PROCESSES IN NUTRIENT BASED PHYTOPLANKTON ECOLOGY

by

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ABSTRACT

Fluctuations in the free intracellular amino acid pools following an ammonia perturbation to ammonium limited *Skeletonema costatum* and *Gymnodinium simplex* provides evidence which suggests that the enzyme glutamine synthetase (EC.6.3.1.2) acts as the primary ammonium assimilating enzyme in marine phytoplankton under nitrogen limitation.

Limiting nutrient patchiness (ammonium) is examined as a factor affecting both phytoplankton physiology and competition. It is shown that temporal patchiness in the supply of the limiting nutrient sets up periodicities in cellular carbon fixation and *in vivo* chlorophyll *a* fluorescence. Populations grown in a patchy limiting nutrient environment appear better adapted to take up nutrient pulses than do populations grown under conditions of homogeneous distributions of the limiting nutrients. It is also shown that the patchiness of the limiting nutrient effects the outcome of species competition with the winners being those species best able to optimize uptake under that particular patchy regime.

A theoretical framework is developed to explore the effects of limiting nutrient patchiness on phytoplankton growth. This work shows that the degree of patchiness in the environment can affect individual growth rates and thus alter community structure even though there is no change in the average ambient nutrient concentration. In addition the apparent K_s' for growth, for patch adapted populations, may be lowered significantly by making the distribution of the nutrient patchy with respect to time.

A qualitative model is proposed relating nutrient supply, light and temperature and their effects on phytoplankton community structure.

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INTRODUCTION

1) Marine phytoplankton and food chain ecology

Marine phytoplankton are the first step in many marine food chains. These microalgae require light, carbon dioxide, inorganic ions (NO_3^-, NO_2^-, NH_4^+, $PO_4^{\equiv}$, $SiO_4^{\equiv}$) and sometimes trace amounts of organic compounds to be able to grow and produce the primary particulate material in the sea. They are heavily grazed by herbivorous zooplankton which in turn are prey to other organisms with higher trophic status. The morphology and size of the phytoplankton determines to a large extent which organisms can consume them, and consequently they play an improtant role in mediating the resulting food chain. This idea was succinctly described by Ryther (1969) in a classic paper discussing three marine food chains: the oceanic, coastal and upwelling, and their potential for fish production. The oceanic community is characterized by low productivity ($\sim$50 $gC \cdot m^{-2} \cdot yr^{-1}$) and a standing stock of primary producers composed mainly of small flagellates. The coastal food chain, termed the continental shelf food chain by Parsons and Takahashi (1973), has an average productivity of $\sim$100 $gC \cdot m^{-2} \cdot yr^{-1}$ with an assemblage of primary producers consisting of large diatoms, dinoflagellates and nanoplankton. Upwelling ecosystems are characterized by Ryther's upwelling community. The primary productivity of these regions averages $\sim$300 $gC \cdot m^{-2} \cdot yr^{-1}$ to which the macrophytoplankton are the greatest contributors.

The differences in these three food chains, mediated in a large part by the primary producers present, result in large differences in the total fish production (harvestable resource) that can be supported by the system.

Greve and Parsons (1977) proposed that the very nature of the phytoplankton in a system may affect not only the production of higher trophic levels, as was argued by Ryther (1969), but also the species composition. Greve and Parsons suggested that there are two principal pathways for transfer of energy in a

marine food web. The first pathway proceeds from nanophytoplankton to ctenophores or medusae, while the second starts with large diatoms and terminates with fish.

The composition or community structure of the primary producers in the world's ocean thus appears to be of paramount importance in determining the pathways and ultimate yield of marine food chains. The large economic importance attributed to marine fisheries makes it important to understand the factors controlling the ability of different phytoplankton groups to attain dominance in the seas.

The control of dominance in a phytoplankton community depends upon the net growth rates of the populations in the community. The net growth rate is the difference between the instantaneous rate of increase of the population and the loss terms due to grazing, sinking and advection. The population with the highest net growth will eventually attain dominance in the system. The instantaneous growth rate (μ) of a population is thus of great ecological importance.

The instantaneous growth rate is affected by many factors including temperature, light and nutrients. It is generally accepted that temperature defines or limits the maximum 'potential' growth rate of a population (Eppley, 1972). Other factors such as nutrients and light will determine the growth rate attained if either of these is limiting.

The major objective of the work reported in this thesis was to examine various aspects of nutrient limited growth of marine phytoplankton, with particular reference to inorganic nitrogen. In the final chapter there is an attempt made to integrate the nutrient effects with other factors such as temperature and light in a simple conceptual scheme.

2) Nitrogen in the marine ecosystem

Nitrogen in the sea is found in inorganic, organic and particulate pools. The dissolved inorganic nitrogen pool (DIN) is composed mainly of molecular nitrogen, nitrate, nitrite and ammonium, whereas the dissolved organic nitrogen pool (DON) primarily consists of urea, amino acids, creatine, peptides and nucleotides. Upon incorporation into phytoplankton or bacterial biomass the nitrogen enters the particulate pool (PON). The fluxes between these pools are controlled by biological, chemical and physical factors. The amount of nitrogen in a pool at any time is a function of the assimilation, regeneration and transformation processes occurring in the system.

2.1) Nitrogen cycling in the sea:

A schematic representation of nitrogen cycling in the sea is given in Figure 1.

a) Physical processes: Molecular nitrogen is in constant exchange between the sea surface and the atmosphere in accordance with Henry's Law (Vaccaro, 1965). For the most part the surface of the oceans seems to be saturated with molecular nitrogen (Fox, 1909; Rakestraw & Emmel, 1938; Benson & Parker, 1961). Molecular nitrogen is of little importance in the biological cycling of nutrients except where it is used in the processes of nitrogen fixation by blue-green algae (Dugdale et al., 1964; Carpenter & McCarthy, 1975).

Fixed nitrogen, in the form of ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) can also enter the sea from the atmosphere most often in association with rain. The amount of this fixed nitrogen varies considerably. Walsh et al. (1978) measured concentrations ($\mu g\text{-at}\cdot\ell^{-1}$) of 0.03 NO_2^-, 5.57 NH_4^+ and 14.18 NO_3^- in the rain water from the New York Bight. This nitrogen flux would account for about 1% of the annual phytoplankton nitrogen budget in the Bight. The concentration of nitrogenous nutrients in rainwater may vary with the proximity to land and the extent of input into the air by the respective land mass.

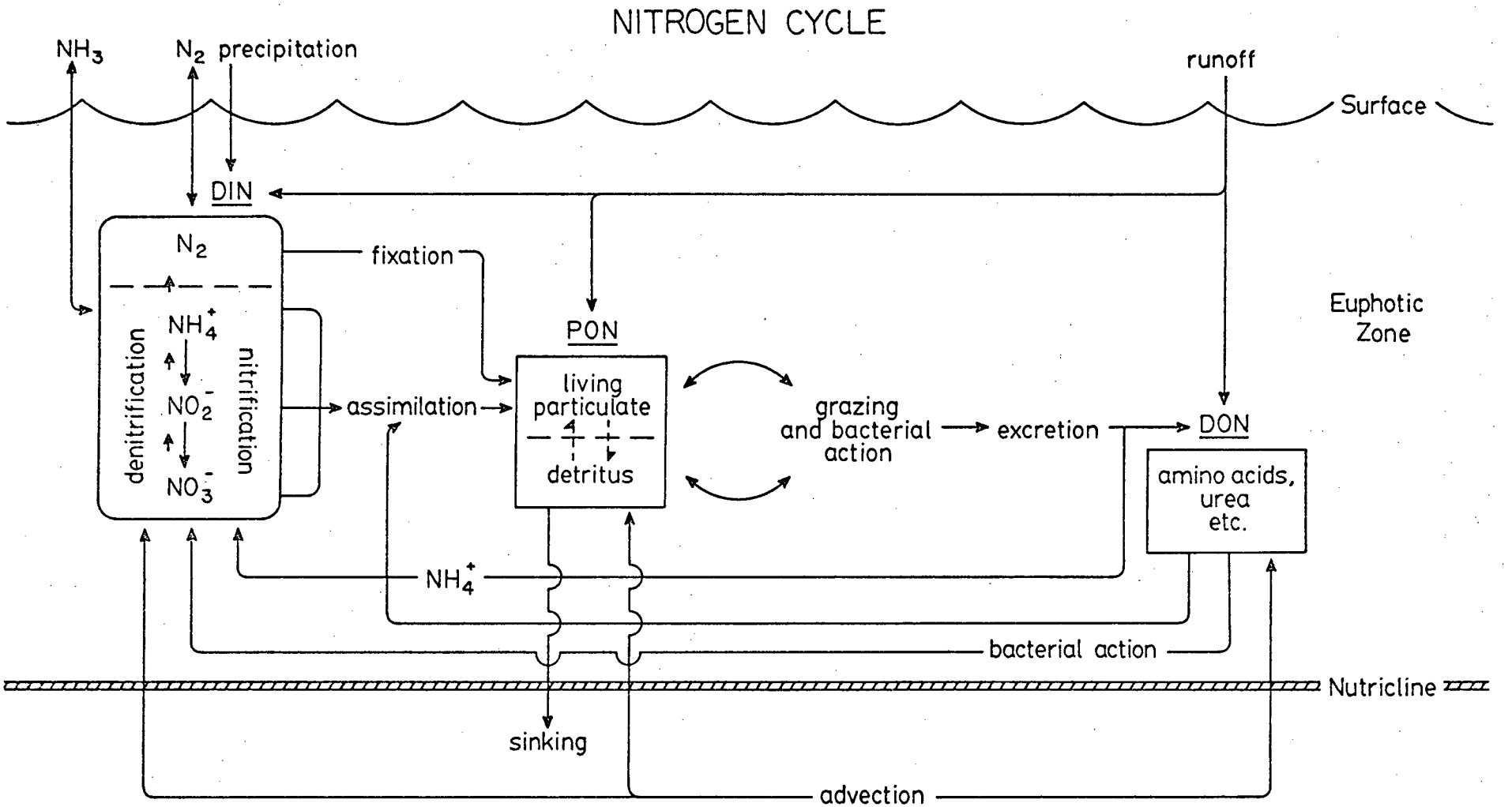


Figure 1. The nitrogen cycle in the sea.

River input of fixed nitrogen can be significant in coastal and estuarine systems. Walsh et al. (1978) using the data of Bowman (1977) and Riley (1959) estimated that the nitrogen flux in the New York Bight, as a result of river input, to be about 3 μg-at $N \cdot l^{-1} \cdot yr^{-1}$, or approximately 8% of yearly productivity demand. Such allocthanous input would be insignificant in a system such as an oligotrophic oceanic gyre.

A major proportion of nitrogenous inputs into the euphotic zone is a result of storm action. During these periods of disturbance, large quantities of deep nutrient-rich water are mixed up into the surface waters (Walsh et al., 1978). Upwelling of nutrient-rich, deep water is a very important process in many areas of the world. Such upwelling occurs at divergences which are most often found in association with the eastern boundary currents (Dugdale, 1976; Wooster & Reid, 1963). The upwelling process off the coast of Peru is of such magnitude that this area represents one of the most productive areas in the world oceans. Other factors such as turbulence across the nutricline also contribute to the physically controlled fluxes.

Losses of nitrogen from the system are primarily a result of the sinking of particulate material including phytoplankton (Smayda, 1970), fecal material and detritus. Other losses occur through organismal migration and advection.

b) Biological processes: Molecular nitrogen can enter the nitrogen cycle via the process of biological fixation. The most commonly studied nitrogen fixing organisms in the seas are the blue-green algae and specifically, members of the genus *Oscillatoria (Trichodesmium)* and some very small coccoid species (Watson, pers. comm.). Initially it was suggested that N_2 fixation could be an important component of the nitrogen flux in oceanic systems (Dugdale et al., 1964; Goering et al., 1966). Other workers studying the Sargasso Sea and the central North Pacific showed that N_2 fixation makes an insignificant contribution to the nitrogen budget (Carpenter & McCarthy, 1975; Mague et al.,

1974). However, Carpenter and Price (1977) have shown that in the eastern Caribbean Sea, N_2 fixation by *Oscillatoria* sp. may be a very significant component of nitrogenous fluxes. This apparently contradictory evidence probably results from the spacial and temporal patchiness of the N_2 fixing organisms.

The uptake of dissolved inorganic and organic nitrogen is controlled by numerous factors; the concentration of the nutrient in solution (Dugdale, 1969), the irradiance (MacIsaac & Dugdale, 1972), temperature (Goldman, 1977; Harrison, 1974) and the other nitrogenous nutrients present (Wheeler et al., 1974). The uptake process will be described in detail in the next section.

The nitrogenous compounds which are taken up are incorporated into primary particulate materials and then become susceptible to grazing pressure. The particulate nitrogen may be either assimilated and remain in the particulate pool or eventually be excreted as ammonium, DON, or fecal material. Zooplankton excretion is a very significant component of nitrogen flux in marine ecosystems. Walsh et al., (1978) showed that zooplankton excretion accounted for ∿35% of the annual nitrogen flux through the euphotic zone in the New York Bight. Recent work by Goldman et al. (1979) suggested that even though ambient nutrient concentrations in oligotrophic areas of the sea may be undetectable, it is possible that phytoplankton growth rates are not nutrient limited. This may be due to a dynamic balance between nutrient regeneration and assimilation resulting in fluxes through the dissolved nutrient pool that are high enough to support high growth rates. Ammonium resulting from regeneration can be converted to NO_2^- and NO_3^- via bacterial nitrification (Vaccaro, 1965).

The uptake and assimilation of dissolved inorganic nitrogen by phytoplankton, with specific reference to ammonium are the major subjects of this thesis. This is an important component of the phytoplankton nitrogen budget, where a major portion of the nitrogen flux into a system may be due to ammonium

regeneration (Dugdale & Goering, 1967; Dugdale, 1976; Harrison, 1978).

3) Kinetics of Nitrogen uptake

The uptake rate of fixed nitrogen (NH_4^+, NO_2^-, NO_3^-, amino acids or urea) appears to be related to the concentration of the substrate in solution by the Michaelis-Menten (1913) hyperbola which is described by the following equation:

$$V = V_{max} \frac{[S]}{K_s + [S]}$$

where V = nutrient uptake rate (hr^{-1})

V_{max} = maximal nutrient uptake rate (hr^{-1})

S = concentration of the nutrient ($\mu g \cdot at \cdot \ell^{-1}$)

K_s = concentration of the nutrient at which $V = 1/2\ V_{max}$ ($\mu g \cdot at \cdot \ell^{-1}$)

Numerous studies have shown the applicability of this expression to nitrogen uptake by marine phytoplankton (Eppley et al., 1969; Eppley & Renger, 1974; Caperon & Meyer, 1972a & b). Nutrient uptake is thought to be enzyme controlled and therefore it is not surprising that there is a strong temperature effect on the process. Eppley (1972) reported a Q_{10} of 1.88 for phytoplankton growth. In a nutrient saturated system or under conditions when uptake equals growth, this value would also apply to nutrient uptake.

Phytoplankton derive almost all their energy from light. It is not surprising that uptake of many nutrients shows a strong dependence on light. MacIsaac and Dugdale (1972) showed the dependence of NO_3^- uptake on light. They described a relationship between uptake and irradiance through a half-saturation constant for light thus:

$$V = V_{max} \frac{[I]}{K_{Lt} + [I]}$$

where V = nutrient uptake rate (hr^{-1})

V_{max} = maximal uptake rate of the limiting nutrient (hr^{-1})

[I] = Irradiance (% surface radiation)

K_{Lt} = Irradiance at which V = 1/2 V_{max}

Inorganic forms of nitrogen, especially NH_4^+, are generally preferred to organic forms (Rees & Syrett, 1979; Wheeler et al., 1974; Wheeler, 1977). When ammonium is added to a nitrate-grown phytoplankton population, nitrate uptake is suppressed (Eppley et al., 1969; Conway, 1977). The potential uptake rate of organic nitrogen increases rapidly upon depletion of inorganic nitrogen and the strongly selective uptake characteristics disappear (Wheeler et al., 1974).

Numerous studies have been conducted over the last decade on the kinetics of nutritional ion uptake in marine phytoplankton. The ability of an organism to take up a limiting nutrient rapidly is of great survival value. The half-saturation constant for nutrient uptake is a measure of the affinity of the organism for the limiting nutrient. In environments where the ambient nutrient concentration is low, a low half-saturation constant (high affinity) enables the organism to continue taking up nutrients at high rates and is therefore considered a competitive advantage (Dugdale, 1976). MacIsaac and Dugdale (1969, 1972), Eppley et al. (1973) and Carpenter and Guillard (1971) have shown that K_s values were higher in coastal (eutrophic) than in oceanic (oligotrophic) phytoplankton assemblages.

The ability of an organism to take up a nutrient is dependent upon both V_{max} and K_s and thus the determination of these values is important to the understanding of the organism's functional relationship to its environment. It is now apparent that these uptake kinetic parameters are more complicated than originally thought. These so-called "constants" are in fact variables dependent on the nutrient-limited growth rate of the population (Eppley & Renger, 1974; Conway & Harrison, 1977; McCarthy & Goldman, 1979). In addition

to this complication many different methods of measuring nutrient uptake kinetics have been employed with no clear understanding as to how the results of various methods compare. Several methods of determining uptake kinetics on the same steady state population are presented in Appendix II. The results reflect a greater degree of complexity in the determination and meaning of the uptake kinetic parameters V_{max} and K_s than has hitherto been expected.

4) Pathways of Nitrogen assimilation

The first step in the growth process occurs when a limiting nutrient is taken up and assimilated. The pathways by which assimilation occurs, their ability to scavenge low levels of the limiting nutrient, and the metabolic cost of operating the pathway are all important factors in determining phytoplankton ability to compete for a limiting resource.

Both nitrate and nitrite are reduced intracellularly to ammonium by the enzymes, nitrate reductase and nitrite reductase (Morris, 1974) before further assimilation can occur. Thus discussions of ammonium assimilation also include important steps in the assimilation of nitrate and nitrite. Even some organic forms of nitrogen are converted both extracellularly (Belmont and Miller, 1965; Saubert, 1957) and intracellularly (Stewart, 1977; Keys et al., 1978) to ammonium. It is clear that the pathway by which ammonium is assimilated into organic plant constituents is a major feature of nitrogenous biochemistry in plants. A discussion of the regulation of nitrate and nitrite reduction is not included in this thesis and the reader is referred to Brown and Johnson (1977) for a comprehensive review of this subject.

The study of ammonium assimilation in phytoplankton lags behind similar studies in higher plants (Miflin & Lea, 1976) and bacteria (Stadman & Ginsburg, 1974). In these two groups of organisms it is thought that the enzyme responsible for primary ammonium assimilation, under conditions of nitrogen limitation, is glutamine synthetase (GS; E.C. 6.3.1.2) which catalyzes the conversion

of the amino acid glutamate to glutamine in the presence of NH_4^+, ATP and Mg^{++}.

Glutamine can then participate in the synthesis of many cellular nitrogenous compounds (Prusiner & Stadtman, 1973). The most common fate is the production of two molecules of glutamate from one molecule of glutamine and α-ketoglutarate in the presence of NAD(P)H. The enzyme catalyzing this reaction is glutamine :2 oxoglutarate aminotransferase (GOGAT; E.C. 2.6.1.53) also known as glutamate synthase. The coupling of GS and GOGAT results in a cycle using one molecule of NH_4^+, α-ketoglutarate, ATP and $NAD(P)H+H^+$ and yielding one molecule of glutamate, ADP, Pi and $NAD(P)^+$. The glutamate which is produced in these reactions can be used to supply an amino group to a wide range of compounds. The most common reaction is that of transamination in which the glutamate amino group is transferred to a α-keto acid (Miflin & Lea, 1977).

The discovery of GOGAT has been very recent (Miflin & Lea, 1976). Early work on ammonium assimilation focused on the enzyme, glutamate dehydrogenase (GDH) which catalyzes the reductive amination of α-ketoglutarate to produce glutamate. Early evidence for this pathway came from work on *Candida utilus* (Sims & Folkes, 1964; Folkes & Sims, 1974) which is one of the few organisms that truly lacks a GOGAT system (Miflin & Lea, 1976), and from reports that GDH can catalyze the *in vitro* assimilation of ammonium into amino acids (Miflin & Lea, 1976).

