169	93.0	1.2±0.7	0.048	2.6
06/16/88	0.5	0.69	9156	94.0	--	0.048	-
06/16/88	2.5	0.69	8541	92.0	--	0.048	-
06/16/88	6.5	0.69	7183	85.0	1.3±0.8	0.048	4.2
06/16/88	12.5	0.69	6050	82.0	--	0.054	-
06/17/88	24.0	0.69	4758	82.0	--	0.058	2.7
06/17/88	31.0	0.69	4017	79.0	1.5±0.7	0.059	2.7
06/18/88	48.0	0.69	2591	67.0	1.4±0.7	0.052	-
06/19/88	72.5	0.69	1992	68.0	1.2±0.5	0.046	2.4
06/20/88	96.5	0.69	1397	56.0	1.4±1.1	0.038	3.4
06/21/88	119.5	0.69	1217	53.0	1.0±0.6	0.036	-

APPENDIX 3

DATA FROM DUAL SPECIES CONTINUOUS CULTURES

Culture #D1 (pH=7.5, P=964 nM, C=48 μM, C/P=50)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5) S. capricorn.	R.rubra	ABSORB (686 nm)
10/12/86		0.45	--	-	4.4±5.0	4.7±5.0	0.480
10/14/86		0.45	57	6.0	5.0±6.0	4.5±3.0	0.445
10/16/86		0.45	45	4.7	2.4±2.0	2.7±3.0	0.408
10/18/86		0.45	50	5.2	--	--	0.300

Culture #D6 (pH=7.4, P=970 nM, C=78 μM, C/P=80)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5) S. capricorn.	R.rubra	ABSORB (686 nm)
08/28/87		0.54	339	35.5	--	--	--
08/30/87		0.52	299	30.8	--	--	--
09/01/87		0.52	284	29.3	--	--	--

Culture #D8 (pH=7.4, P=970 nM, C=97 μM, C/P=100)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$)	#CELLS/ml (mean±SDx10^5) S. capricorn.	R.rubra	ABSORB (686 nm)
12/15/87		0.41	288	29.7	--	--	0.048
12/17/87		0.39	197	20.3	--	--	0.060
12/21/87		0.70	68	7.0	--	--	--
12/24/87		0.70	72	7.4	3.5±3.5	2.4±1.7	--

Culture #D10 (pH=7.5, P=970 nM, C=157 μM, C/P=162)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5) S. capricorn.	R.rubra	ABSORB (686 nm)	CELL CARBON (mg/l)
05/30/88		0.72	28	3.0	1.3±1.3	2.8±2.4	0.145	4.2
06/02/88		0.72	64	6.6	1.1±1.1	1.7±1.1	0.135	4.1
06/06/88		0.72	60	6.2	1.1±0.5	2.0±1.1	0.141	-
06/08/88		0.72	48	4.9	--	--	0.144	-

Culture #D10b (pH=7.5, P=970 nM, C=15

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)
06/19/88		0.72	264
06/20/88		0.72	197
06/21/88		0.72	158
06/22/88		0.72	135
06/23/88		0.72	95
06/24/88		0.72	74
06/26/88		0.72	60
06/27/88		0.72	87
06/28/88		0.72	92
- PERTURBED WITH 7762nM P -			
06/28/88	0.0	0.72	7771
06/28/88	0.5	0.72	6893
06/28/88	1.3	0.72	5912
06/28/88	2.0	0.72	4967
06/28/88	4.0	0.72	3533
06/28/88	6.0	0.72	2756
06/28/88	12.5	0.72	1008
06/29/88	21.5	0.72	201
06/29/88	30.5	0.72	122
06/30/88	46.5	0.72	144
06/30/88	54.0	0.72	125
07/01/89	71.0	0.72	141
07/02/88	95.0	0.72	205
07/03/88	120.5	0.72	365

7 μM, C/P=162)