It may be kinetically advantageous for an organism to possess glutamine synthetase when ammonium levels are low. The half-saturation constant (K_s) of GS for ammonium is in the micromole range, whereas the K_s for GDH is in the millimole range (Falkowski & Rivkin, 1976; Miflin & Lea, 1976; Ahmed et al., 1977). Conversely, it requires more energy to synthesize a molecule of glutamate via the GS/GOGAT pathway than via the GDH pathway (i.e., GS requires 1 ATP and GOGAT requires 1 NAD(P)H $\tilde{=}$ 3 ATP). The GS/GOGAT system may be viewed

as a high energy/high affinity system and GDH as a low energy/low affinity system. Recent research with higher plants suggests that GDH is used for assimilation when ammonium is in excess whereas the GS/GOGAT system operates when ammonium is low (Stewart & Rhodes, 1977a). The two systems appear to be reciprocally regulated through intracellular glutamine concentration. When the glutamine concentration is low (low ambient nitrogen), the GS/GOGAT system is stimulated and GDH is repressed. When glutamine is high (high ambient nitrogen) GDH is derepressed and GS/GOGAT repressed.

There have been few reports on the pathways of ammonia assimilation in marine phytoplankton. In an early phytoplankton study (Eppley & Rogers, 1970), and in the early plant and bacterial studies, glutamate dehydrogenase was the only system that was assayed. Further work on GDH in marine phytoplankton was reported by Ahmed et al. (1977).

Falkowski and Rivkin (1976) drew attention to the fact that GDH, with its low NH_4^+ affinity, would be a poor ammonium scavenger under conditions of low ambient nitrogen concentrations and suggested that the large intracellular ammonium pools (Eppley & Rogers, 1970) had been over-estimated due to nucleotide deamination during the extraction procedure. They also demonstrated that the GS/GOGAT system was operating in marine phytoplankton. Its high affinity for ammonium coupled with high *in vitro* activity indicated that this pathway could be an important assimilatory pathway under nitrogen limitation. Other recent enzymological studies have supported the contention that the GS/GOGAT system is important in the assimilation of ammonium (Edge & Ricketts, 1978).

The evidence for use of the GS/GOGAT system under conditions of nitrogen limitation in marine phytoplankton is restricted to *in vitro* enzyme kinetic observations. Results in Chapter I describe the pathway of ammonia assimilation in two species of marine phytoplankton (*Gymnodinium simplex* (Dinophyceae) and *Skeletonema costatum* (Bacillariophyceae)) determined using *in vivo* methods.

The fluctuations in the free intracellular amino acid pools of the nitrogen-limited, chemostat-grown phytoplankton were monitored following an ammonium perturbation. The results are then used to elucidate the apparent pathway of primary ammonium assimilation (Turpin & Harrison, 1978).

5) Nutrient-based competition

Nutrient uptake is only the first step in the growth process. The nutrients which are taken up must be used efficiently if a population is to grow. Monod (1942) proposed a simple model relating the growth rate of micro-organisms to the concentration of the limiting nutrient according to the formula:

$$\mu = \mu_{max} \frac{[S]}{K_s + [S]}$$

μ = growth rate (hr^{-1})

μ_{max} = maximum growth (hr^{-1})

[S] = substrate concentration ($\mu g\text{-at} \cdot \ell^{-1}$)

K_s = the substrate concentration promoting 1/2 maximal growth ($\mu g\text{-at} \cdot \ell^{-1}$)

This model has been most useful in studying the processes of nutrient-based competition. A major weakness is that it assumes that the specific growth rate ($time^{-1}$) is equal to the specific uptake rate of the limiting nutrient. This assumption appears only to be true under conditions of steady state growth. Another problem is that the model assumes a constant cell yield per mole of limiting nutrient. Recent studies on nutrient-limited growth kinetics of marine phytoplankton have shown that this assumption is invalid because the amount of limiting nutrient per cell varies over the growth range of the population (Caperon & Meyer, 1972a & b; Eppley & Renger, 1974; Paasche, 1973; Droop, 1970; Fuhs, 1969; Goldman & McCarthy, 1978). Many workers (Droop, 1968; Goldman & McCarthy, 1978) have shown that the amount of limiting nutrient per cell (Q) varies in response to the nutrient limited growth rate according to

the relationship proposed by Droop (1968) where:

$$\mu = \bar{\mu}\,(1 - Q_{min}/Q)$$

μ = growth rate (hr^{-1})

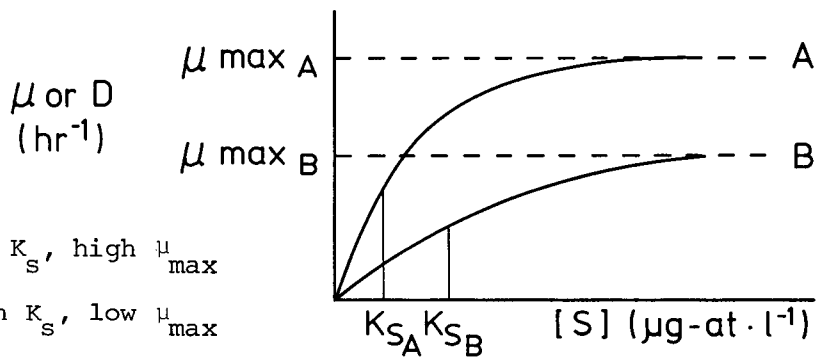
$\bar{\mu}$ = growth rate when $Q \to \infty$ (hr^{-1})

Q = cell quota ($\mu g\text{-at}\cdot cell^{-1}$)

Q_{min} = minimum quota needed for growth to proceed ($\mu g\text{-at}\cdot cell^{-1}$)

a) Competition in a homogeneous environment: Harder et al. (1977) reviewed the processes involved during competition between microorganisms grown in continuous culture. Two general cases of single nutrient-based competition are defined by the Monod model. In the first case, one of two species competing for a limiting resource has both the higher μ_{max} and the lower K_s for growth (as represented by species A below).

Case 1 for Monod Competition



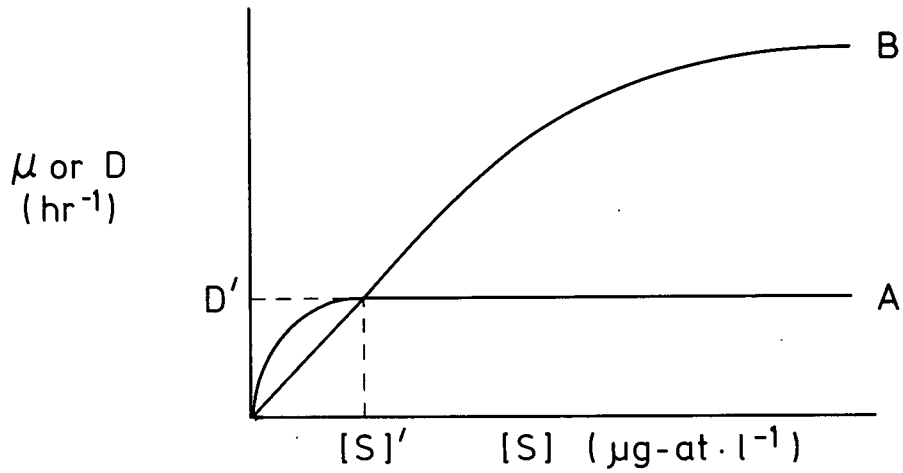
Species A - low K_s, high μ_{max}

Species B - high K_s, low μ_{max}

Regardless of the ambient substrate concentration, species A is always able to outgrow species B. Similarily, in a continuous culture, at any dilution rate (D) (see Appendix I), species A will maintain an ambient nutrient concentration lower than that required for species B to maintain a growth rate equal or greater than the dilution rate and hence B will wash out.

In the second case, the growth vs. substrate curves of the two competitors cross so that, at lower substrate concentrations or dilution rates, species A will win, but at higher concentrations or dilution rates species B will win. This condition is attained when species A has a low μ_{max} and a low K_s for growth, whereas species B has a high μ_{max} and a high K_s. Such a situation is described below:

Case 2 for Monod Competition



Species A - low K_s, low μ_{max}

Species B - high K_s, high μ_{max}

In this scheme, D' is the dilution rate at which species A and B maintain the same ambient substrate concentration while S' is the substrate concentration where the growth rates of species A and B are equal. In this system three results are theoretically possible when the two species are competing for the same limiting resource:

(1) $\mu_A = \mu_B$ when $[S] = [S]'$

(2) $\mu_A < \mu_B$ when $[S] > [S]'$

(3) $\mu_A > \mu_B$ when $[S] < [S]'$

And similarily, in a chemostat when:

(1) $\mu_A = \mu_B$ when $D = D'$

(2) $\mu_A = \mu_B$ when $D < D'$

(3) $\mu_A = \mu_B$ when $D > D'$

Simple models of this type have been used to explain results obtained in phytoplankton competition studies (Michelson et al., 1979; Titman, 1976; Tilman, 1977) and at the same time to provide a simple model for interpreting nutrient-based competition in the sea.

The theory of competition for a single limiting nutrient was expanded by Taylor and Williams (1975) to include many potentially limiting resources. Subsequent experimental work by Tilman (1977; Titman, 1976) confirmed this multiple resource-based competition theory. He established that when two resources are potentially limiting, the growth rate of the organism is described by the concentration of the most limiting nutrient, assuming the validity of the Monod expression. There thus exists a sharp switch from limitation by one nutrient, to limitation by another (Rhee, 1978). The relative supply rate of the two potentially limiting nutrients at this switch-over point can be determined. At the point where limitation switches from one nutrient to another, growth should be limited equally by both nutrients as described below (Titman, 1976):

$$\mu_1 = \mu_2$$

or,

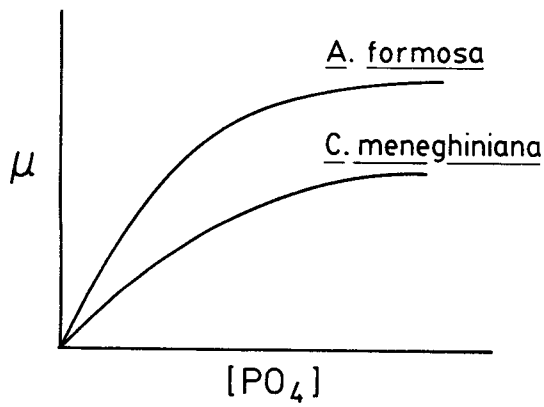
$$\frac{[S_1]}{K_s + [S_1]} \tilde{=} \frac{[S_2]}{K_2 + [S_2]}$$

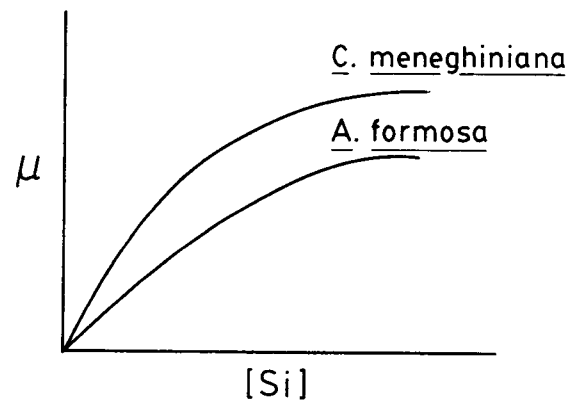
or,

$$\frac{[S_1]}{[S_2]} = \frac{K_1}{K_2}$$

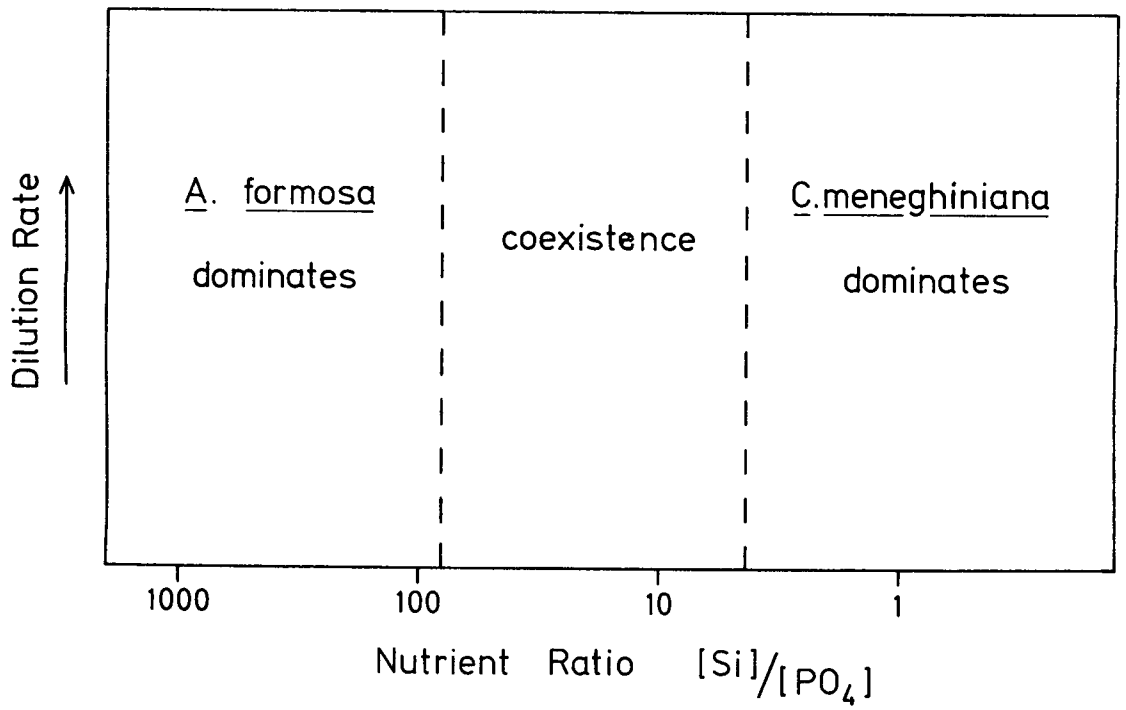
The ratio of nutrients needed to obtain a growth rate which is equally limited by each nutrient is therefore given by the ratio of the half-saturation constants for growth for the two nutrients in question. If the ratio of substrates in the system is greater than the ratio of half-saturation constants, the population is limited by the nutrient S_2. If the ratio of substrates is less than the ratio of half-saturation constants, the population is limited by S_1. As a result, along a continuum of the ratio of two potentially limiting nutrients, there exists a region within which a population will be S_1-limited and another in which it is S_2-limited. Consequently when two species are cultured together there is a range of substrate supply ratio over which species A would be limited by S_1 and species B by S_2 (providing K_1/K_2 for the two species are not identical). In this region the populations are limited by different resources and hence competitive exclusion does not take place. Outside this region, when both species are limited by the same resource, competition can be explained on the basis of Monod competition.

Tilman (1977) used two species, *Asterionella formosa* and *Cyclotella meneghiniana*. Under phosphate limitation, *A. formosa* out-competed *C. meneghiniana* at all substrate concentrations. Conversely, *C. meneghiniana* out competed *A. formosa* under all cases of silicate limitation. The respective μ vs. [S] curves are reproduced below:





The ratios of K_{Si}/K_{PO_4} for *A. formosa* and *C. meneghiniana* are 97 and 5.6, respectively. Therefore, at Si/PO_4 ratios greater than 97, both species were PO_4-limited and *A. formosa* won. At ratios less than 5.6, both species were Si-limited and *C. meneghiniana* won. In the zone between 97 and 5.6, coexistence occurred. The outcome of competition is described diagrammatically below.



In Tilman's work the dilution rate of the continuous cultures essentially had no effect on the result of competition. This can be interpreted as an example of "Case 1" Monod competition, in other words the u vs. [S] curves for the two species do not cross. The examples of "Case 2" Monod competition discussed earlier suggest that further insights into nutrient based competition may be obtained by observing systems in which both the dilution rate, or substrate concentration, and the ratio of potential limiting resources are important. This problem is approached conceptually in Chapter VI of this thesis.

b) Competition in a fluctuating environment: It was assumed in the preceding discussion of nutrient-based competition that there were both temporal and spacial homogeneity in the environment. There have been few attempts to understand the importance of fluctuations in nutrient supply that must exit in nature.

The first report of microalgal responses to fluctuating nutrient regimes was by Caperon (1969). The long-term growth response of *Isochrysis galbana* was followed in response to numerous dilution rate changes. Caperon (1969) showed that the results could be interpreted in a model that included a time lag response between the nutrient concentration and growth. The incorporation of a time lag provided explanations of a number of the transient responses observed by Caperon (1969).

Grenney et al. (1973) showed that a more complex growth model, which included external nutrient concentration and three intracellular nitrogen pools, also accommodated Caperon's (1969) data. Grenney et al. (1973) used their model to explain the effect of fluctuating nutrient supply rates (dilution rates) on the outcome of phytoplankton competition. They showed that with certain low frequency (weeks) dilution rate changes, co-existence between competing species could occur. Over the period of oscillation, however, the population densities of all species fluctuated markedly. This study indicated the potential importance of low frequency resource supply fluctuations upon phytoplankton community structure. Three further questions arise. 1) What if the supply rate remains constant but the temporal or spacial distribution of the resource varies? 2) How does this affect the outcome of competition? 3) Is there an optimal degree of patchiness of the limiting nutrient for a given species?

Chapters II, III, IV and V are concerned with these questions from experimental and modelling viewpoints. In Chapters II - IV, results are presented from experiments with phytoplankton grown in chemostats either as unialgal or mixed species cultures. The average nutrient flux through each system was kept constant but the temporal patchiness of the limiting resource varied with respect to time. The effects of competition and the physiology of the population are monitored to determine the importance of limiting nutrient patchiness on the physiology, growth and competitive advantage of the various populations.

Chapter VII reports an approach to the problem of competition for limiting nutrients when the distribution was patchy with respect to time. Growth curves for hypothetical species were generated that were dependent on the average substrate concentration in the system and the degree of patchiness of that substrate.

6) Purpose

This thesis contains results of several experimental approaches to phytoplankton physiological ecology. This makes it, in some respects, very general in nature. An attempt has been made to synthesize the work into a cohesive study on the biochemical, physiological and ecological levels of phytoplankton responses to resource fluctuations.

The study of marine phytoplankton at steady state has left many unanswered questions about their functional relationship to the environment. Since these organisms live in an environment which is fluctuating, it is important to study the effects of these fluctuations on their physiology and growth.

Chapter I

EVIDENCE FOR THE GLUTAMINE SYNTHETASE PATHWAY OF AMMONIUM ASSIMILATION

1) Summary

An ammonium limited chemostat culture of *Gymnodinium simplex* (Lohm.) Kofoid et Swezy was perturbed with ammonium and fluctuations in the free intracellular amino acid pools were followed 80 min. The steady-state value of glutamate was 2.07×10^{-15} mol·cell^{-1} and of glutamine was 0.31×10^{-15} mol·cell^{-1}. Five minutes after the perturbation, a substantial rise in glutamine was observed with corresponding decrease in glutamate. A similar experiment was performed with an ammonium limited culture of *Skeletonema costatum* (Grev.) Cleve. Two and one-half minutes after the perturbation, free intracellular glutamate had decreased by 0.22×10^{-15} mol·cell^{-1} and glutamine had increased by 0.15×10^{-15} mol·cell^{-1}. These observations are considered a result of glutamine synthetase acting as the primary ammonium assimilating enzyme.

2) Introduction

The mechanisms of ammonium assimilation in marine phytoplankton and higher plants have received increasing attention over the past few years. Initially it was thought that glutamate dehydrogenase (GDH; EC. 1.4.1.3) was primarily responsible for ammonium assimilation (Basham & Kirk, 1964; Folkes & Sim, 1974; Sims & Folkes, 1964). Recent evidence indicates that the glutamine synthetase (GS; EC 6.3.1.2)/glutamate synthase (GOGAT; EC 2.6.1.53) system may be of primary importance in ammonium incorporation, especially under ammonium limitation (Arima & Kumazawa, 1977, Falkowski & Rivkin, 1976; Tempest et al., 1973). The pathways of nitrogen assimilation in plants have been reviewed by Miflin and Lea (1976). Work by Sims and Folkes (1964) and Basham and Kirk (1964) coupled with the *in vitro* ability of GDH to synthesize glutamate from α-ketoglutarate and ammonium were the main reasons for considering GDH as the primary ammonium assimilating enzyme. Further work on the kinetics of GDH in marine phytoplankton revealed that it had a low affinity for ammonium. Eppley and Rogers (1970) showed that in the marine diatom *Ditylum brightwellii* (West) Grunow, GDH had a K_m for ammonium of 10 mM, whereas the intracellular ammonium pool concentration was between 5 and 10 mM. Falkowski and Rivkin (1976) found that the K_m of GDH for ammonium in the marine diatom *Skeletonema costatum* (Grev.) Cleve was 28 mM, indicating an unreasonably low affinity for ammonium if this enzyme was in fact responsible for primary ammonium assimilation. They also suggested that the levels of intracellular ammonium pools could easily be overestimated due to contamination and nucleotide deamination. Such an overestimation could have resulted in the erroneous conclusion that intracellular ammonium levels were within the K_m range of GDH. In other marine phytoplankton species, Ahmed et al. (1977) found the ammonium K_m for GDH was between 4.5-10 mM.

Tempest et al. (1973) reported that ammonium limited bacteria showed an initial increase in glutamine directly following an ammonia perturbation. They concluded that the GS pathway of ammonium assimilation was operating but they did not show an initial drop in glutamate and a corresponding rise in glutamine. Such a covariance would be expected, over very short time intervals, if the GS pathway was responsible for ammonium assimilation (equation 1).

$$\text{glutamate} + NH_4^+ + ATP \xrightarrow[\text{divalent cation}]{GS} \text{glutamine} + ADP + Pi \qquad (1)$$

Other workers have shown that GS has a lower ammonium K_m than GDH, thereby suggesting that GS is kinetically more favorable for ammonium assimilation (Falkowski & Rivkin, 1976; Stewart & Rhodes, 1977).

In vitro enzyme kinetic results which provide evidence that the GS/GOGAT pathway operates but do not prove that this is the enzyme system functioning *in vivo*. Information regarding the products of ammonium assimilation is needed.

This chapter reports the results of studies on the response of ammonium limited chemostat cultures of *Gymnodinium simplex* and *Skeletonema costatum* to an ammonium perturbation. The fluctuations in the free amino acid pools are used to elucidate the pathway of primary ammonium assimilation in these organisms under ammonium limitation.

3) Materials and Methods

Gymnodinium simplex (NEPCC-119; Northeast Pacific Culture Collection, Department of Oceanography, The University of British Columbia) and *Skeletonema costatum* (NEPCC-18b) were isolated from Pacific Ocean water samples taken on May 16, 1973 at 48°38'N, 126°00'W and June 20, 1977 from Patricia Bay, B.C., respectively. The two species were grown in ammonium-limited chemostats in artificial seawater at 18°C as described by Davis et al. (1973). *G. simplex* was maintained in a 6-liter boiling flask at a

dilution rate of 0.25 d^{-1}. *S. costatum* was maintained in a 2-liter boiling flask at a dilution rate of 1.0 d^{-1}. The cultures were continuously stirred at 60 rpm with a magnetic stirrer. Continuous illumination was supplied by four fluorescent bulbs, three high-output Vitalite (Durotest) bulbs and one daylight Powertube VHO Sylvania. This light was filtered through a sheet of blue Plexiglas (No. 2069, Rohm and Haas, Philadelphia), 0.3 cm thick to simulate the spectrum of 5 m underwater light for coastal areas. Total irradiance was 150 $\mu Ein \cdot m^{-2} \cdot s^{-1}$. The inflow nutrient concentrations were 10 $\mu g\text{-at} \cdot \ell^{-1}$ ammonium, 3.2 $\mu g\text{-at} \cdot \ell^{-1}$ phosphate, 4.2 $\mu g\text{-at} \cdot \ell^{-1}$ silicate. In the *S. costatum* cultures, silicate was added at 35 $\mu g\text{-at} \cdot \ell^{-1}$. Vitamins and trace metals were added as in f medium (Guillard & Ryther, 1962) but at a reduced concentration of f/20.

Nutrient analysis was carried out using a Technicon Autoanalyzer, and methods previously described by Davis et al. (1973).

Cell numbers were determined by using an inverted microscope. The cells were killed with a drop of Lugol's iodine solution before counting. Culture fluorescence was measured by a Turner (Palo Alto, Calif.) Model 111 fluorometer. Fluorescence, and cell and nutrient concentrations were monitored daily and steady-state was obtained when there was no trend in these parameters over a 5-day period.

The ammonium perturbation consisted of shutting off the pump and then quickly injecting 3 ml of 10 mM ammonium chloride into the steady-state ammonium limited chemostat culture which resulted in a sudden increase in ammonium concentration from 0.2 to ca. 5 μM. The disappearance of ammonium from the medium (i.e., the uptake) was followed during the experiment. Samples for amino acid analysis were collected at 5, 10, 40 and 80 min after the perturbation. Values at time zero were the steady-state values immediately prior to the perturbation.

For amino acid extraction and analysis, one liter of culture (500 ml in

the case of *S. costatum*) was filtered onto a 47 mm glass fiber filter (Reeve Angel) at 190 mm Hg negative pressure. After filtration, the cells and filter were washed with 10 ml 30% sodium formate solution to remove any seawater contaminants. At the predetermined sample time the filter was plunged into boiling 90% ethanol contained in 15 ml screw top centrifuge tubes. The time required to filter, wash and kill the cells was 1.5 min. The filter was broken up by vigorous shaking and vortex mixing (Vortex-Genie, Fischer, Bohemia, N.Y.). The filter paper-ethanol suspension was then centrifuged and the supernatant place in 100 ml rotary evaporator flasks. The extraction of the filter and cells in boiling 90% ethanol was repeated three times and the combined extracts were evaporated to dryness. A special reflux tube (Buchi NS24/40) for amino acid analysis was used to avoid sample contamination which could occur from carryover of previous samples prepared on the same machine. The tube was rinsed with 90% ethanol before each analysis to minimize contamination.

Amino acid analysis was carried out using a Beckman 120C Amino Acid Analyzer. Acidic and neutral amino acids were separated using Li^+ form resin which provided resolution of asparagine and glutamine. The basic amino acids were separated using a 16 x 0.9 cm bed of Na^+ form resin. Operating procedures were those outlined in the Beckman Procedures Manual, A-TB-044, May 1967.

When a standard solution containing 19 amino acids was extracted using the same procedures, the extraction efficiency ranged from 95-99%; glutamate and glutamine were >99%, whereas asparagine was ca. 95%.

4) Results

Gymnodinium simplex:

The steady-state cell density was 25 x 10^6 cell$\cdot l^{-1}$ and the concentration of ammonium and phosphate was 0.2 and 1.5 $\mu g\text{-at}\cdot \ell^{-1}$, respectively. The quantities of the free intracellular amino acids following the ammonium perturbation are shown in Table I.

TABLE I. Quantities of intracellular free amino acids in ammonium limited *Gymnodinium simplex* at time zero and after ammonium perturbation: confidence limits of analysis are ±3%.

Amino acids (10^{-15} mol·cell^{-1})	Time (min)				
	0	5	10	40	80
alanine	0.75	0.72	1.21	1.65	0.85
arginine	++[a]		+++[a]	++	+[a]
asparagine	++	+	+++	+++	+
aspartate	++	++	++	+++	++
cysteine	-[b]	-	-	-	-
glycine	0.39	0.45	1.18	0.65	0.55
glutamine	0.31	0.78	0.95	1.20	0.81
glutamate	2.07	1.31	1.72	1.97	1.87
histidine	+++	+++	0.26	+++	++
isoleucine	+	++	0.16	+	-
leucine	+	++	0.26	+	+
lysine	0.31	0.17	0.51	0.50	0.27
methionine	-	+	+	+	-
phenylalanine	+	+	+++	+	+
proline	-	-	-	-	-
serine	0.74	0.50	1.51	0.56	0.52
threonine	++	++	+++	++	++
tryptophan	-	-	-	-	-
tyrosine	+	++	+++	+	+
valine	-	++	-	-	+

[a] +, ++, +++ = increasing level of detectability.

[b] - = not detectable.

Glutamate was the most predominant free amino acid under steady-state conditions of ammonium limitation with levels of 2.07×10^{-15} mol·cell^{-1}. The other major amino acids, at steady state, in order of decreasing abundance were alanine, serine, glycine, glutamine and lysine. Five minutes after the perturbation the levels of glutamate had dropped by 0.76×10^{-15} mol·cell^{-1}, whereas glutamate had increased by 0.47×10^{-15} mol·cell^{-1}. The decreasing glutamate concentration recovered after 10 min coupled with a further increase in glutamine (Table I, Fig. 2). The levels of both glutamate and glutamine increased up to 40 min and then decreased slightly.

The other major amino acids remained at more or less constant levels for the first 5 min following the perturbation. There was a marked increase by 10 min and then a continual decrease with time (Table I, Fig. 3). Ammonium concentration in the culture medium remained above 4 μM throughout the perturbation experiment.

Skeletonema costatum:

The levels of two free amino acids, glutamate and glutamine, in *S. costatum* before and 2.5 min following the ammonium perturbation are given in Table II. During this time period glutamate dropped by 0.22×10^{-15} mol·cell^{-1} and glutamine rose by 0.15×10^{-15} mol·cell^{-1}.

TABLE II. Quantities of intracellular free amino acids, glutamate and glutamine, in ammonium limited *Skeletonema costatum* at time zero and after ammonium perturbation: confidence limits of analysis are ±3%.

Amino acids (10^{-15} mol·cell^{-1})	Time (min)	
	0	2.5
glutamate	1.28	1.06
glutamine	0.79	0.94

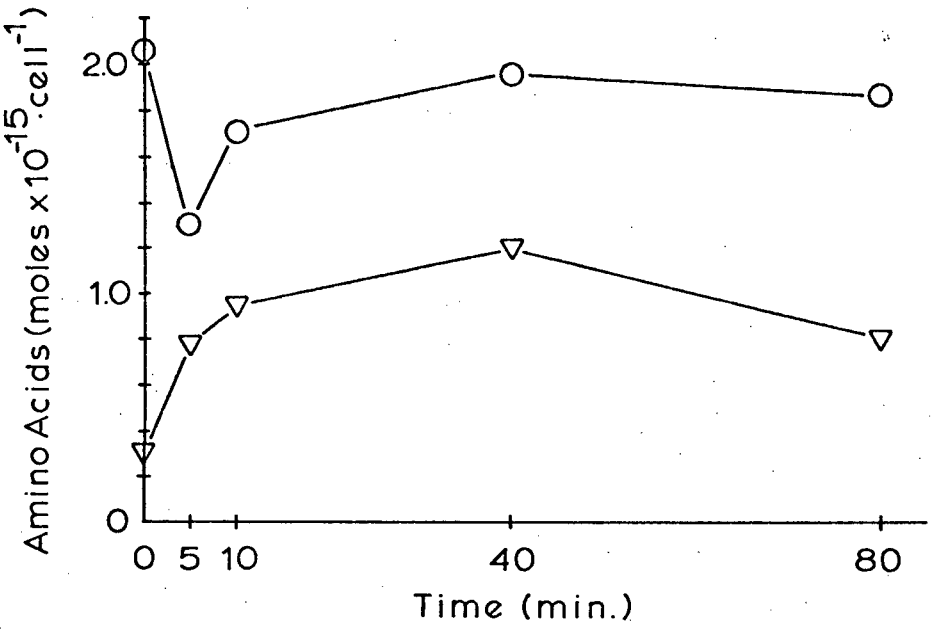


Figure 2. Fluctuations in level of free intracellular amino acid pools in response to addition of ammonium (perturbation at T=0) to ammonium limited *Gymnodinium simplex*: T=0 values are steady-state values immediately prior to perturbation: O = glutamate, ∇ = glutamine.

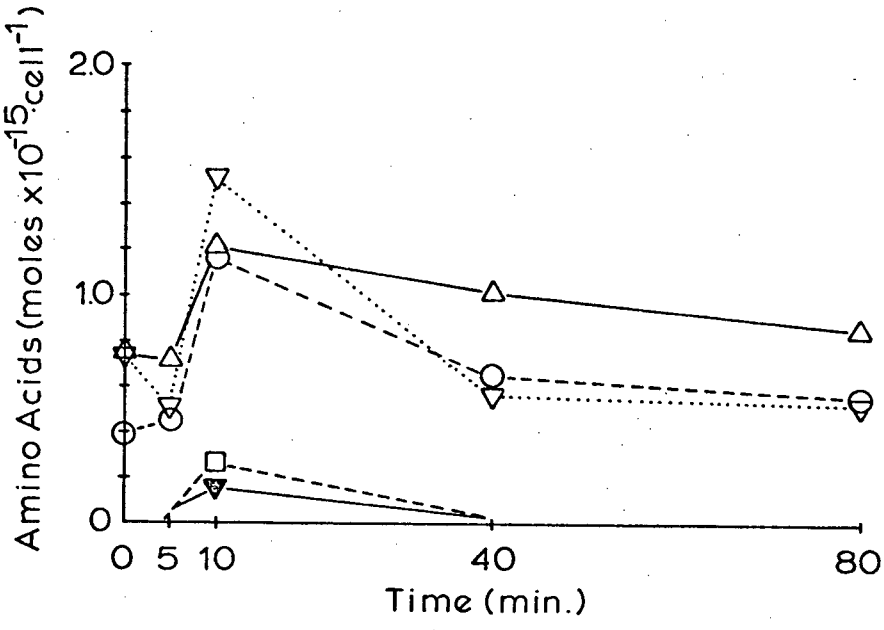


Figure 3. Fluctuations in level of free intracellular amino acid pools in response to addition of ammonium (perturbation at T=0) to ammonium limited *Gymnodinium simplex*: T = 0 values are steady-state values immediately prior to perturbation: ∇ = serine, O = glycine, Δ = alanine, ▼ = isoleucine, and ◘ = leucine.

5) Discussion

If the GS pathway is active in these two marine phytoplankton, we would expect (Equation 1) an equal molar decrease in glutamate and increase in glutamine immediately following the perturbation (i.e., a short enough time so that the GOGAT and transaminase systems would cause minimal interference). This is what was observed. The decrease in glutamate 5 min after perturbation for *G. simplex* was 0.76×10^{-15} mol·cell^{-1}. This leaves 0.29×10^{-15} mol·cell^{-1} of glutamate unaccounted for if an equal molar GS reaction occurs (Equation 1). Likewise in *S. costatum*, 0.07×10^{-15} mol·cell^{-1} of glutamate was unaccounted for. This discrepancy can be partially explained by noting the slight increase in the levels of other amino acids (glycine, leucine, tyrosine, isoleucine) in *G. simplex* cells, over the same period. The biosynthesis of these amino acids results in loss of glutamate by the transaminase enzymes. Further interference could arise from the production of glutamate by glutamate synthase but, as a result of close agreement between the observed and expected results after 5 min, the contribution of GOGAT to the glutamate pool by that time was probably small. After 10 min the contribution of GOGAT to the glutamate pool appeared to be substantial as indicated by the increased glutamate levels. Amino acids other than glutamate and glutamine were not quantitated in the *S. costatum* experiment.

If the GDH was responsible for the primary assimilation of ammonium in this organism we would expect to see an initial rise in the levels of glutamate rather than a drop. The reaction mediated by glutamate dehydrogenase is:

$$\alpha\text{-ketoglutarate} + NH_3 + NAD(P)H + H^+ \xrightleftharpoons{GDH} \text{glutamate} + NAD(P)^+ + H_2O \quad (2)$$

It would also be impossible to account for the rapid rise in glutamine concentration if the GDH system was solely responsible for ammonium assimilation. Glutamate synthase, the enzyme responsible for the interconversion of glutamate and glutamine is essentially irreversible, favoring the formation of glutamate from glutamine and α-ketoglutarate (Woolfolk et al., 1966). The reaction catalyzed by GOGAT is:

$$\text{glutamine} + \alpha\text{-ketoglutarate} + NAD(P)H + H^+ \xrightarrow{GOGAT} 2 \text{ glutamate} + NAD(P)^+ \quad (3)$$

In *G. simplex*, the responses of the other major amino acids to the perturbation, are markedly different from those exhibited by glutamate and glutamine. The relatively constant levels of the other amino acids from 0 to 5 min are consistent with the GS pathway being responsible for primary ammonium assimilation in this organism. The rapid increase in the levels of these amino acids 10 min after the perturbation reflects the rapid transfer of the amino nitrogen throughout the amino acid pool as a result of transaminase activity. Interestingly, glutamate never rose above its steady-state level. This implies that glutamate utilization increased rapidly following the perturbation.

The decrease in the amino acid levels near the end of the time series was not due to ammonium limitation as the ammonium levels never dropped below 4 $\mu g\text{-at} \cdot \ell^{-1}$ in the external medium. This level is well above the K_s for ammonium uptake for this organism (Turpin, unpubl.). The decrease, best exhibited in the amino acids other than glutamate and glutamine, is undoubtedly due to some form of limitation. It is possible, due to the low steady-state

growth rate (1/5 μ_{max}) of the organism that there was a reduction in the activity of many enzyme systems. This low sub-maximal growth rate could result in the inability of the enzyme systems responsible for carbohydrate utilization and α-ketoglutarate production to provide an adequate source of carbon skeletons needed to allow the initial rapid ammonium assimilation. The possible intracellular competition for ATP between ammonium uptake and assimilation and CO_2 fixation could also result in decrease of available carbon skeletons (Falkowski & Stone, 1975). Either of these possibilities coupled with amino acid utilization, would account for the observed decrease in the amino acid levels.

The reason that a drop in glutamate, in response to an ammonium addition, was not observed by other workers (Tempest et al., 1973) may be due to the high metabolic rates of the bacteria used and the inability to obtain adequate short-term time series data relative to the organism's metabolic rate. The experimental conditions were such that the organism's metabolic rate was slow enough to allow resolution of the intracellular glutamate drop.

Chapter II

LIMITING NUTRIENT PATCHINESS AS A FACTOR IN PHYTOPLANKTON ECOLOGY

1) Summary

The effect of limiting nutrient patchiness on community structure and species succession was examined in natural phytoplankton communities held in ammonium limited continuous culture at a dilution rate of 0.3 day^{-1}. Under a homogeneous distribution of the limiting nutrient members of genus *Chaetoceros* dominated but when ammonium was added daily (patchy distribution), *Skeletonema* dominated. Intermediate patchiness gave rise to an assemblage dominated by both *Chaetoceros* and *Skeletonema*. The nutrient uptake ability of each assemblage was determined three weeks after experiment initiation. Each assemblage was best able to optimize uptake of ammonium under its particular patchy nutrient regime. Optimization of a patchy environment took place by an increased maximal uptake rate (V_{max}) while optimization of a homogeneous environment appeared to take place by increased substrate affinity (i.e., low K_s). It is also shown that coexistence of two populations might be expected due to the patchiness of a single limiting nutrient. The importance of patchiness in relation to other factors which determine community structure is discussed.

2) Introduction

The importance of nutrients in limiting phytoplankton growth in aquatic systems has long been realized. There are several mechanisms by which ambient nutrient concentrations may control phytoplankton growth and hence community structure. Species specific growth and nutrient uptake kinetics and associated parameters (V_{max}, μ_{max} and K_s) have been proposed by Dugdale (1967) and shown by Eppley et al. (1969) to be important in explaining species succession. Titman (1976) and Tilman (1977) showed that community structure can be affected by different resource limitations (silicate and phosphate) which act on different populations within the community. This idea has also been developed in simulation models of species competition (Taylor & Williams, 1975). Grenney et al. (1973) suggested through their modelling efforts, that low frequency resource fluctuations may result in an unstable coexistence of phytoplankton populations limited by a single resource. Stross and Pemrick (1974) and Chisholm and Stross (1976) have provided a case for niche separation based on periodicity in nutrient uptake kinetics. Mickelson et al. (1979) observed changes in the outcome of competition between marine diatoms as a result of changes in continuous culture dilution rate, or in a more ecological sense, the specific flux of the limiting nutrient. In this experimental system, species selection occurs on the basis of growth kinetics.