%P_{SOL})	#CELLS/ml (mean±SDx10^5) S. capricorn.	R.rubra	ABSORB (686 nm)	CELL CARBON (mg/l)
27.2	0.5±0.1	0.7±0.5	0.049	3.3
20.3	0.2±0.2	0.8±0.5	0.043	4.8
16.3	0.3±0.2	0.9±0.4	0.057	-
13.9	0.5±0.2	1.2±0.7	0.081	2.1
9.8	1.0±0.3	1.8±0.7	0.104	3.5
7.6	--	--	0.128	-
6.2	1.2±0.7	1.4±0.9	0.137	3.9
8.9	0.7±0.4	0.8±0.5	0.130	3.3
9.5	0.8±0.4	1.0±0.8	0.110	3.5
89.0	1.1±0.5	1.1±0.4	0.114	3.6
80.5	--	--	0.115	-
69.1	--	--	0.115	-
59.7	--	--	0.115	4.4
45.5	--	--	0.115	-
36.7	0.9±0.4	1.3±0.8	0.115	2.5
16.2	1.3±0.7	1.2±0.7	0.131	-
4.3	1.9±1.6	1.2±0.8	0.206	-
3.4	3.0±1.9	1.9±1.2	0.248	2.9
4.9	5.3±2.4	1.8±1.2	0.321	7.6
4.9	5.4±3.3	1.2±0.9	0.303	6.6
7.5	3.0±1.7	0.7±0.6	0.243	5.3
14.5	1.4±0.6	0.2±0.2	0.146	4.0
31.0	0.9±0.3	0.1±0.2	0.070	3.3

Culture #D11 (pH=7.4, P=970 nM, C=118 μM, C/P=122)

Absorbance measured with 4 cm cells.

DATE	TIME AFTER PERTURBATION (hr)	$\mu(d^{-1})$	P_{SOL} (nM)	$\%P_{SOL}$	#CELLS/ml (mean±SDx10^5) S. capricorn.	R.rubra	ABSORB (686 nm)	CELL CARBON (mg/l)
06/28/88		0.66	160	16.5	1.2±0.6	1.1±0.6	0.124	-
06/30/88		0.66	202	20.8	0.7±1.1	0.7±0.3	0.082	3.1
07/03/88		0.66	187	19.3	0.4±0.4	0.2±0.1	0.083	2.6
07/06/88		0.66	129	13.3	1.1±0.4	0.4±0.2	0.103	3.2
07/07/88		0.66	190	19.6	0.5±0.6	0.1±0.1	0.097	3.5
- PERTURBED WITH 7762 nM P -								
07/07/88	0.0	0.66	7256	83.1	0.7±0.4	0.3±0.2	0.102	-
07/07/88	0.5	0.66	6977	81.6	--	--	0.101	4.1
07/07/88	1.5	0.66	6202	74.3	1.0±0.5	0.3±0.2	0.115	-
07/07/88	3.5	0.66	5478	68.7	--	--	0.096	3.6
07/07/88	6.5	0.66	4468	61.1	--	--	0.105	-
07/07/88	10.0	0.66	3271	49.4	1.3±0.7	0.3±0.2	0.114	-
07/08/88	21.0	0.66	604	11.8	1.9±0.9	0.5±0.4	0.168	5.3
07/08/88	29.5	0.66	223	5.1	1.8±1.2	0.3±0.2	0.206	-
07/09/88	47.0	0.66	91	2.9	5.0±2.3	0.6±0.3	0.312	7.3
07/10/88	69.0	0.66	72	3.3	3.7±1.7	0.7±0.5	0.283	6.5
07/11/88	93.0	0.66	56	3.5	2.5±1.2	0.5±0.5	0.228	6.1
07/12/88	121.0	0.66	85	6.7	1.5±1.0	0.5±0.2	0.148	4.3
07/13/88	146.0	0.66	109	9.7	0.7±0.6	0.4±0.3	0.114	4.5

REFERENCES

Ahlgren, G. 1980. Effects on algal growth rates by multiple nutrient limitation. Arch. Hydrobiol. 89:43-53.

Aiba, S. 1982. Growth kinetics of photosynsthetic microooganisms. Adv. Biochem. Engin. 23:119-125.

Alldredge, A.L. and Y. Cohen. 1987. Can microscale chemical patches persist in the sea? Microelectrode study of marine snow, fecal pellets. Science. 235: 689-691.

Auer, M.T., M.S. Kieser and R.P. Canale. 1986. Identification of critical nutrient levels through field verification of models for phosphorus and phytoplankton growth. Can. J. Aquat. Sci. 43:379-388.

Bader, F.B. 1982. Kinetics of double-substrate limited growth. In Bazin, M.J. (ed) pp.2-31 Microbial Population Dynamics. CRC Press, Inc. Boca Raton, FL.

Barford, J.P., N.B. Pamment and R.J. Hall. 1982. Lag phases and Transients. In Bazin, M.J. (ed) pp.55-89 Microbial Population Dynamics. CRC Press, Inc. Boca Raton, FL.

Bartsch, A.F. (Chairman). 1971. Algal assay procedurce bottle test, p.11-15. National Eutrophication Research Program, Environmental Protection Agency, Washington, D.C.

Boos, W. 1987. Introduction: mechanisms and energetics of phosphate transport in other microorganisms. pp. 185. In Torriani-Gormi, A. (ed). Phosphate metabolism and cellular regulation in microorganisms. American Society for Microbiology, Washington, D.C.

Borst-Pauwels, G.W.F.H. and P.H.J. Peters. 1987. Phosphate uptake in *Saccharomyces cerevisiae*. pp. 205-211 In Torriani-Gorini, A. (ed0. Phosphate metabolism and cellular regulation in microorganisms. American Society for Microbiology, Washington, D.C.

Braddock, J.F., H.V. Luong and E.J. Brown. 1984. Growth kinetics of *Thiobacillus ferrooxidans* isolated from arsenic mine drainage. Appl. Environ. Microbiol. 48:48-55.

Bratbak, G. and T.F. Thingstad. 1985. Phytoplankton-bacteria interactions: an apparent paradox? Analysis of a model system with both competition commensalism. Mar. Ecol. Prog. Ser. 25:23-30

Broecker, W.S. and T.H. Peng. 1982. Tracers in the sea. Lamont-Doherty Geological Observatory. Palisades, NY. 690pp.

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

Brown, E.J., D.K. Button and D.S. Lang. 1981. Competition between heterotrophic and autotrophic microplankton for dissolved nutrients. Microb. Ecol. 7:199-206.

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

Button, D.K. 1969. Effect of clay on the availability of dilute organic nutrients to steady-state heterotrophic populations. Limnol. Oceanogr. 14:95-100.

Button, D.K. 1978. On the theory of nutrient concentration control of microbial growth rates. Deep-Sea Res. 25:1163-1177.

Button, D.K. 1985. Kinetics of nutrient-limited transport microbial grown. Microbiol. Rev. 49:270-297.

Button, D.K. 1986. Affinity of organisms for substrate. Limnol. Oceanogr. 31:453-456.

Button, D.K., S.S. Dunker and M.L. Morse. 1973. Continuous culture of *Rhodotorula rubra*: kinetics of phosphate-arsenate uptake, inhibition and phosphate limited growth. J. Bacteriol. 113:599-611.

Caperon, J. 1967. Population growth in micro-organisms limited by food supply. Ecology 48:715-722.

Caperon, J. 1968. Population growth response of *Isochrysis galbana* to variable nitrate environment. Ecology 49:866-872.

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

Chisholm, S.W., R.J. Olson, E.R. Zettler, R. Goericke, J.B. Waterbury and N.A. Welschmeyer. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature 334:340-343.

Collos, Y. 1980. Transient situations in nitrate assimilation by marine diatoms. 1. Changes in uptake parameters during nitrogen starvation. Limnol. Oceanogr. 25:1075-1081.

Collos, Y. 1982a. Transient situations in nitrate assimilation by marine diatoms. 2. Changes in nitrate and nitrite following nitrate perturbation. Limnol. Oceanogr. 27:528-535.

Collos, Y. 1982b. Transient situations in nitrate assimilation by marine diatoms III. Short-term uncoupling of nitrate uptake and reduction. J. Exp. Mar. Biol. Ecol. 62:285-295.