All the preceding studies considered only a homogeneous distribution of the limiting nutrient. There have been no studies on the effect of temporal patchiness of the limiting nutrient on phytoplankton competition and growth. The ability of various populations, or individuals of a population, to utilize a patchy resource could be instrumental in mediating resource competition and hence species succession in aquatic systems.

This study was designed to answer two questions: 1) Can limiting nutrient patchiness influence phytoplankton succession and community

structure? 2) If so, are there physiological differences (i.e., nutrient uptake ability) among the resulting assemblages that would allow for optimal use of the limiting nutrient in a particular patchy regime?

Continuous culture systems have been used extensively by microbiologists (Jannasch, 1967, 1968a & b; Meers, 1971, 1973; Veldkamp & Kuenen, 1973; Harder et al., 1977) to analyze factors influencing microbial selection in mixed species systems. This technique has been adapted for the study of competition in phytoplankton communities (Dunstan & Menzel, 1971; Titman, 1976; Tilman, 1977; Mickelson et al., 1979; Harrison & Davis, 1979).

Nitrogen is the most frequent nutrient limiting plant growth in the sea. Of its inorganic forms, ammonium is the most readily regenerated (Dugdale, 1976; Harrison, 1978). I chose to examine the effects of ammonium patchiness on ammonium limited natural phytoplankton assemblages maintained in continuous culture.

3) Material and Methods

The experiments were conducted at the CEPEX (Controlled Ecosystem Population Experiment) site at Saanich Inlet, Vancouver Island, British Columbia, Canada. Experiment 1 was conducted in July 1978 and was duplicated (Experiment 2) in August 1978.

3.1) Inoculum:

A natural assemblage of marine phytoplankton was obtained from a large controlled ecosystem enclosure (CEE) as described by Menzel and Case (1977). Assemblages for Experiment 1 and 2 were obtained from a sample integrated from 4-8 m in CEE 78-2, on July 10 and August 9, 1978, respectively, and filtered through 153 µm Nitex netting to remove any large zooplankton. The inoculum for Experiment 2 was allowed to grow for 4 days and then a small inoculum of *Skeletonema costatum* (Grev.) Cleve, *Thalassiosira nordenskioldii* Cleve, *Chaetoceros socialis* Lauder and *C. constrictus* Gran was added so that

these species, which were absent from the natural sample but present in the inoculum for Experiment 1, could be observed in the succession sequence.

3.2) Incubation:

Cultures were maintained in outdoor continuous cultures. Three liter, borosilicate, flat bottom, boiling flasks were placed in a water-filled Plexiglas incubator system similar to that described by Davis et al. (1973). Temperature was maintained at 13 ± 1°C by a cooling unit (Haws HR4-20). Natural sunlight was attenuated and spectrally corrected to simulate Jerlov Type 3 coastal water at 5 m (Holmes, 1957) by surrounding the incubator with 1/8" blue Plexiglas (Rohm and Hass, #2069) (Davis et al., 1973). As the culture vessels were submerged in flowing water, infra-red wavelengths were also removed.

3.3) Inflow medium:

Two hundred liters of Saanich Inlet surface water were collected on July 11, 1978. The water was filter-sterilized using a 147 mm, 0.45 μm Millipore filter and stored in a 200-liter Nalgene barrel in the dark in a cold room for the duration of the experiment. Nutrient analyses indicated that the water had 0.5 $\mu g\text{-at}\cdot\ell^{-1}$ total inorganic nitrogen (NO_3^-, NO_2^-, NH_4^+) 7.5 $\mu g\text{-at}\cdot\ell^{-1}$ SiO_4^{-4}, and 0.4 $\mu g\text{-at}\cdot\ell^{-1}$ PO_4^{-3}. This water was used as a stock supply throughout the experiment. Aliquots were removed and enriched to the desired inflow concentrations as needed. Samples of the inflow medium were taken regularly to check nutrient concentrations.

3.4) Patchiness regimes:

Three continuous cultures were set up as outlined below, with a constant dilution rate of 0.3 day^{-1} maintained by piston pumps (Fluid Metering, Inc.). Each culture received the same amount of ammonium each day and only the temporal distribution of ammonium varied, from continual addition, 8 additions/day to 1 addition/day.

a) System 1: Continual addition (Fig. 4). Inflow medium for Experiment 1 was enriched to 10 μg-at.ℓ^{-1} ammonium chloride, 3 μg-at.ℓ^{-1} potassium phosphate (monobasic) and 20-45 μg-at·ℓ^{-1} sodium silicate. Vitamins and trace metals were added as f/25 (Guillard & Ryther, 1962). The inflow medium during Experiment 2 was identical to Experiment 1, except PO_4^{-3} and SiO_4^{-4} were increased to 3.5 and 50 μg-at·ℓ^{-1}, respectively.

b) System 2: 8 additions/day (Fig. 4). Dilution rate and nutrient concentrations in the inflow medium were identical to System 1 except the latter contained no added NH_4Cl. The NH_4Cl additions were controlled by a separate pump which was turned on and off with a specially modified timer (Cincinnati, Model 422). Additions were 1 min in duration (0.94 ml of 1.2 mM NH_4Cl) and occurred every 3 hr starting at 2400 hr. This resulted in the daily nitrogen flux being identical to that of System 1 (i.e., 3 μg-at·ℓ^{-1}·day^{-1}). This system, with its independent flow of seawater and non-limiting nutrients, assured that the only variable between the two systems was the temporal distribution of ammonium.

c) System 3: 1 addition/day (Fig. 4). This system was similar to System 2, except the NH_4Cl addition was at 0100 hr and consisted of 7.5 ml of 1.2 mM NH_4Cl over a 1 min interval. The daily ammonium flux through this system was the same as the other systems (3 μg-at·ℓ^{-1}·day^{-1}).

3.5) Measurements:

Culture effluents were collected daily and preserved for identification and enumeration in Lugol's iodine. An inverted microscope was used for enumeration of samples. Fluorescence was monitored with a fluorometer (Turner Model 111) equipped with a high sensitivity door. Nutrients in culture effluents were analyzed with a Technicon Autoanalyzer using methods previously described (Davis et al., 1973).

After approximately 3 weeks of exposure to the nutrient regimes, the responses of the resulting assemblages to a nutrient pulse or perturbation

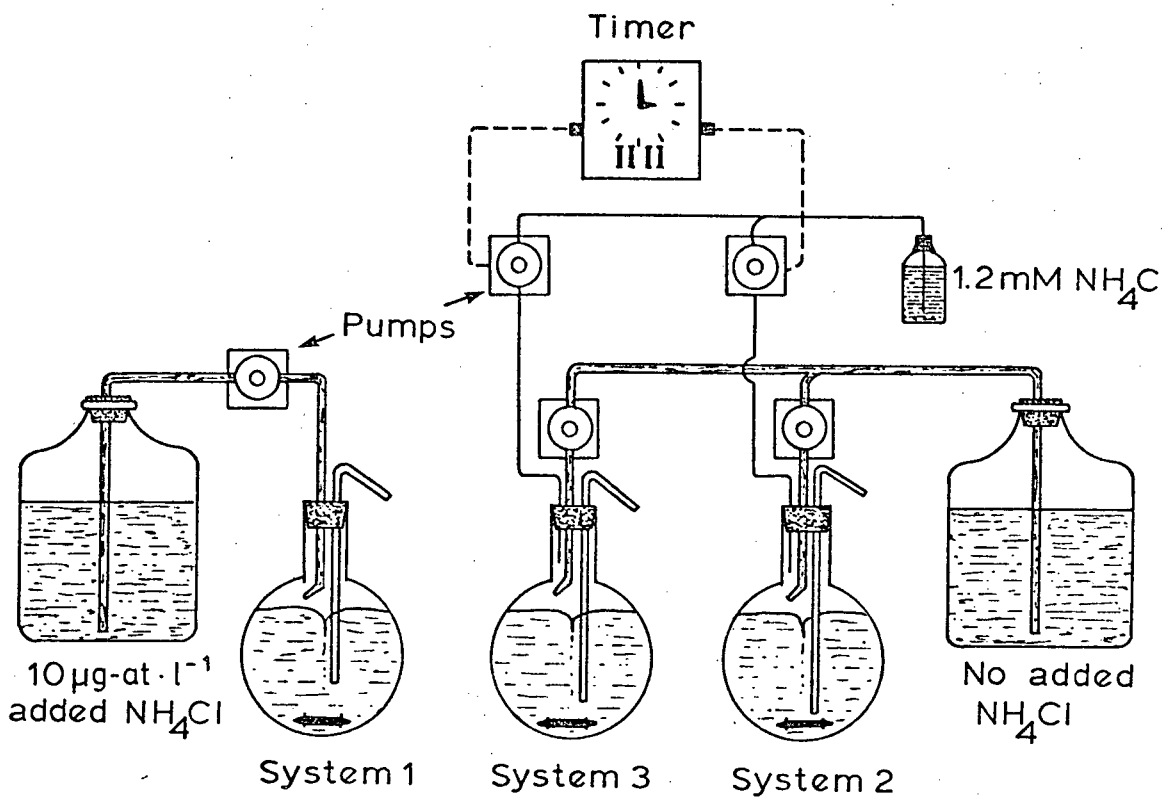


Figure 4. A schematic representation of the three culture systems. All systems were continuous flow (D = 0.3 d^{-1}). System 1 received NH_4Cl continually in the inflow medium. System 2 received 8 additions/day, while System 3 received 1 addition/day of NH_4Cl. The flux of NH_4Cl through all Systems was 3 µg-at·ℓ^{-1}.day^{-1}. The reactors were in a water bath (13 $\pm$ 1°C) as described in the text.

(Caperon & Meyer, (1972b) was determined. Nutrient disappearance was followed with a Technicon Autoanalyzer. The ability of each phytoplankton assemblage to respond to the various patchiness regimes was determined in this manner.

4) Results

4.1) Experiment 1:

a) Ambient nutrients: The continuous flow pumps were started after the inoculum had grown as a batch culture for 1 day. On day 2, ambient ammonia concentrations (measured each day between 1000-1200 hr) reached a maximum of 1.3, 0.8 and 0.9 $\mu g\text{-}at \cdot \ell^{-1}$ for Systems 1, 2 and 3, respectively. For the rest of the experiment, ambient ammonium concentration never rose above 0.6 $\mu g\text{-}at \cdot \ell^{-1}$.

Silicate concentrations in the inflow medium were raised periodically throughout the experiment to keep the ambient levels well above silicate limitations (Fig. 5). After day 7 there was a distinct trend in the ambient silicate concentration with System 1 having the highest concentration followed by 2 and 3, respectively (Fig. 5).

b) Community structure: The composition of the initial community is illustrated in Table III. Species present were *Skeletonema costatum*, *Chaetoceros simplex* Ostenfeld, *C. simile* Cleve, *C. compressus* Lauder, *C. constrictus*, *C. debilis* Cleve, *Thalassiosira rotula* Meunier, *T. nordenskioldii*, *Nitzschia longissima* (de Brebisson ex Kutzing) Ralfs, *N. pungens* Grunow, *N. delicatissima* Cleve, *N. palea* (Kutzing) W. Smith, *Stephanopyxis turris* (Grev.) Ralfs, *Cerataulina bergonii* (H. Peragallo) Schutt, small flagellates and a few representatives of other diatom genera such as *Rhizosolenia* and *Leptocylindrus*.

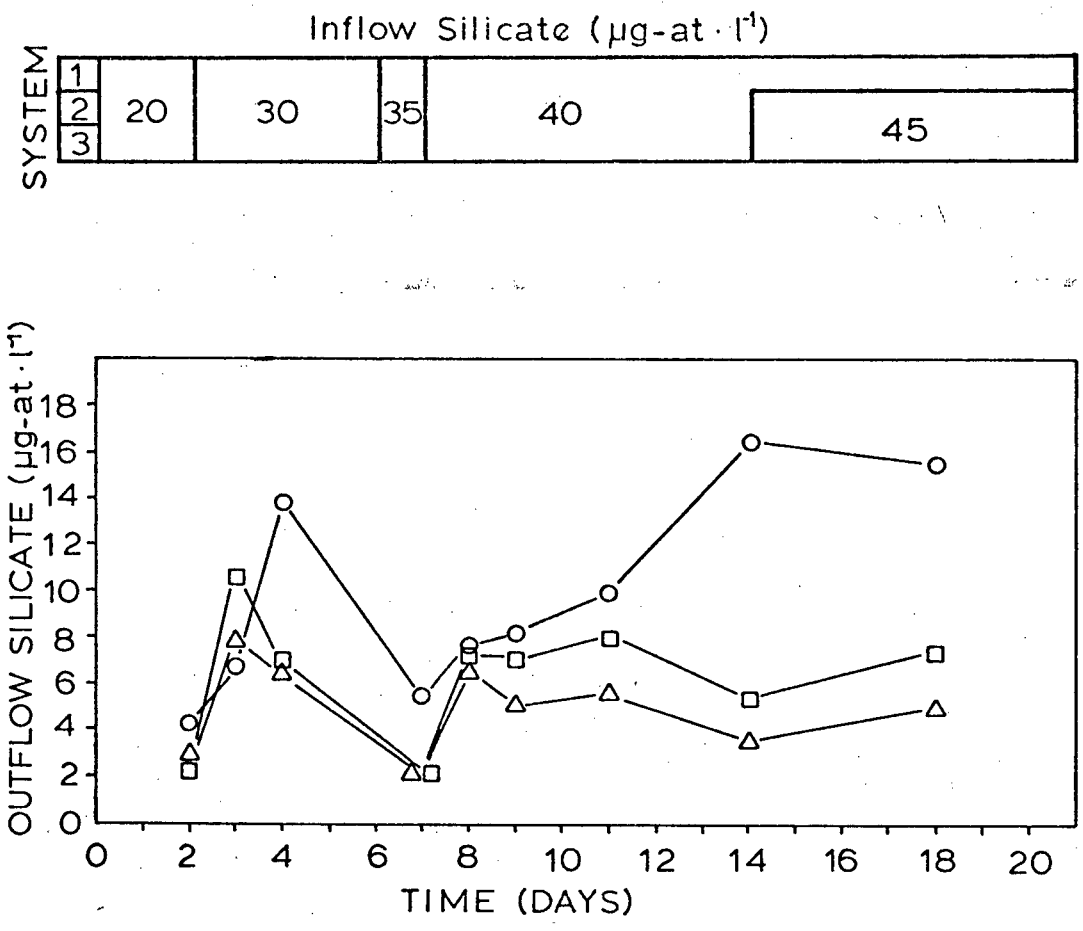


Figure 5. Inflow and outflow silicate concentrations (Exp. 1). System 1, (O); System 2, (□); System 3, (Δ).

Table III. The compostion of the initial inocula for Experiments 1 and 2.

	Exp. 1 ($x10^6$ cell$\cdot l^{-1}$)	Exp. 2 ($x10^6$ cell$\cdot l^{-1}$)
Skeletonema	0.63	1.4
Chaetoceros	3.5	3.4
Thalassiosira	0.92	5.9
Stephanopyxis	--	2.4
Cerataulina	--	0.7
Nitzschia	0.96	1.4
Flagellates	1.6	8.6
Others	1.4	--
TOTAL	9.01	23.5

The community structure in all 3 systems remained constant for the first 3 days as evidenced by: 1) the domination by the genus *Chaetoceros*, and, 2) the constant relative abundances of *Skeletonema* and *Chaetoceros* (Figs. 6A, B, C & 7A,B).

By day 5, *Skeletonema* was approximately 5 times more numerous in the patchy systems (2 & 3) than it was in System 1 (Fig. 6A,B,C). From day 9 to the termination of Experiment 1, *Skeletonema* and *Chaetoceros* accounted for 65% to 85% of the total cell numbers in all three treatments. The primary difference among the final assemblages was the relative proportions of *Chaetoceros* and *Skeletonema* present.

Chaetoceros continued to dominate System 1 until the end of the experiment (Figs. 6A & 7B). System 2 (8 additions/day) was dominated by *Chaetoceros* until day 16 after which *Skeletonema* was the most abundant. *Chaetoceros* was dominant until day 9 in System 3 (1 addition/day) with *Skeletonema* dominating thereafter.

An ammonium limited semi-continuous culture was started at the same time as Experiment 1. It was diluted once a day and had the same turnover rate and ammonium flux as System 3, and thus, this semi-continuous culture served as a replicate. After 3 weeks both Systems 3 and the semi-continuous culture replicated very well (Figs. 7A & B) when considering the treatment differences.

c) Nutrient uptake: Uptake rates were measured at the end of each experiment. The ability of each culture to procure ammonium under the ammonium addition regimes of the other systems was determined. For example, System 3 received an addition of ammonium each day at 0100 hr bringing the reactor concentration to 3 $\mu g\text{-at} \cdot \ell^{-1}$. Therefore, this addition was made to aliquots of each of the 3 cultures at 0100 hr and the disappearance of ammonium in the dark was followed.

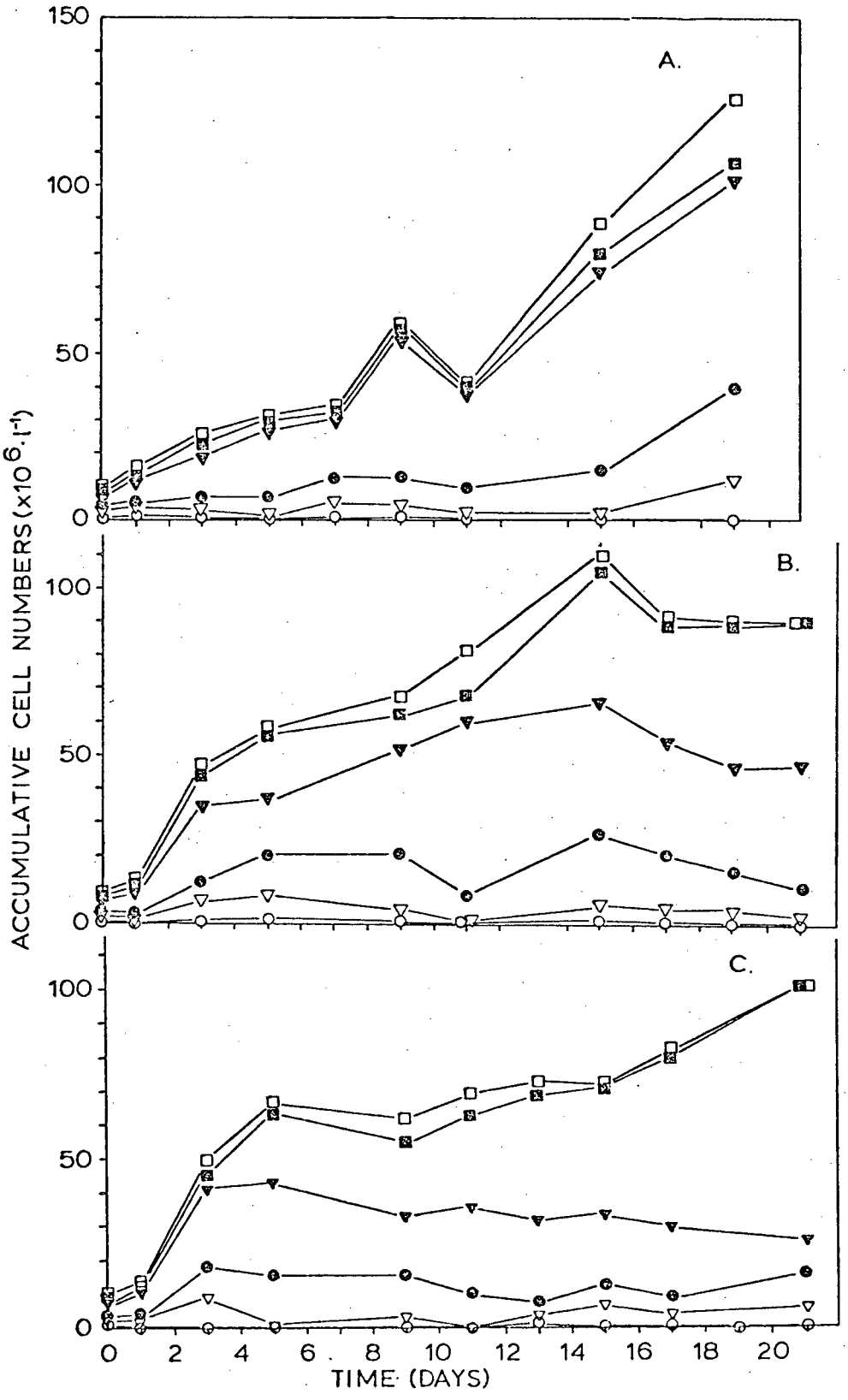


Figure 6. Accumulative cell numbers (Exp. 1) *Thalassiosira*, (O); Flagellates, (▽); *Nitzschia*, (O); *Chaetoceros*, (▽); *Skeletonema*, (■); Total numbers, (□): A, System 1: B, System 2: C, System 3.