Collos, Y. 1984. Transient situations in nitrate assimilation by marine diatoms. V. Interspecific variability in biomass and uptake during nitrogen starvation and resupply. Mar. Ecol. Prog. Ser. 17:25-31.

Condrey, R.E. 1982. The chemostat and Blackman kinetics. Biotechnol. Bioeng. 24:1705-1709.

Conway, H.L. and P.J. Harrison. 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation IV. Transient response of *Chaetoceros debilis, Skeletonema costatum* and *Thalassiosira gravida* to a single addition of the limiting nutrient. Mar. Biol. 43:33-43.

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

Cunningham, A. and P. Maas. 1982. The growth dynamics of unicellular algae. In Bazin, M.J. (ed) pp. 167-188. Microbial Population Dynamics. CRC Press, Inc. Boca Raton, FL.

Currie, D.J. 1986. Does orthophosphate uptake supply sufficient phosphorus to phytoplankton to sustain their growth? Can J. Fish. Aquat. Sci. 43:1482-1487.

Currie, D.J. and J. Kalff. 1984a. Can bacteria outcompete phytoplankton for phosphorus? A chemostat test. Microb. Ecol. 10:205-216.

Currie, D.J. and J. Kalff. 1984b. A comparison of the ability of freshwater algae and bacteria to acquire and retain phosphorus. Limnol. Oceanogr. 29:298-310.

Currie, D.J. and J. Kalff. 1984c. The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in freshwater. Limnol. Oceanogr. 29:311-321.

Currie, D.J., E. Bentzen and J. Kalff. 1986. Does algal-bacterial phosphorus partitioning vary among lakes? A comparative study of orthophosphate uptake and alkaline phosphatase activity in freshwater. Can. J. Fish. Aquat. Sci. 43:311-318.

DePinto, J.V., T.C. Young, J.S. Bonner and P.W. Rodgers. 1986. Microbial recycling of phytoplankton phosphorus. Can. J. Fish. Aquat. Sci. 43:336-342.

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

Droop, M.R. 1974. The nutrient status of algal cells in continuous culture. J. Mar. Biol. Assn. U.K. 54:825-855.

Droop, M.R. 1983. 25 years of algal growth kinetics. Botanica Marina. 26:99-112.

Dugdale, P.C. 1967. Nutrient limitation of the sea: dynamics, identification and significance. Limnol. Oceanogr. 12:685-695.

Dugdale, R.C. and J.J. Goering. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. 12:196-206.

Elrifi, I.R. and D.H. Turpin. 1985. Steady-state luxury consumption and the concept of optimum nutrient ratios: A study with phosphate and nitrate limited *Selenastrum minutum* (Chlorophyta) J. Phycol. 21:592-602

Eppley, R.W., E.H. Renger and W.G. Harrison. 1979. Nitrate and phytoplankton production in southern California coastal water. Limnol. Oceanogr. 29:483-494.

Faust, M.A. and D.L. Correll. 1976. Comparison of bacterial and algal utilization of orthophosphate in an estuarine environment. Mar. Biol. 34:151-162.

Fenchel, T. and T.H. Blackburn. 1979. Bacteria and mineral cycling. Academic Press, London. 225pp.

Froelich, P.N. 1988. Kinetic control of dissolved phosphate in natural rivers and estuaries: a primer on the phosphate buffer mechanism. Limnol. Oceanogr. 33:649-668.

Fuhs, G.W. 1969. Phosphorus content and rate of growth in the diatom *Cyclotella nana* and *Thalassiosira fluviatilis*. J. Phycol. 5:312-321.

Fuhs, G.W., S.D. Demmerle, E. Canelli and M. Chen. 1972. Characterizations of phosphorus limited plankton algae. pp. 113-133. In G.E. Likens (ed). The limiting nutrient concept, Limnology and Oceanography Special Proceedings.

Goldman, J.C. and P.M. Glibert. 1983. Inorganic nitrogen uptake by phytoplankton. pp.233-274. In E.J. Carpenter and D.G. Capone (ed). Nitrogen in the marine environment. Academic Press, New York.

Gotham, I.J. and G.-Y. Rhee. 1981. Comparative kinetic studies of phosphate-limited growth and phosphate uptake in phytoplankton in continuous culture. J. Phycol. 17:257-265.

Harder, W., J.G. Kuenen and A. Matin. 1977. A review: microbial selection in continuous culture. J. Appl. Bacteriol. 43:1-24.

Harrison, W.G. 1980. Nutrient regeneration and primary production in the sea. In Fackowsk, P.G. (ed). Primary productivity in the sea. Plenum Press, NY.

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

Hecky R.E. and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. Limnol. Oceanogr. 33:796-822.

Hobbie, J.E., R.J. Daley and S. Jasper. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33:1225-1228.

Holligan, P.M., R.D. Pingree and G. T. Mardell. 1985. Oceanic solitons, nutrient pulses and phytoplankton growth. Nature.314:348-350.

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

Jackson, G.A. 1980. Phytoplankton growth and zooplankton grazing in oligotrophic oceans. Nature. 284:439-441.

Kilham, P. and R.E. Hecky. 1988. Comparative ecology of marine and freshwater phytoplankton. Limnol. Oceanogr. 33:776-795.

Krempin, D.W., S.M. McGrath, J. Beeler Soo Hoo and C.W. Sullivan. 1981. Orthophosphate uptake by phytoplankton and bacterioplankton from the Los Angeles Harbor and Southern California Coastal waters. Mar. Biol. 64:23-33.

Lang, D.S. 1980. Phosphorus metabolism of several aquatic microorganisms. M.S. Thesis, University of Alaska, Fairbanks, 75pp.

Lang, D.S. and E.J. Brown. 1981. Phosphorus-limited growth of a green alga and a blue-green alga. Appl. Environ. Microbiol. 42:1002-1009.

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

Law, A.T., B.R. Robertson, S.S. Dunker and D.K. Button. 1976. On describing microbial growth kinetics from continuous culture data: some general considerations, observations and concepts. Microb. Ecol. 2:261-283.

Lehman, J.T. and D. Scavia. 1982. Microscale patchiness of nutrients in plankton communities. Science. 216:729-730.

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

McCarthy, J.J., W.R. Taylor and J.L. Taft. 1977. Nitrogenase nutrition of the plankton in Chesapeake Bay. I. Nutrient availability and phytoplankton preferences. Limnol. Oceanogr. 22:996-1010.

Meffert, M.E. and J. Overbeck. 1979. Regulation of bacterial growth by algal release products. Arch. Hydrobiol. 87:118-121.

Molot, L.A. 1981. The concept of microbial affinity for limiting nutrients in steady state and rhythmic systems. Ph.D. Thesis, University of Alaska, Fairbanks, 86pp.

Molot, L.A. and E.J. Brown. 1986. Method for determining the temporal response of microbial phosphate transport affinity. Appl. Environ. Microbiol. 51:524-531.

Monod, J. 1942. Recherches sur la croissance des cultures bacteriennes. Actualites scientifiques et industrielles. Hermann, ed., Paris.

Morel, F.M.M. 1987. Kinetics of nutrient uptake and growth in phytoplankton. J. Phycol. 23:137-150.

Nalewajko, C. and D.R.S. Lean. 1980. Phosphorus. pp.235-258. In Morris, I(ed). The physiological ecology of phytoplankton (studies in Ecology Vol. 7) Univ. of CA press. Berkeley, CA.

Nyholm, N. 1977. Kinetics of phosphate limited algal growth. Biotechnol. and Bioeng. 19:467-492.

Nyholm, N. 1978. Dynamics of phosphate limited algal growth: simulation of phosphate shocks. J. Theor. Biol. 70:415-425.

Paasche, E. 1973. Silicon and the ecology of marine plankton diatoms. II. Silicate-uptake kinetics in five diatom species. Mar. Biol. 19:262-269.

Parsons, T.R., M. Takahashi and B. Hargrave. 1984. Biological Oceanographic Processes 3rd ed. Pergamon Press. Oxford. 330pp.

Parsons, T.R. and Harrison, P.J. 1983. Nutrient cycling in aquatic ecosystems, marine environment. Encyclopedia of Plant Physiology 12D. 77-105.