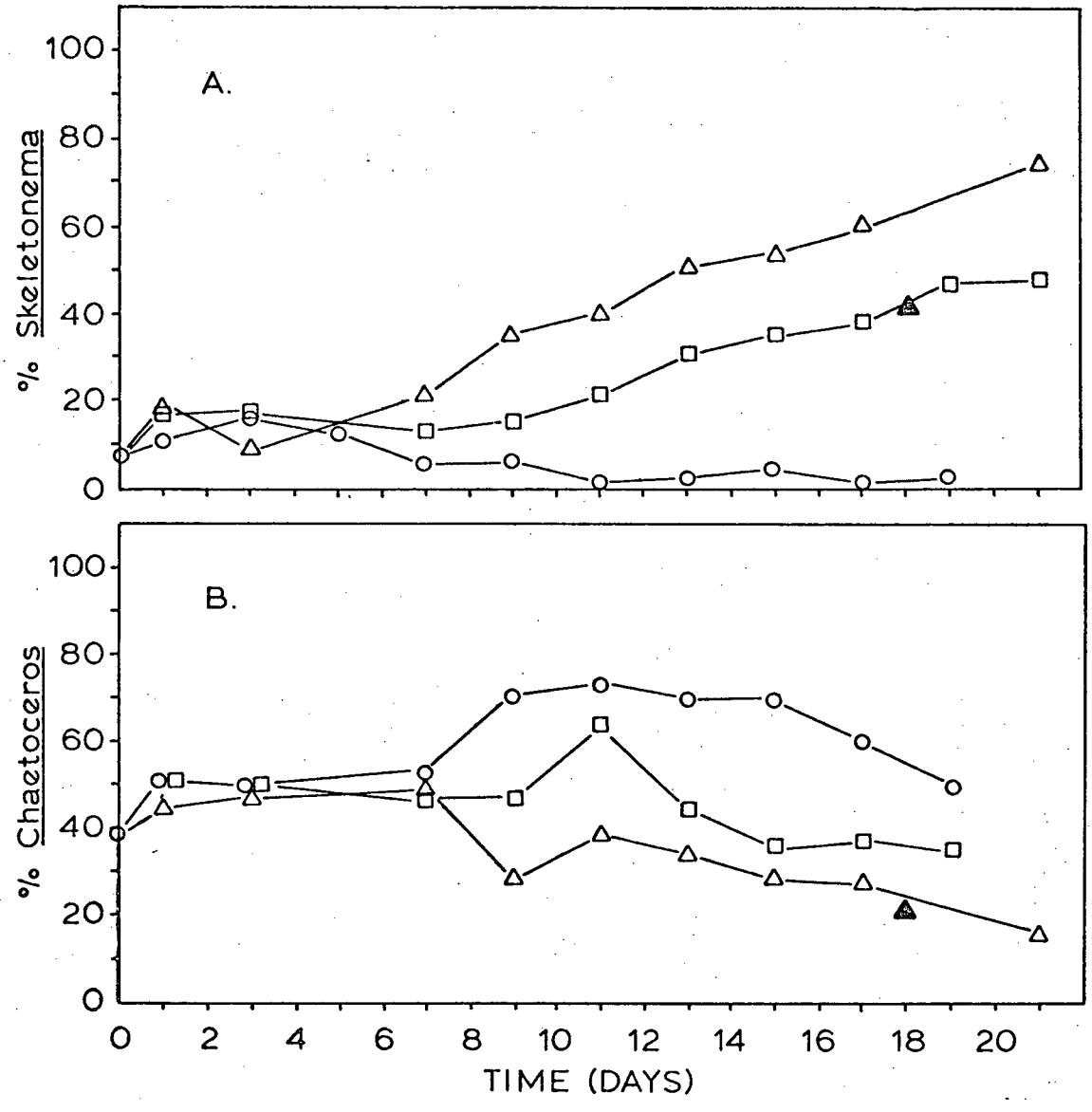


Figure 7. Relative cell numbers (Exp. 1). System 1, (o); System 2, (□); System 3, (Δ); semi-continuous culture (▲) (see text): A, percent *Skeletonema*: B, percent *Chaetoceros*.

The decreases in ammonium concentration with time for the 3 systems is seen in Fig. 8. The system previously exposed to this once a day addition (System 3) had the highest uptake. System 2 was exposed to pulses of lower concentration but still exhibited far higher uptake than did System 1 which was subject to a relatively homogeneous ammonium distribution over the preceding 3 weeks.

This uptake experiment was repeated during the day at 1200 hr. The pattern of uptake and magnitude of the rates during the day (Fig. 9) were essentially the same as those at night, with System 3>2>1, in ranked order of ability to take up the large pulse of the limiting nutrient.

The uptake characteristics of all cultures when exposed to the addition regime of System 2 were examined by repeating the perturbation experiments with a smaller nutrient addition (0.38 $\mu g\text{-at}\cdot\ell^{-1}$) so that the ambient ammonium concentration was equivalent to the additions to System 2. At this low NH_4Cl concentration, the uptake of System 2 was so rapid that no ambient ammonium was detected 2 min after the addition. It was, however, still detectable in aliquots from Systems 1 and 3. In another experiment to further assess their rapid uptake abilities, a larger addition of NH_4Cl (1 $\mu g\text{-at}\cdot\ell^{-1}$) was added to another aliquot from each system. The ambient NH_4^+ concentration was 0.36 $\mu g\text{-at}\cdot\ell^{-1}$ 2 min after the perturbation of System 2. Both Systems 1 and 3 had substantially more ammonium remaining after a similar 2 min incubation.

4.2) Experiment 2:

A duplication experiment was initiated a few days after the termination of Experiment 1. Systems 2 and 3 were lost in an accident. The differences in the species composition between this inoculum and that of Experiment 1 are shown in Table III. The community structure of System 1, expressed as relative number of *Skeletonema* and *Chaetoceros*, is shown in Figure 10. The fact that *Chaetoceros* dominated over *Skeletonema* throughout the experiment

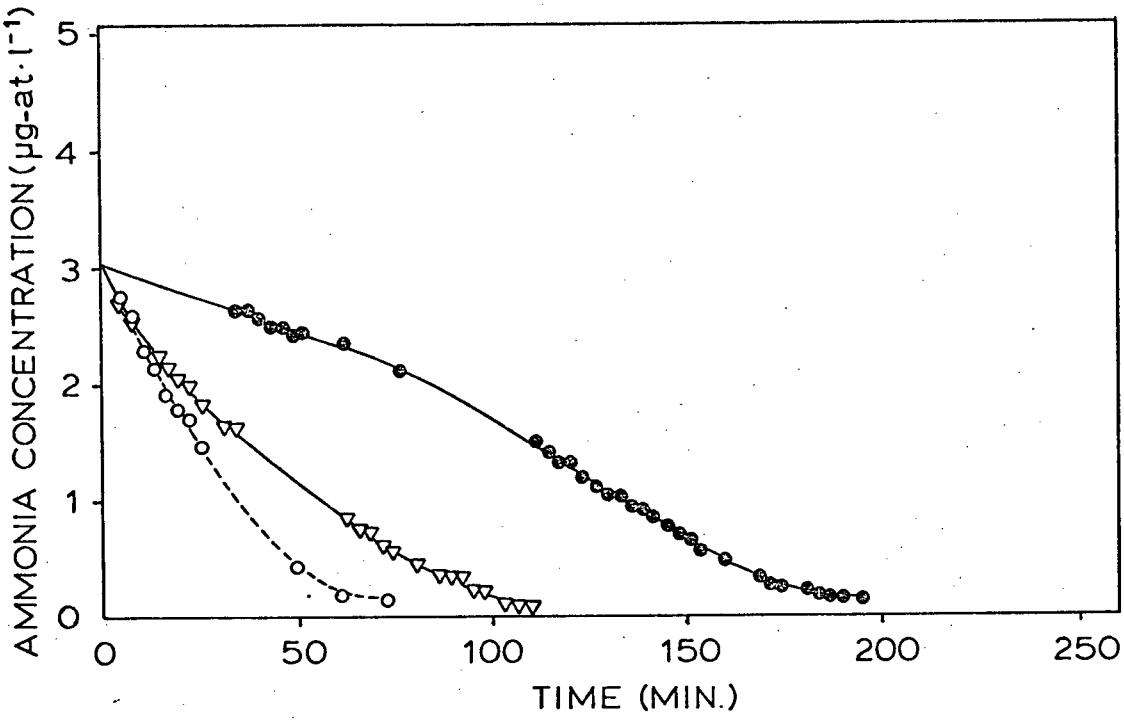


Figure 8. Disappearance of ammonium with time, following an ammonium perturbation at 0100 hr (Exp. 1). System 1, (●); System 2, (▽); System 3, (○).

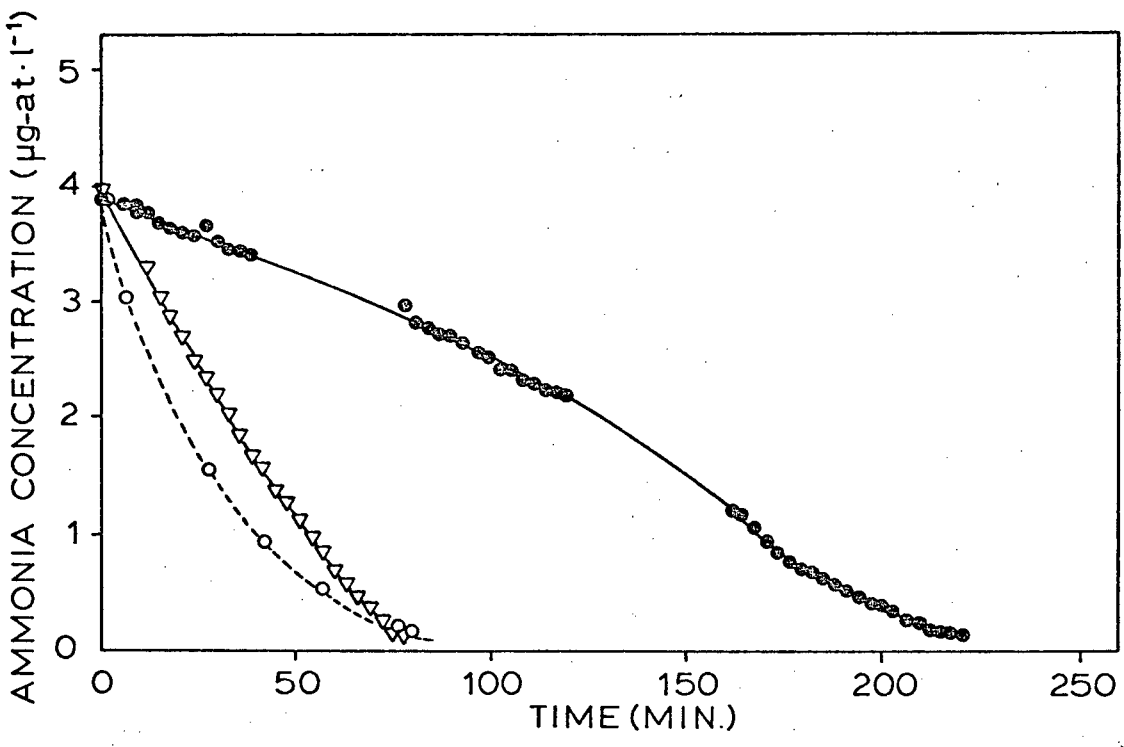


Figure 9. Disappearance of ammonium with time, following an ammonium perturbation at 1200 hr (Exp. 1). System 1, (●); System 2, (▽); System 3, (0).

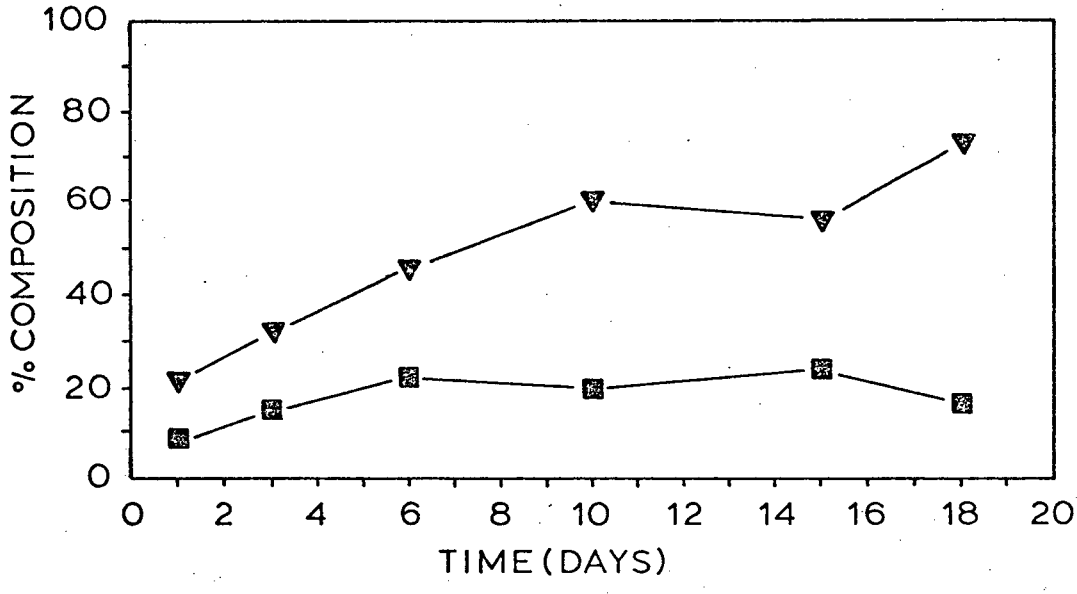


Figure 10. Relative diatom numbers (Exp. 2, System 1): percent *Skeletonema* (■) and *Chaetoceros* (▼).

points to the high degree of consistency in these data, even though both the initial inoculum (Table III) and the irradiance (Fig. 11) differed markedly from that in Experiment 1.

5) Discussion

The primary difference in community structure among the final assemblages was in the proportion of *Skeletonema* and *Chaetoceros* present. Differences in final composition must, therefore, have been mediated through differences in competitive ability for the limiting nutrient over the range of patchiness presented.

The reason that System 2 took up low concentration pulses faster than System 3 (and yet the opposite holds true for high nutrient concentration pulses) may be due to the complexity of the uptake mechanism itself. Conway et al. (1976) and Conway and Harrison (1977) showed that after addition of the limiting nutrient to a phytoplankton culture there is often an initial, short-lived, rapid uptake, followed by a more constant, possibly internally or feedback controlled uptake rate. Due to the dual nature of the uptake mechanism, the preceding observations could be explained if System 2 had the more rapid initial uptake rate, but System 3 had the more rapid internally controlled or long term uptake rate (see also Appendix II).

The high uptake rate of the assemblages exposed to a patchy regime indicated selection of populations best able to procure the limiting nutrient in its patchy distribution.

It can be inferred that assemblage 1 with its low maximal uptake rate must therefore compensate by having a lower K_s for ammonia than either of the other assemblages. If this were not the case, assemblage 1 could not have been selected for on the basis of nutrient uptake and growth ability. Mickelson et al. (1979) have used the same reasoning to rank growth kinetics of 3 species of diatoms based on their competitive abilities.

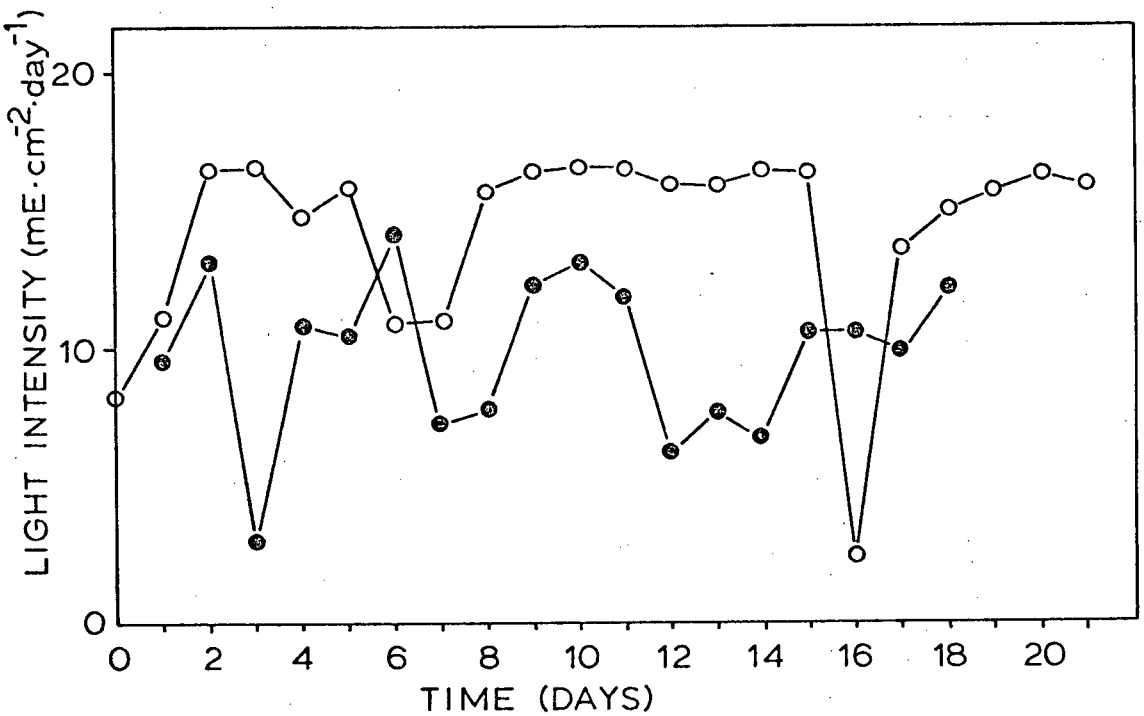


Figure 11. Surface light intensity (400-700 nm). A light filter (see text) was used to reduce it by 50% during experiments. Experiment 1, (O); Experiment 2, (●).

The results obtained here are consistent with other published work on phytoplankton physiology and competition. Conway and Harrison (1977) showed that when ammonium limited *Skeletonema costatum* was perturbed with an addition it was able to take up the pulse more rapidly than a similar culture of *Chaetoceros debilis*. Mickelson et al. (1979) showed that in ammonium limited continuous cultures (with a homogeneous ammonium distribution) *Chaetoceros* can, in some cases, out-compete *Skeletonema*.

5.1) Replicability:

In any study it is important to draw attention to the reproducability and consistency of the results. In this study, three treatments (3 variations in the temporal distribution of the limiting nutrient) were employed. There are three sets of observations that indicate consistency among treatments:

First is the trend in community structure, with assemblage 1 being dominated by *Chaetoceros*; assemblage 2 codominated by *Chaetoceros* and *Skeletonema* and assemblage 3 by *Skeletonema*; second, the trend in ambient silicate concentrations, and third, the trend in community physiology.

Replicability was demonstrated by a semi-continuous culture (daily dilution) most closely approximating System 3. The resulting trends in community structure were very consistent (Fig. 7) in spite of the treatment differences (semi-continuous dilution, daytime ammonia addition compared with continuous dilution, nighttime ammonia addition). The duplication of System 1 in Experiment 2 showed the same trend in community structure as System 1 in Experiment 1, even though the inoculum and light conditions varied greatly.

The internal consistency of the data and the replication and duplication of predominant trends indicates the consistency of the results as a function of the given treatments.

5.2) Ecological considerations:

Limiting nutrient patchiness can occur by many mechanisms. The regeneration of nutrients in the euphotic zone has been shown to be an important source of nutrients for phytoplankton growth (Eppley et al., 1973; Dugdale, 1976). Since nutrient regeneration may occur at point sources, concentration gradients and patches may be maintained for some time due to the low contribution of turbulence to dissipation at small size scales. Upwelling, runoff, advection, diel zooplankton migration and other phenomena can cause large scale temporal and spacial nutrient patchiness. Nutrient patchiness would then appear to be a phenomenon that occurs on scales from micrometers (bacteria, zooplankton) to kilometers (upwelling and runoff).

These experiments showed that, under identical daily nutrient fluxes, the outcome of competition between the two dominant populations, *Skeletonema* and *Chaetoceros*, was mediated by the patchiness of the limiting nutrient. Figure 12 shows a simple competition scheme where the competitive advantage of each group is expressed as a function of patchiness. The two curves should intersect and the point of intersection represents the degree of patchiness mediating coexistence of the two groups. This could account for apparent long term coexistence seen in natural systems where more than one organism is limited by a single resource.

Optimization of a patchy limiting nutrient environment, over the range tested, appears to occur by an enhanced maximal uptake rate (V_{max}) while adaptation to a homogeneous limiting nutrient system appears to be more a function of substrate affinity (K_s). Neither of these adaptive mechanisms should be considered mutually exclusive as uptake is still a function of both V_{max} and K_s, at any nonsaturating substrate concentration.

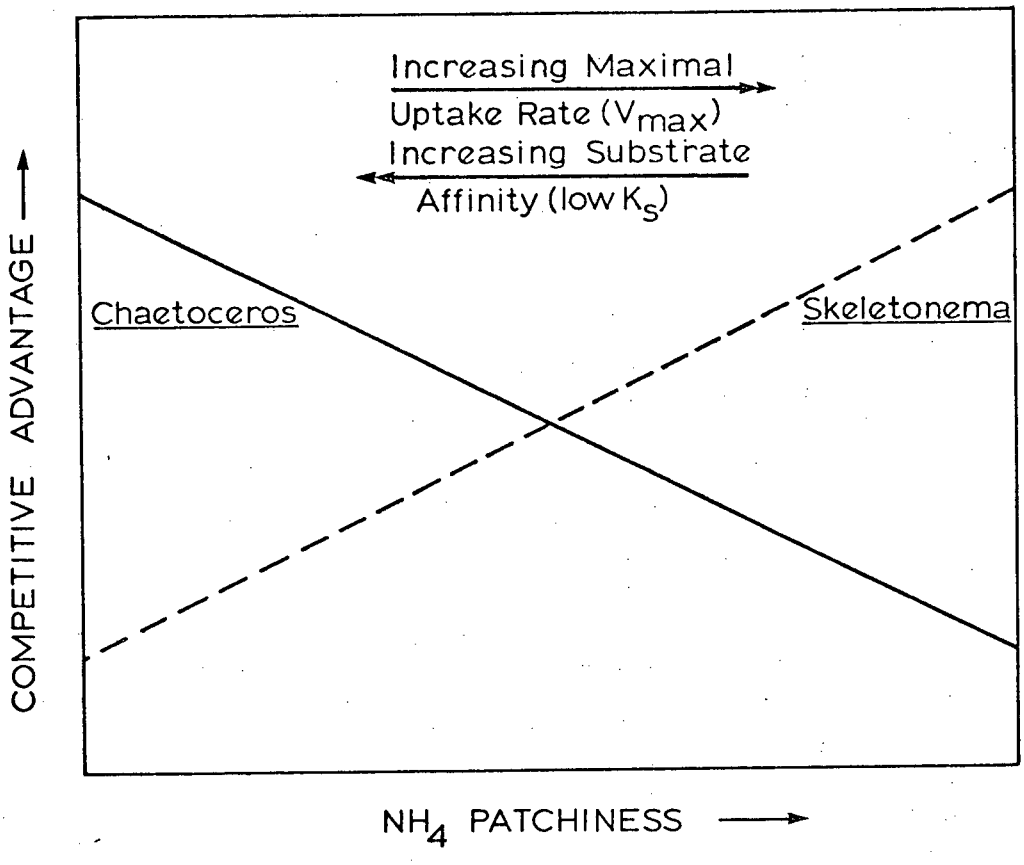


Figure 12. Possible changes in competitive advantage between *Chaetoceros* and *Skeletonema* as a function of ammonium patchiness.

There are two factors of importance in the outcome of competition for a patchy resource. The first are differences in interspecific nutrient uptake ability. These inherent differences in the genetic make-up of different phytoplankton species give rise to overall differences in competitive ability (Doyle, 1975). Superimposed on this variability is intraspecific variability mediated by such factors as life cycle stage (Davis et al., 1973), growth rate (Eppley & Renger, 1974; Turpin, unpubl.), past history (Chapter 4), light quantity and quality and temperature. When a shift in community structure is observed, however, it can be concluded that the interspecific differences are of greater importance.

The importance of temperature (Eppley, 1972) and light (Ryther, 1956) in the establishment of upper limits for phytoplankton growth and affecting competition is well documented (Goldman & Ryther, 1976). Under conditions of controlled light and temperature it has been shown that the specific flux of the limiting resource can affect the outcome of competition (Meers, 1971; Harder et al., 1977; Mickelson, In Press; Mickelson et al., 1979; Harrison & Davis, 1979). A schematic representation of the effects of the specific nutrient flux of the limiting nutrient on community structure is given in Fig. 13. Once the general community structure has been set by the specific flux of the limiting nutrient factors such as patchiness, fine-tune the system with respect to determining population dominance. The specific flux in these systems was such that it selected for fast-growing centric diatoms. The patchiness imposed, determined which centrics would dominate.

At the same time that one nutrient is limiting for some species, other species may be limited by other nutrients (Titman, 1976; Tilman, 1977; Rhee, 1978). The same argument for community fine-tuning by patchiness can be used for populations limited by any resource. The importance of differential sinking and herbivore grazing (Steele & Frost, 1977) cannot be ignored as

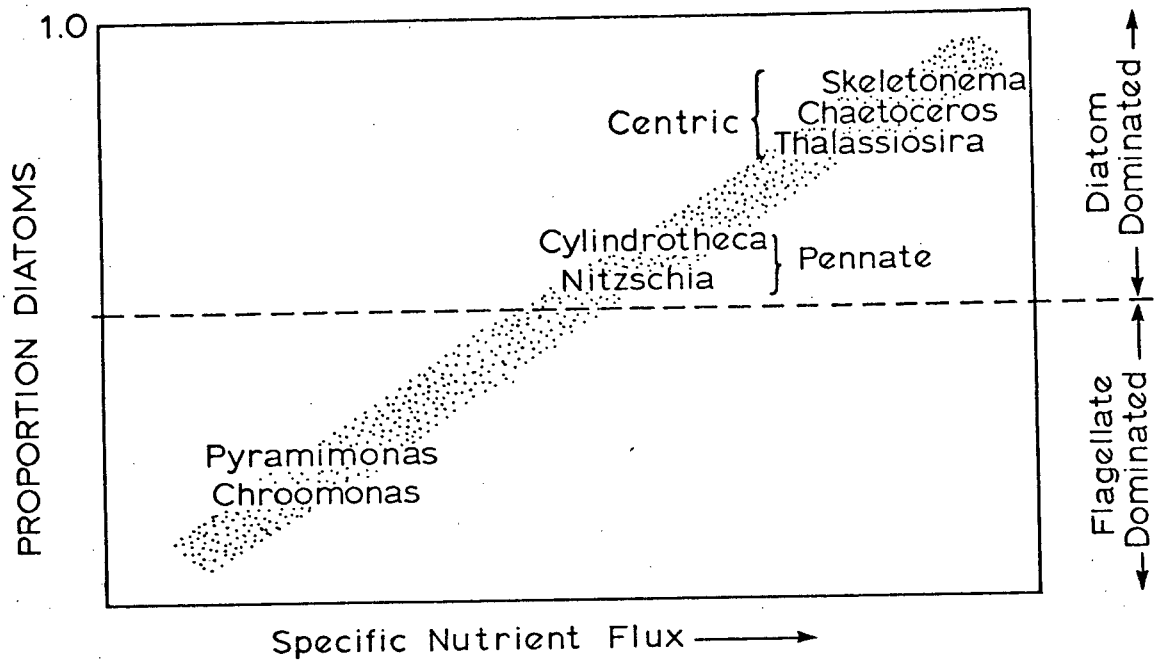


Figure 13. A schematic representation of the possible relationship between specific nutrient flux (nitrogen) and community structure.

as factors that would have to be included in any model accounting for phytoplankton diversity.

Based on earlier arguments that coexistence could be expected on a single resource due to patchiness, one would expect the maximum theoretical number of coexisting species to be equal to two times the number of limiting resources. When considering all these previously mentioned factors in addition to diversity maintenance by allelopathy (DeFreitas & Frederickson, 1978) and the contemporaneous disequilibrium hypothesis of Richerson et al. (1970), there is seemingly little reason to invoke a "paradox of the plankton" (Hutchison, 1961).

There are several potential practical applications of limiting nutrient patchiness in manipulating phytoplankton communities. By using the correct frequency of nutrient additions coupled with correct nutrient fluxes and ratios, favorable species may be selected for use in aquaculture systems. The Great Central Lake fertilization project (Takahashi & Nash, 1973) has resulted in enhanced fish yields following areal bombing of the lake with nutrients (Stockner, pers. comm.). Alteration of the frequency of bombing could lead to selection of more favorable primary producers which in turn might result in enhanced herbivore production and increases in fish yields.

The recent work by Marra (1978a; 1978b) indicated the potential importance of fluctuating light regimes in terms of primary productivity. Both Marra's work and this study seem to show that interpretation of growth only as a function of constant environmental conditions will not lead to the accurate understanding and prediction of phytoplankton dynamics in the sea. The environment of a phytoplankton cell is continually fluctuating and, therefore, work should begin to focus on understanding the growth dynamics of these organisms under fluctuating environmental conditions.

Chapter III

CELL SIZE MANIPULATION IN PHYTOPLANKTON ASSEMBLAGES

1) Summary

In cultures of natural phytoplankton and a mixed culture of diatoms, the mean cell diameter that was selected for in species competition experiments was related to the time between limiting nutrient (ammonium) additions (i.e., temporal patchiness). The mean cell size increased as the frequency of the nutrient addition decreased. The possibility that limiting nutrient patchiness may be of some importance in cell size selection in nature is also discussed.

2) Introduction

Factors regulating the cell size of phytoplankton in the sea have been discussed (Malone, 1971; Semina, 1971, 1972; Parsons & Takahashi, 1973b; Laws 1975) and there has been considerable controversy as to their importance (Parsons & Takahashi, 1973b; Hecky & Kilham, 1974; Parsons & Takahashi, 1974; Malone, 1975; Parsons & Takahashi, 1975). Phytoplankton cell size has been suggested to be important in determining trophic level structure and the efficiency of food chain energy transfer (Parsons et al., 1967; Ryther, 1969; Parsons & LeBrasseur, 1970; Greve & Parsons, 1977). If this hypothesis is to be tested it will require the manipulation of cell size in natural phytoplankton assemblages. This is not possible at present.

This chapter reports how cell size was manipulated in diatom-dominated laboratory cultures and in cultures of natural phytoplankton populations, by changing the temporal patchiness of the limiting nutrient (ammonium).

3) Methods

A natural phytoplankton sample taken from 4-8 m in a controlled ecosystem enclosure (CEE) at the CEPEX site (Menzel & Case, 1977) was filtered through a 150 μm mesh to remove any large zooplankton. Diatoms predominated in the sample and the most numerous species were *Thalassiosira nordenskioldii*, *Chaetoceros* spp. and *Skeletonema costatum*. Unidentified small flagellates made up $< 40\%$ of the sample cell numbers. Further details of the composition of this initial sample are given in Experiment 2 in Chapter 2, p. 41.

Cultures were grown in 3-liter flasks and incubated at $13 \pm 1^{\circ}C$ in a water-filled Plexiglas incubator system (Ch. 3). Natural sunlight was attenuated by 50% and spectrally corrected by surrounding the incubator with 1/8" blue Plexiglas. Details of the incident radiation during the experimental period are given in Experiment 2 in Chapter 2, p. 50.

The culture medium consisted of a 200-liter sample collected from 0-4 m in the CEE and filter-sterilized using a membrane filter (0.45 μm). This water was found to be nitrogen depleted (<0.5 $\mu g\text{-at}\cdot\ell^{-1}$) and was enriched to give final concentrations of ammonium chloride, 10 $\mu g\text{-at}\cdot\ell^{-1}$; potassium phosphate (monobasic), 3 $\mu g\text{-at}\cdot\ell^{-1}$; silicate, 50 $\mu g\text{-at}\cdot\ell^{-1}$ and vitamins and trace metals to f/25 (Guillard & Ryther, 1962).

The natural phytoplankton sample described above was used to inoculate three outdoor cultures (Exp. 1) in which the limiting nutrient, ammonium, was added continuously in one culture and semi-continuously at different time intervals in the other two cultures. Culture 1 was a continuous flow culture with a dilution rate of 0.3 day^{-1}. Culture 2 was a semi-continuous culture, diluted every day (dilution rate = 0.3 day^{-1}) with inflow medium in which the ammonium enrichment of 10 $\mu g\text{-at}\cdot\ell^{-1}$ was omitted. This culture received its ammonium supply as a discrete pulse of 9 $\mu g\text{-at}\cdot\ell^{-1}$ every 3 days. Culture 3 was identical to culture 2 except that 21 $\mu g\text{-at}\cdot\ell^{-1}$ ammonium was added once every 7 days. A comparison of the treatments used in all three cultures indicates that both the dilution rate and the limiting nutrient flux (21 μg-at $NH_4^+\cdot week^{-1}$) were identical in all three cultures and only the frequency of additions (temporal distribution) of the limiting nutrient varied.

A similar experiment (Exp. 2) was conducted in the laboratory as a test of duplication. Cultures were grown in a light regime of 16L:8D and an irradiance of 150 $\mu Ein\cdot m^{-2}\cdot sec^{-1}$. Unialgal cultures of *Chaetoceros* sp. (#277), *Skeletonema costatum* (#18b) and *Thalassiosira nordenskioldii* (#252) (Northeast Pacific Culture Collection, Department of Oceanography, U.B.C.) were mixed together and maintained in three cultures as described in Experiment 1, with the exception of culture 1 which was a semi-continuous culture, diluted daily. Since the temperature was higher (18°C) in this experiment, the dilution rate was increased to 0.5 day^{-1} in order to achieve a similar degree of nitrogen limitation as used in Experiment 1. Nitrogen fluxes were

adjusted so the weekly ammonium flux (35 $\mu g\text{-}at \cdot \ell^{-1}$) through all cultures was identical. Samples of the inflow medium were taken regularly to check expected nutrient concentrations and samples from the cultures were frequently analyzed to determine ambient nutrient concentrations. After three weeks of treatment, the organisms present in both Experiments 1 and 2 were identified and counted. Cell volumes of the most dominant species were calculated from measurements of cell dimensions of 50 cells, using an eyepiece micrometer and an inverted microscrope.

4) Results and Discussion

Nutrient analyses of the culture effluent indicated that the ammonium was undetectable in the continuous flow culture. In culture 2, the 9 $\mu g\text{-}at \cdot \ell^{-1}$ addition of ammonium fell below detectable levels by the end of the first day and as a result it was N-starved for the following 2 days until another pulse was given. Culture 3 depleted the 21 $\mu g\text{-}at \cdot \ell^{-1}$ ammonium addition in two days, resulting in a 5-day period of N-staryation before the next weekly ammonium pulse.

Figure 14 shows the mean cell diameter of the dominant species (>85% of culture biomass) from Experiments 1 and 2 as a function of the time between ammonium additions. There is a significant increase (t-test, p=.01) in cell size over the range of treatments. Experiment 1, System 1 (continual addition) was dominated by *Chaetoceros* sp., System 2 by *Skeletonema costatum* and System 3 by *Thalassiosira nordenskioldii*.

It is also of interest to note that in addition to the trend of increasing cell size with low frequency patchiness, the large cells, such as *Thalassiosira* and *Skeletonema*, formed chains, whereas the small *Chaetoceros* remained single-celled.

Experiment 2 demonstrated the same trend as Experiment 1 with the mean cell diameter increasing with the time between ammonium additions (Fig. 14).

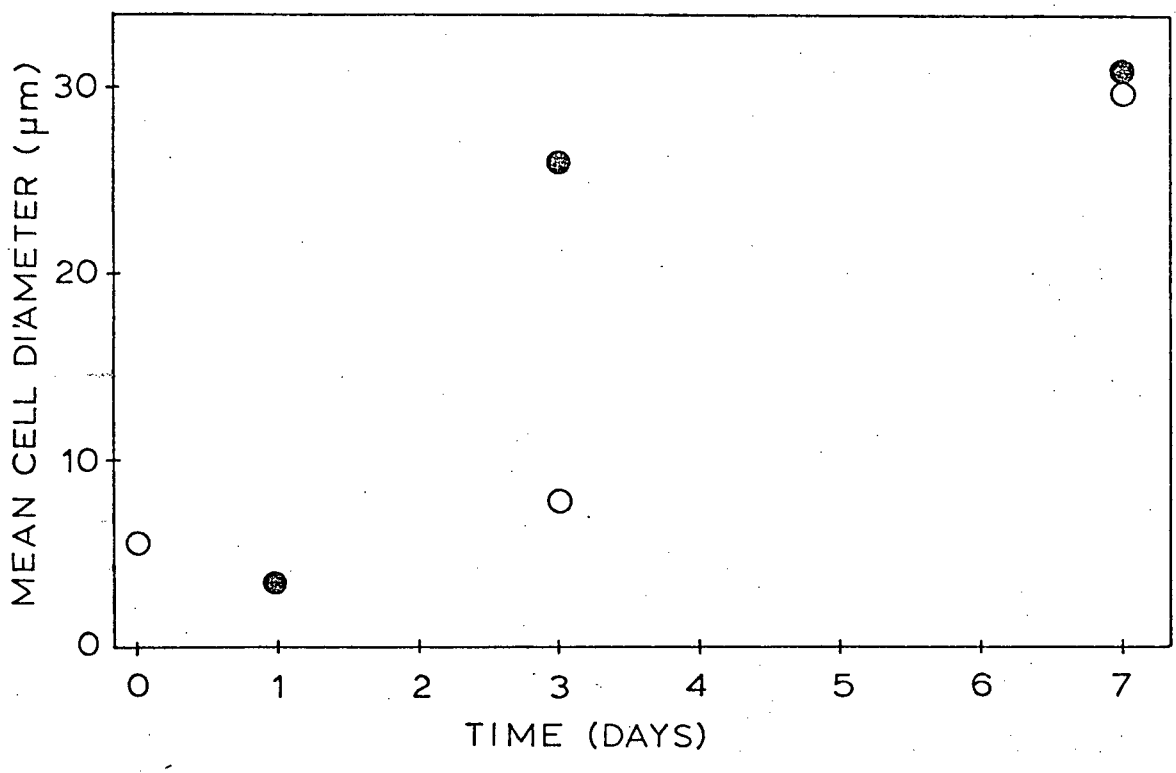


Figure 14. Mean cell diameter as a function of the time between ammonium additions for Exp. 1 (O) and Exp. 2 (●). The mean cell diameters between each treatment in each experiment were significantly different ($p = .01$). Mean cell diameter was calculated from the mean cell volume assuming an equivalent sphere.

Skeletonema, however, washed out of all cultures soon after the experiment was initiated. This was probably due to the poor condition of the inoculum[1]. Culture 1 was dominated by *Chaetoceros* sp.; in culture 2, *Chaetoceros* sp. and *Thalassiosira nordenskioldii* codominated, while culture 3 was dominated by *T. nordenskoldii*.