Pasciak, W.J. and J. Gavis 1974. Transport limitation of nutrient uptake in phytoplankton. Limnol. Oceanogr. 19:

Quarmby, L. M., D.H. Turpin and P.J. Harrison. 1982. Physiological responses of two marine diatoms to pulsed additions of ammonmium. J. Exp. Mar. Biol. Ecol. 63:173-181.

Raymont, J.E.G. 1980. Plankton and productivity in the oceans. 2nd. ed. vol. I. Phytoplankton. pp. 304-305 and 336-337. Pergamon Press. Oxford. 489pp.

Rhee, G.-Y. 1972. Competition between an alga and an aquatic bacterium for phosphate. Limnol. Oceanogr. 17:505-514.

Rhee, G.-Y. 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. J. Hycol. 9:494-506.

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

Rhee, G.-Y. 1978. Effects of N:P atomic ratios and nitrate limitation on algae growth cell compostion and nitrate uptake. Limnil. Oceanogr. 23:10-25.

Rhee, G.-Y. 1980. Continous culture in phytoplankton ecology. Adv. Aquat. Microbiol. 2:151-203.

Rhee, G.-Y. and I.J. Gotham. 1980. Optimum N:P ratios and coexistence planktonic algae. J. Phycol. 16:486-489.

Richerson, P., R. Armstrong and R.C. Goldman. 1970. Contemporaneous disequilibrium, a new hypothesis to explain "the paradox of the plankton." Proc. Nat. Acad. Sci. 67:1710-1714.

Riegman, R. and L.R. Mur. 1984. Regulation of phosphate uptake kinetics in *Oscillatoria agardhii*. Arch. Microbiol. 139:28-32.

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

Sakshaug, E., A. Kjersti, S. Myklestad and Y. Olsen. 1983. Nutrient status of phytoplankton communities in Norwegian waters (marine, brackish, and fresh) as revealed by their chemical composition. J. Plankton Res. 5:175-196.

Scavia, D., G.L. Fahnenstiel, J.A. Davis and R.G. Kreis, Jr. 1984. Small-scale nutrient patchiness: some consequences and a new encounter mechanism. Limnol. Oceanogr. 29:785-793.

Schindler, D.W. 1975. Whole-lake eutrophication experiments with phosphorus, nitrogen and carbon. Verh. Int. Verein. Limnol. 19:3221-3231.

Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes. Science 195:260-262.

Smith, R.E. and J. Kalff. 1982. Size-dependent phosphorus uptake kinetics and cell quota in phytolankton. J. Phycol. 18:275-284.

Smith, R.E. and J. Kalff. 1983. Competion for phosphorus among co-occurring freshwater phytoplankton. Limnol. Oceanogr. 28:448-464.

Sommer, U. 1884. The paradox of the palnkton: fluctuations of phosphorus availability maintain diversity of phytoplankton in flow-through cultures. Limnol. Oceanogr. 29:633-636.

Sommer, U. 1985a. Seasonal succession of phytoplankton in Lake Constance. BioScience 35:351-357.

Sommer, U. 1985b. Comparison between steady state and non-steady state competition: experiments with natural phytoplankton. Limnol. Oceanogr. 30:335-346.

Sommer, U. and S.S. Kilham. 1985. Phytoplankton natural community competition experiments: a reinterpretation. Limnol. Oceanogr. 30:436-440.

Stanier, R.Y., E.A. Adelberg and J. Ingraham. 1976. The microbial world. 4th ed. Prentice-Hall, Inc., Englewood Cliffs, N.J. 871 pp.

Sterner, R.W. 1986. Herbivores' direct and indirect effects on algae populations. Science. 231:605-607.

Suttle, C.A. and P.J. Harrison, 1986. Phosphate uptake rates of phytoplankton assemblages grown at different dilution rates in semicontinuous culture. Can. J. Fish. Aquat. Sci. 43:1474-1481.

Suttle, C.A. and P.J. Harrison. 1988a. Ammonium and phosphate uptake rates, N:P supply ratios, and evidence for N and P limitation in some oligotrophic lakes. Limnol. Oceanogr. 33:186-202.