These results are consistent with other recent studies. Nitrogen additions were made on a weekly basis to the nitrogen-limited CEE community from which the initial inoculum for Experiment 1 was taken. The dominant phytoplankters over a 60-day period were generally large-celled diatoms, such as *Stephanopyxis turris, Cerataulina bergonii* and *Thalassionema nitzschiodes* (Parsley & Davis, pers. comm.). Mickelson (in press) found that over a wide range of dilution rates (continual limiting nutrient addition) in nitrogen-limited continuous cultures, small cells dominated. He suggested that cell size must be determined, therefore, by factors other than nutrient supply rates. Data from Eppley et al. (1969) suggest that small cells have a distinct advantage in situations in which there is a constant supply of the limiting nutrient because of their lower K_s values compared to large cells. When the nutrient supply becomes patchy, large cells apparently gain the advantage. Even though respiration rates were not determined in this study, it is tempting to suggest that in a low-frequency patchy environment, small cells, with a higher specific respiration rate (Laws, 1975; Banse, 1976) tend to "burn themselves up" before the appearance of the next nutrient pulse. If respiration is a significant factor in species selection, the competition scheme that has been proposed for high-frequency limiting nutrient patchiness (Turpin & Harrison, 1979; Ch. 3) could be expanded to include the possible effect of size-dependent respiration losses at lower frequency

[1] The *Skeletonema* inoculum appeared to be in poor health. Cells were very thin, chains were short and often clumped. This was in contrast to the large vigorous cells seen in the natural sample.

patchiness (Fig. 15).

The results of these indoor and outdoor culture studies indicate that by varying the frequency of addition of the limiting nutrient, natural phytoplankton populations can be manipulated to produce communities dominated by either large or small cells. Since nutrient flux is a coarse tuning variable tending to regulate selection between phytoplankton groups (Turpin & Harrison, 1979; Ch. 3), it may be possible to study cell size selection within flagellates by using a much lower dilution rate than was used in this study.

The role of pulsed nutrient supplies in determining cell size in natural systems is not known. The importance of grazing (McAllister et al., 1960; Parsons et al., 1967; Malone, 1971; Steele & Frost, 1977) and sinking (Semina, 1972) in controlling cell size is not to be denied. Nevertheless, it does seem possible, that a wide range in temporal patchiness of the nutrient supply may control selective cell size growth in the sea.

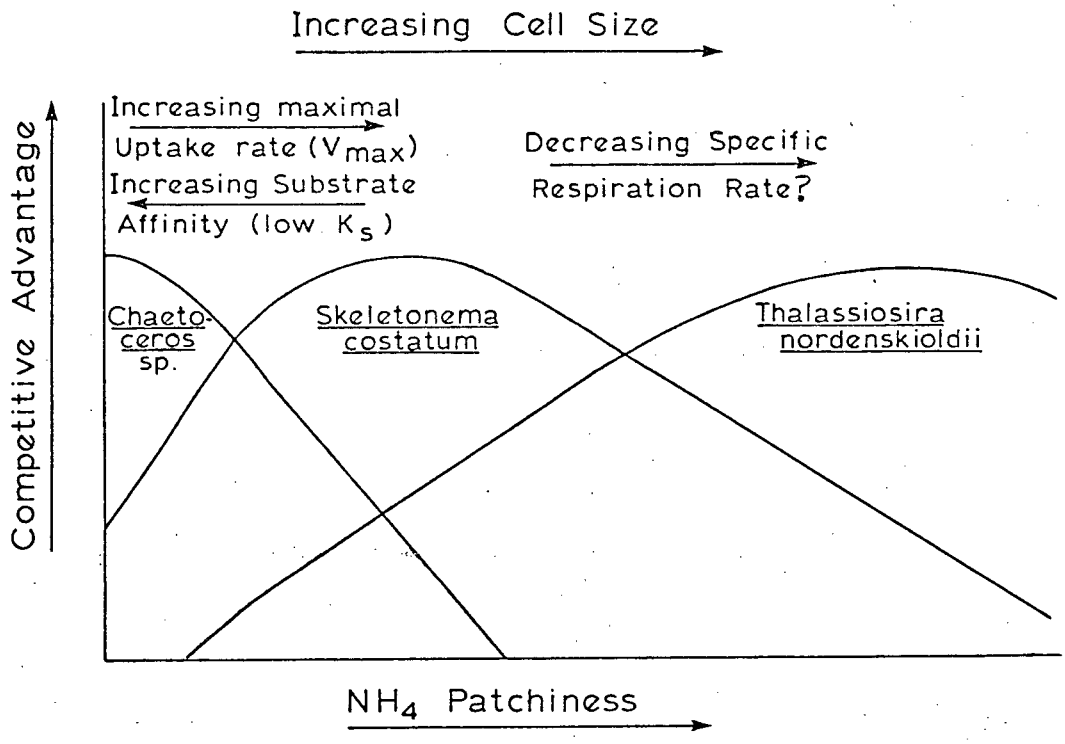


Figure 15. Possible changes in competitive advantage between three marine phytoplankton as a function of ammonium patchiness.

Chapter IV

RESPONSE OF AMMONIUM LIMITED *Skeletonema costatum* AND *Chaetoceros gracile* TO LIMITING NUTRIENT PATCHINESS

1) Summary

The effects of pulsed ammonium additions on the ammonia limited marine diatoms, *Skeletonema costatum* and *Chaetoceros gracile* were examined. It was found that ammonium patchiness produced periodicities in carbon assimilation and *in vivo* fluorescence. Changes in nutrient uptake ability under varying limiting nutrient patchiness regimes indicate that a given population may be able to adapt its nutrient uptake characteristics, thereby optimzing the temporal distribution of the limiting resource. *S. costatum* shows a greater ability to utilize a pulse of ammonium than *C. gracile*. This evidence is consistent with the outcome of the competition studies in Chapter 2.

2) Introduction

In Chapters II and III, limiting nutrient patchiness was examined as a factor affecting the outcome of competition in phytoplankton assemblages. It was shown that the temporal distribution of the limiting nutrient affected the outcome of competition and the resulting physiological characteristics of the community. The observation that the assemblage dominating under patchy conditions had a higher V_{max} than the one dominating under homogeneous conditions, suggested that interspecific variability in the nutrient uptake characteristics was of great importance in affecting competition. The remaining questions were: to what extent can a given species adapt to a given patchy environment, and to what degree does intraspecific variability allow for optimization of a patchy environment?

In attempts to answer these questions, two species of marine phytoplankton were grown in unialgal continuous cultures under a range of ammonium patchiness conditions. Their physiological response to these treatments was monitored through nutrient uptake and photosynthesis experiments, in addition to the monitoring of culture fluorescence and cell numbers. The marine diatoms, *Skeletonema costatum* and *Chaetoceros gracile* were studied. to gain insight into the physiological mechanisms contributing to the outcome of competition observed in studies in Chapter 2. In those studies *S. costatum* dominated under the patchy conditions and a small *Chaetoceros* sp., similar to *C. gracile*, dominated under the homogenous nutrient conditions.

3) Materials and Methods

The inocula were obtained from the Northeast Pacific Culture Collection (NEPCC) at The University of British Columbia (*Skeletonema costatum*, NEPCC 18b; *Chaetoceros gracile*, NEPCC 294) and were grown in 6-liter continuous cultures at a dilution rate of 0.6 d^{-1} under conditions described in Chapter 1. Inflow medium was artificial seawater (Davis et al., 1973) enriched to f/2 (Guillard

& Ryther, 1962) with phosphate and silicate and to f/25 with vitamin and trace metals. The limiting nutrient, ammonium was added as ammonium chloride to a concentration of 30 $\mu g\text{-at}\cdot\ell^{-1}$. This established a daily ammonium flux through the cultures of 18 $\mu g\text{-atN}\cdot\ell^{-1}\cdot day^{-1}$. The cultures were grown at 18°C and the irradiance was 150 $\mu Ein\cdot m^{-2}\cdot s^{-1}$, continuous light (Ch. 1).

Once steady-state had been attained, the continuous cultures described above were divided into two x 2-liter flasks with a dilution rate of 0.6 d^{-1}. System 1 for both species had the same inflow medium as the parent culture, whereas System 2 had no added ammonia in the inflow medium. System 2, for both species, received a single daily ammonia addition at 1300 hr, consisting of 12.7 ml of 2.83 mM ammonium chloride solution. This resulted in an identical ammonia flux through both Systems 1 and 2 of 18 $\mu g\text{-atN}\cdot\ell^{-1}\cdot day^{-1}$. The addition was controlled by a specially modified timer (Cincinnati, Model 422) and a calibrated metering pump (Fluid Metering Inc., New Jersey). A diagrammatic representation of these systems appears in Fig. 4 along with an assessment of the experimental design.

3.1) Measurements:

Culture effluents were collected daily and preserved in Lugol's iodine. An inverted microscope was used for enumeration of samples. *In vivo* fluorescence was monitored with a fluorometer (Turner, Model 111) equipped with a high sensitivity door. Nutrients in culture effluents were analyzed with a Technicon Autoanalyzer using methods previously described (Davis et al., 1973).

After two weeks of exposure to the nutrient regimes, the response of the populations to an ammonia pulse or perturbation (Caperon & Meyer, 1972b) was determined. A 250 ml sample was removed from a chemostat and perturbed with 1.59 ml of 2.83 mM ammonium chloride. This raised the ambient concentration of ammonia to 18 $\mu g\text{-at}\cdot\ell^{-1}$, the same concentration that was obtained

immediately after the once per day pulse in System 2. Since System 2 cultures were acclimated to the pulse occurring at 1300 hr, the uptake experiments for these cultures were carried out at this same time. Ammonium disappearance was then followed with an Autoanalyzer. The ability of the various phytoplankton populations to respond to the different patchiness regimes was determined.

Carbon assimilation was measured over a 24 hr period to observe the influence of the daily ammonia addition on photosynthetic activity. This was accomplished by placing 50 ml of the culture in a 50 ml screw top test tube and adding 0.4 ml of 2.5 $\mu Ci \cdot ml^{-1}$ $NaH^{14}CO_3$. The samples from System 1 were incubated for 1 hr, whereas the samples from System 2 were incubated for 8, 3 hr intervals extending over the 24 hr period. The shorter incubation time for System 1 was chosen to minimize the effects of nitrogen starvation that occurs upon removing the sample from this continual ammonium addition system. A longer incubation time could be used for the samples from System 2 because the limiting nutrient (ammonium) was added at only one time each day. Following the incubations, samples were filtered onto 0.45 µm Millipore filters and suspended in 15 ml of Scinti-Verse scintillation fluid. The radioactivity in the samples was determined using a Unilux III liquid scintillation counter.

Average daily C/N assimilation ratios were calculated for all systems after determining the total daily carbon assimilation and the calculated daily nitrogen assimilation. Daily carbon assimilation for System 1 was calculated by multiplying the mean hourly carbon assimilation rate by 24. The daily carbon assimilation for System 2 was calculated by summing the 8, 3 hr incubation values. Average daily nitrogen assimilation was equated to the daily ammonia flux through the culture. Correction was made for the washout of a portion of the ammonium in System 2 (as ammonia was added in a discrete pulse

and, as the uptake of this pulse was not instantaneous some washout occurred). This correction did not have to be applied to System 1 as there was essentially no detectible ammonia in the outflow.

4) Results

4.1) Fluorescence:

Culture fluorescence in the homogeneous cultures remained constant throughout the experiment, whereas it fluctuated markedly in the once a day ammonia addition culture, System 2 (Fig. 16). This fluctuation was most noticeable in *S. costatum*. The minimum culture fluorescence occurred at ∿ 1900 hr, 4 hr after the addition of the ammonium pulse, while the fluorescence maximum occurred at ∿ 0300 hr, 14 hr after the pulse. *C. gracile* (System 2) showed some fluctuation in culture fluorescence with a minimum at ∿ 1700 hr, 6 hr after the ammonium addition, and a maximum at ∿ 0300 hr, 14 hr after the addition.

4.2) Carbon assimilation:

System 1, for both species, showed a constant carbon assimilation rate over the 24 hr period (Fig. 17). In the once a day ammonium addition cultures, carbon assimilation fluctuated in response to the daily ammonium addition. For both species, a maximum was observed at ∿ 0300 hr, 14 hr after the ammonium addition and the minimum occurred directly following the ammonium addition.

4.3) Carbon/Nitrogen assimilation ratios:

The average daily C/N assimilation ratios (by atoms) for *S. costatum* were 14.3±1.2 and 12.8±1.7 for Systems 1 and 2, respectively. This difference was not significant ($p = 0.3$). The average daily C/N assimilation ratios for *C. gracile* were 11.6 ± 1.2 and 10.0 ± 1.8 for Systems 1 and 2, respectively. These differences were again not significant ($p = 0.07$). There was, however, a significant difference between the two species grown in both the homogeneous and patchy conditions ($p = 0.0001$ and $p = 0.03$, respectively).

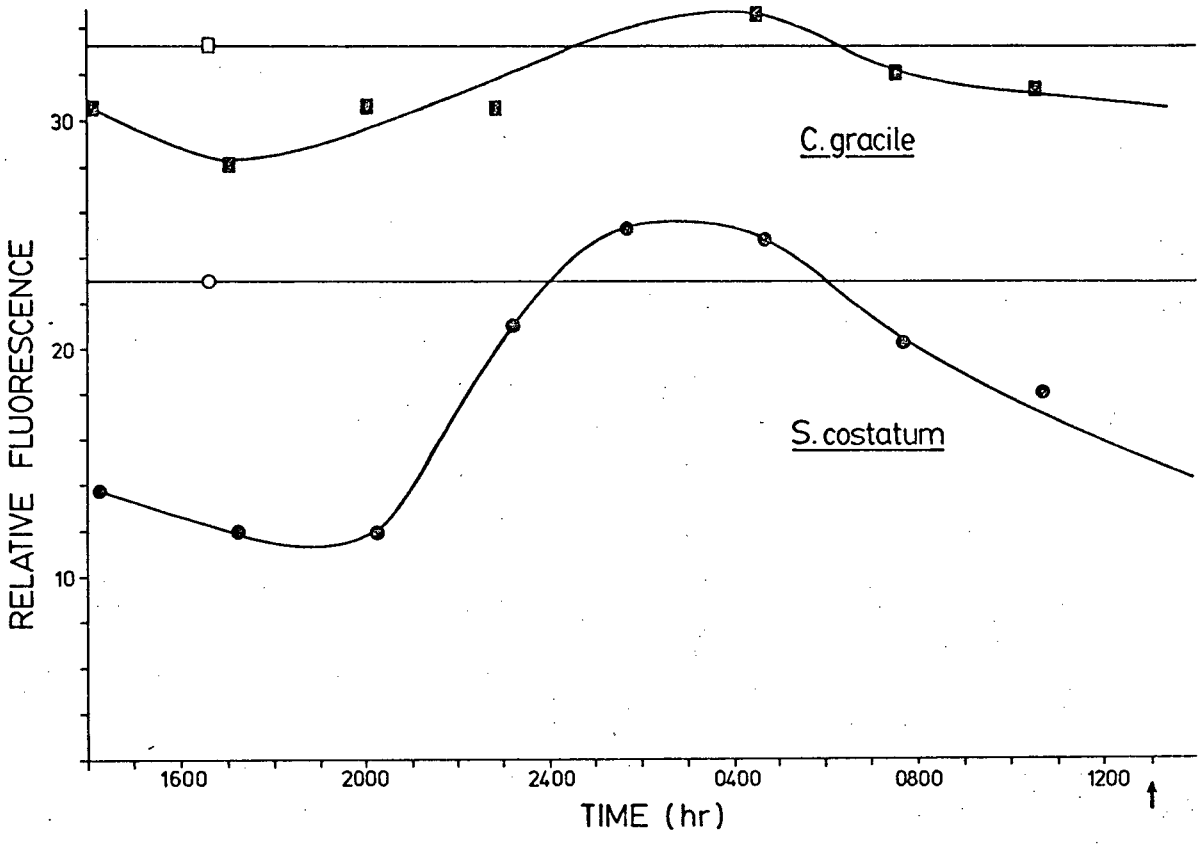


Figure 16. Relative fluorescence over a 24 hr period for *C. gracile* (□, System 1, continuous addition of ammonia; and ■, System 2, daily addition of ammonia) and *S. costatum* (○, System 1; ●, System 2). All systems were continuous flow with a dilution rate of 0.6 d^{-1} and grown under continuous light. The arrow represents the time of the ammonia addition to System 2. The point indicated for System 1 represents the mean of four values taken throughout the day.

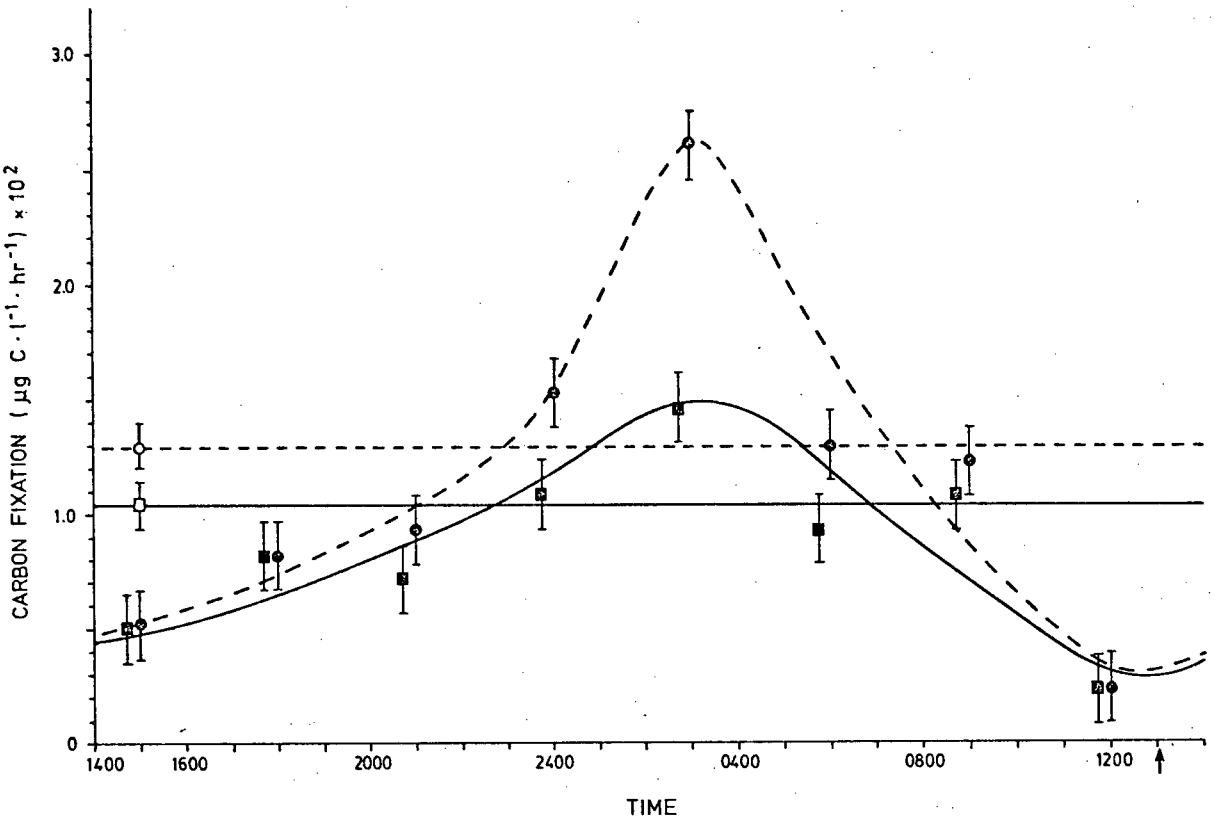


Figure 17. Carbon fixation rate for *S. costatum*, ---- (○, System 1, continual addition of ammonia; and ●, System 2, daily addition of ammonia) and *C. gracile*, —— (■, System 1, continual addition of ammonia; and □, System 2, daily addition of ammonia) ↑, indicates time of ammonia addition to System 2. Bars represent 95% confidence limits.

4.4) Cell numbers:

No trends were apparent in the cell densities in any of the systems. System 1 for *S. costatum* had an average density of $7.3 \pm 0.4 \times 10^8$ cells$\cdot\ell^{-1}$, whereas System 2 exhibited an average density of $7.4 \pm .08 \times 10^8$ cells$\cdot\ell^{-1}$. *C. gracile* (System 1) had an average cell density of $4.7 \pm 0.3 \times 10^8$ cell$\cdot\ell^{-1}$, whereas the average density of System 2 was $4.6 \pm 1.2 \times 10^8$ cell$\cdot\ell^{-1}$.