Suttle, C.A. and P.J. Harrison. 1988b. Ammonium and phosphate uptake kinetics of size-fractionated plankton from an oligotrophic freshwater lake. J. Plank. Res. 10:133-149.

Suttle, C.A. and P.J. Harrison. 1988c. Rapid ammonium uptake by freshwater phytoplankton. J. Phycol. 24:13-16.

Suttle, C.A., J.G. Stockner and P.J. Harrison. 1987. Effects of nutrient pulses on community structure and cell size of a freshwater phytoplankton assemblage in culture. Can. J. Fish. Aquat. Sci. 44:1768-1774.

Suttle, C.A., J.G. Stockner, K.S. Shortreed and P.J. Harrison. 1988. Time-courses of size-fractionated phosphate uptake: are larger cells better competitors for pulses of phosphate than smaller cells? Oecologia. 74:571-576.

Tarapchak, S.J. and L.R. Herche. 1986. Phosphate uptake by microorganisms in lake water: deviations from simple Michaelis-Menten kinetics. Can. J. Fish. Aquat. Sci. 43:319-328.

Tarapchak, S.J. and C. Nalewajko. 1986a. Introduction: phosphorus-plankton dynamics symposium. Can. J. Fish. Aquat. Sci. 43: 293-301.

Tarapchak, S.J. and C. Nalewajko. 1986b. Synopsis: phosphorus-plankton dynamics symposium. Can. J. Fish. Sci. 43:416-419.

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

Technicon Industrial Systems. 1978. Total organic carbon/dissolved organic carbon in water and wastewater. Industrial Method No. 451-76W/A. Technicon Instruments Corporation, Tarrytown, NY.

Tempest, D.W. and O.M. Neijssel. 1978. Eco-physiological aspects of microbial growth in aerobic nutrient-limited environments. pp. 105-153. In Alexander, M.(ed) Advances in microbial Ecology Vol. 2 Plenum Press, NY. pp. 105-153.

Terry, K.L. 1980. Nitrogen and phosphorus requirements of *Pavlova lutheri* in continuous culture. Bot. Mar. 13:757-764.

Terry, K.L., E.A. Laws and D.J. Burns. 1985. Growth rate variation in the N:P requirement ratio of phytoplankton. J. Phycol. 21:323-329.

Thomas, W.H. and A.N. Dodson. 1972. On nitrogen deficiency in tropical Pacific Oceanic phytoplankton. II. Photosynthetic and cellular characteristics of a chemostat-grown diatom. Limnol. Oceanogr. 17:515-523.

Tilman, D. 1980. Resources: a graphical-mechanistic approach to competition and predation. Amer. Nat. 116:362-393.

Tilman, D. and S.S. Kilham. 1976. Phosphate and silicate growth and uptake kinetics of the diatoms *Asterionella formosa* and *Cyclotella meneghiniana* in batch and semi-continuous culture. J. Phycol. 12:373-383.

Tilman, D., S.S. Kilham and P. Kilham. 1982. Phytoplankton community ecology: the role of limiting nutrients. Ann. Rev. Ecol. Syste. 13:349-372.

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

Turpin, D.H. 1986. Growth rate dependent optimum ratios in *Selenastrum minutum* (Chlorophyta): implications for competition, coexistence and stability in phytoplankton communities. J. Phycol. 22:94-102.

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

Turpin, D.H. and P.J. Harrison. 1980. Cell size manipulation in natural marine planktonic, diatom communities. Can. J. Fish. Aquat. Sci. 37: 1193-1195.

Turpin, D.H., J.S. Parslow and P.J. Harrison. 1981. On limiting nutrient patchiness and phytoplankton growth: a conceptual approach. J. Plank. Res. 3:421-431.

Vadstein, O., A. Jensen, Y. Olsen and H. Reinertsen. 1988. Growth and phosphorus status of limnetic phytoplankton and bacteria. Limnol. Oceanogr. 33:489-503.

Williams, F.M. 1971. Dynamics of Microbial populations. pp. 197-267. In Patten, B.C. (ed) Systems Analysis and Simulation Ecology. Academic Press. NY.