4.5) Nutrient uptake:

a) Interspecific differences: The nutrient uptake response of System 1 (homogeneous distribution of the limiting nutrient) to a perturbation equivalent to the daily addition of System 2 is represented in Fig. 18. *S. costatum* exhibited a very rapid initial uptake (V'_{max}; see Appendix II) over the first 3 min following the perturbation, when compared to *C. gracile*. Subsequent uptake (V_i; see Appendix II) is also more rapid for *S. costatum*.

The nutrient uptake response of System 2 (daily addition) to its daily ammonium pulse is shown in Figure 19. The general features of the results are similar to System 1 with *S. costatum* having a higher V'_{max} (3 min) and a continued higher, longer-term uptake, V_i, than *C. gracile*.

b) Intraspecific differences; *C. gracile*: The only difference between the nutrient uptake ability of the homogeneous population and the one addition per day population was in the initial rapid uptake (V'_{max}). In the patchy-grown population, V'_{max} was greater than the homogeneous culture (Fig. 20).

S. costatum: No major difference in nutrient uptake rates was apparent between the two culture conditions (Fig. 21). There appeared to be some smaller differences, however, between the two treatments. System 2 exhibited a higher V_i and, as a consequence, the patch-adapted population could take up a daily pulse of ammonium faster than the homogeneous population (System 1).

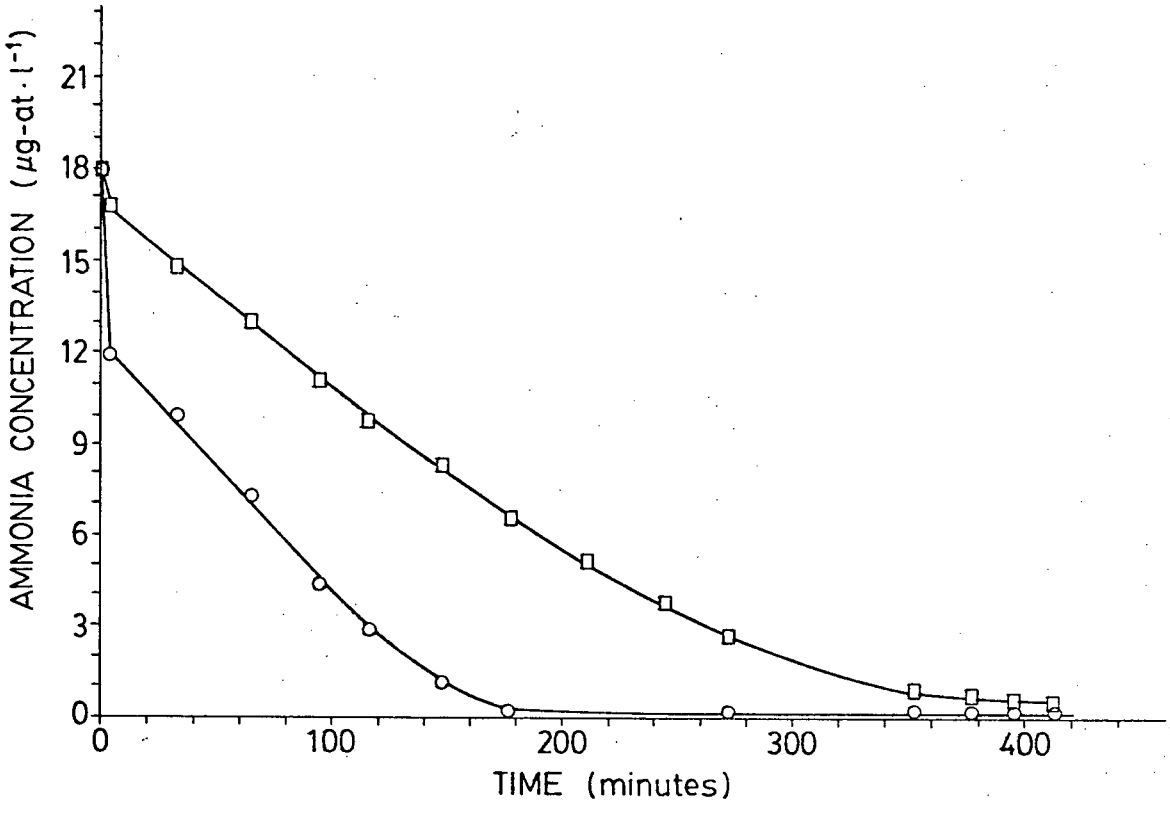


Figure 18. The disappearance of ammonium as a function of time during a perturbation experiment for System 1 (continual addition of ammonium) *C. gracile*, □ , and *S. costatum*, 0 .

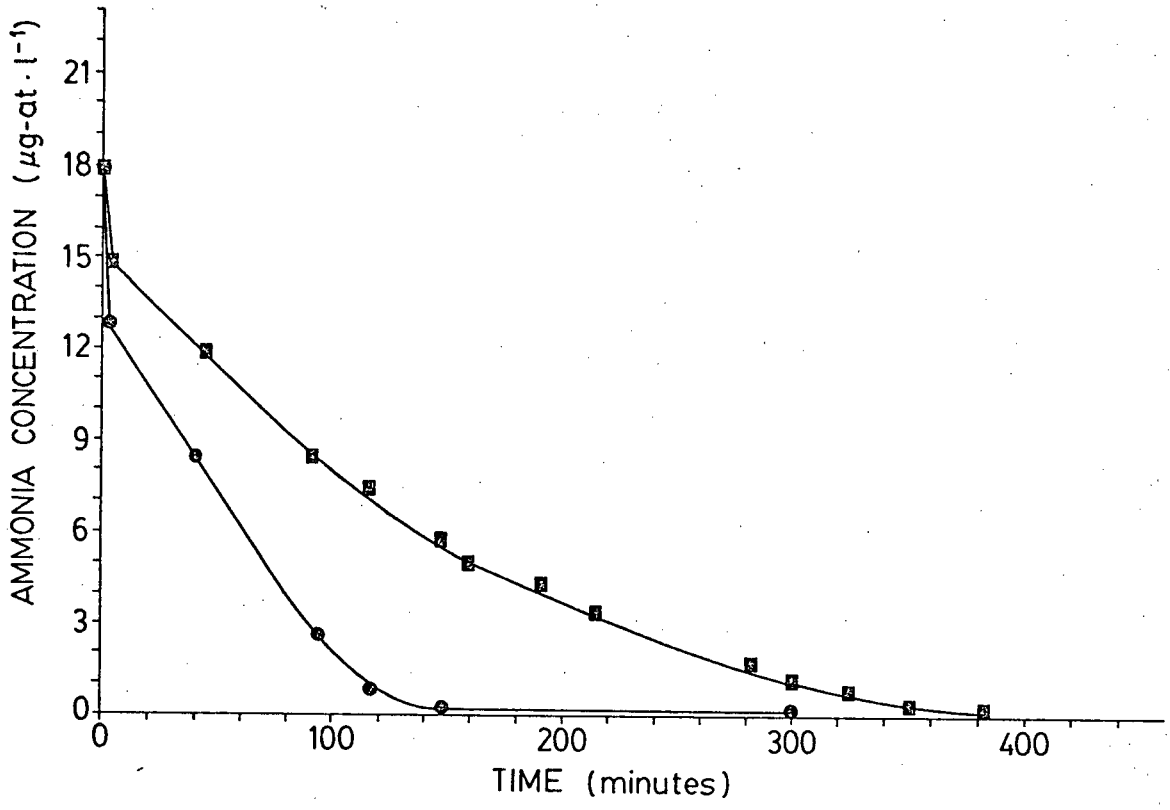


Figure 19. The disappearance of ammonium as a function of time during a perturbation experiment for System 2 (daily addition of ammonium) *C. gracile*, ■, and *S. costatum*, ● .

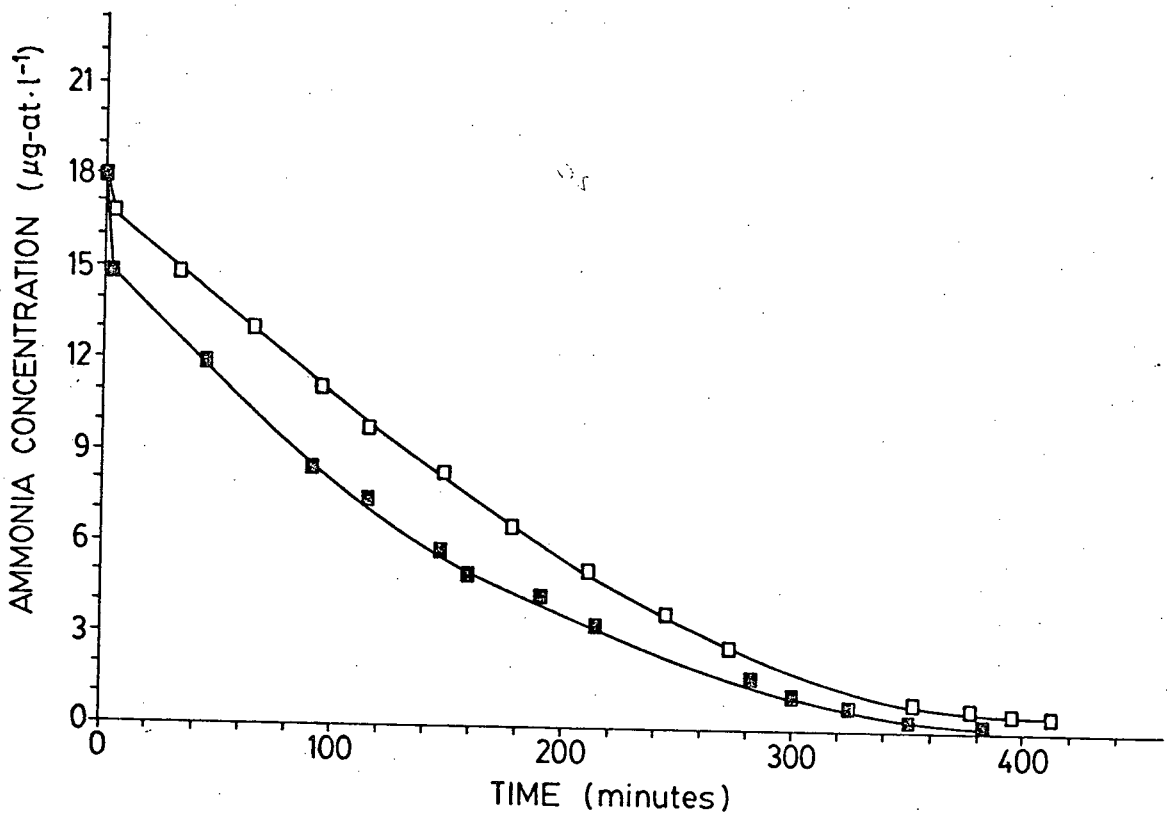


Figure 20. The disappearance of ammonium as a function of time during a perturbation experiment for *C. gracile*, System 1 (continual addition of ammonium □ , and System 2 (daily addition of ammonium) ■ .

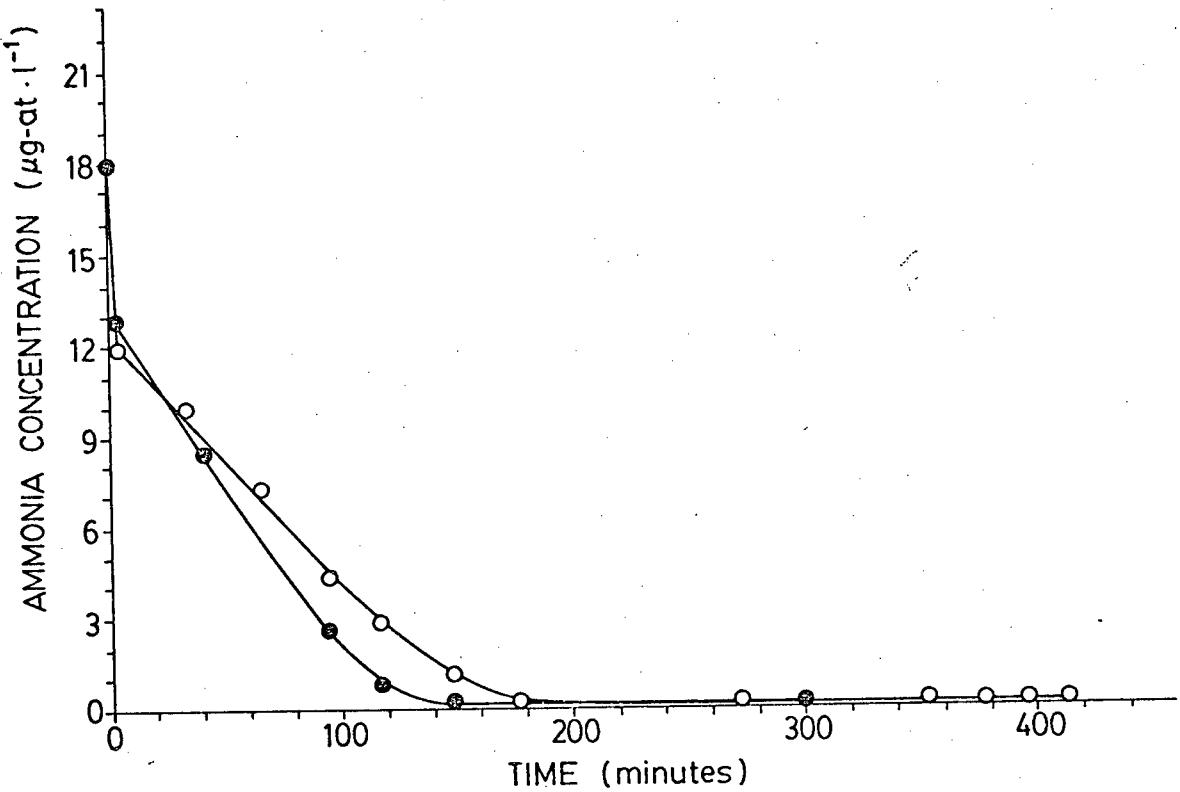


Figure 21. The disappearance of ammonium as a function of time during a perturbation experiment for *S. costatum*, System 1 (continual addition of ammonium), O , and System 2 (daily addition of ammonium), ● .

5) Discussion

Limiting nutrient patchiness (ammonium) initiates a number of physiological periodicities in phytoplankton populations. The periodicity in carbon fixation did not, however, significantly alter the average daily C/N assimilation ratios in the two species tested. The periodicity in carbon fixation could be explained in terms of an intracellular energy allocation mechanism. When the limiting nutrient is available, energy is shunted to the uptake process at the expense of carbon fixation (Falkowski & Stone, 1975). After the available nitrogen has been assimilated, enhanced carbon fixation occurs. Ohmori & Hattori (1978) obtained some biochemical evidence to support this type of energy allocation. They showed that the addition of ammonium to an ammonium limited phytoplankton culture resulted in a rapid drop in intracellular ATP. This ATP drop was shown to be a result of glutamine synthetase activity.

Periodicities in chlorophyll *a* fluorescence could be due to either a change in chlorophyll *a* levels or a change in photosynthetic efficiency. As chlorophyll *a* was not directly measured, it was not possible to determine the underlying physiological mechanism mediating the fluorescence fluctuations.

Under all treatments *S. costatum* had a higher nutrient uptake rate than did *C. gracile*. This is in agreement with the work of Conway and Harrison (1977) who worked on *S. costatum* and *C. debilis*. The two *C. gracile* populations (1 and 2) showed some interesting differences in uptake ability depending on the nutrient past history under which they were grown. The patch-adapted population (System 2) showed a much greater V_{max}' than the homogeneous population, while the subsequent uptake rate, V_i, was the same for both populations. As a result the population grown on a patchy nutrient source was best able to take up a pulse of the limiting nutrient.

S. costatum also showed slight differences in nutrient uptake ability in response to limiting nutrient patchiness. Over the first 3 min the

homogeneous culture had a slightly higher V_{max}' but the V_i was greater in the patch-adapted system. Consequently, the population grown under conditions of once a day ammonia addition was best able to optimize uptake of the nutrient with that patchy temporal distribution.

Other workers (Chisholm & Stross, 1976) have induced periodicities in phytoplankton cellular metabolism by use of light:dark cycles. When *Euglena gracilis* was grown under light:dark cycles, diurnal fluctuations in ^{14}C assimilation and V_{max} for phosphate uptake were observed.

The major differences between the physiology of the populations grown on homogeneous or patchy nutrient distributions was that the pulsed populations established a periodicity in a number of parameters such as chlorophyll *a* fluorescence and carbon assimilation. The observation that cell numbers remained relatively constant throughout the 24 hr cycle in System 2 supported the work of Caperon (1969). He suggested that phytoplankton growth was an integrative result of the nutritional past history of the population over the preceding 24 hr period. Changes in nutrient uptake ability suggested that populations grown on a pulsed nutrient system tended to exhibit higher nutrient uptake rates than those grown on homogeneous distribution of the limiting nutrient. This phenomenon will be explored further in Chapter V and its potential effect on phytoplankton growth and competition will be discussed.

In summary, the results of competition observed in Chapters II and III appear to be due almost entirely to interspecific nutrient uptake differences. The role of intraspecific variability in the competition for the limiting nutrient is minor when compared to the interspecific differences. Nevertheless, the ability of a species to alter its physiology in response to a patchy environment may be important in the optimization of a particular nutrient regime.

Chapter V

LIMITING NUTRIENT PATCHINESS AND PHYTOPLANKTON GROWTH: A CONCEPTUAL APPROACH

1) Summary

A theoretical framework is developed to explore the effects of limiting nutrient patchiness on phytoplankton growth. Growth rate is represented as a function of the average ambient substrate concentration in the medium, the degree of patchiness and the patch duration. Phytoplankton growth, in relation to the external substrate concentration, is mediated by the cell quota for the limiting nutrient.

Two general conclusions can be drawn from this study. First, the degree of patchiness in the environment can affect individual growth rates and thus alter community structure even though there is no change in the average ambient nutrient concentration. Second, for patch-adapted populations, the apparent K_s' for growth can be lowered significantly by making the distribution of the limiting nutrient patchy with respect to time. The insights which this model provides into future experimental methodologies are also discussed.

2) Introduction

The application of the Monod equation (1) (Monod, 1942)

$$\mu = \mu_{max} \frac{[S]}{K_s + [S]} \qquad (1)$$

μ = growth rate (hr^{-1})

μ_{max} = maximum growth rate (hr^{-1})

[S] = substrate concentration ($\mu g\text{-at} \cdot \ell^{-1}$)

K_s = half-saturation constant ($\mu g\text{-at} \cdot \ell^{-1}$)

to nutrient limited phytoplankton growth has generally been unsuccessful in describing growth on nutrients other than carbon (Droop, 1968; Caperon & Meyer, 1972a;Fuhs et al., 1972; Goldman et al., 1974; Harrison et al., 1976; Goldman & McCarthy, 1978). In many of these latter cases nutrient limited growth rates were described better as a function of the cell quota (Droop, 1968; Caperon & Meyer, 1972a;Goldman & McCarthy, 1978).

The preceding studies were all conducted under steady state conditions with a relatively homogeneous distribution of the limiting nutrient. However, it has been shown recently that following an addition of the limiting nutrient to a nutrient limited culture, uptake rate far exceeds growth rate (Conway et al.,1976; Conway & Harrison, 1977; McCarthy & Goldman, 1979). Davis, Breitner and Harrison (1978) provided a model that simulated silicate limited diatom growth at a steady state as well as the transient uptake response to a silicate addition. The degree to which the transient uptake rate exceeded growth was dependent upon the species, the nutrient in question (Conway & Harrison, 1977) and the degree of nutrient limitation (McCarthy & Goldman, 1979; Eppley & Renger, 1974).

The ability of some species to respond more rapidly than other species to a patch of the limiting nutrient could provide a basis for explaining resource

competition and niche separation. Further work (Turpin & Harrison, 1979; Chapters III, IV & V) has shown that this is the case with the temporal distribution of the limiting nutrient causing modification of community structure and physiology.

Nutrient limitation is well documented in many freshwater and marine ecosystems. The cycling of nutrients for utilization by the primary producers occurs by diffusion from the nutrient-rich water below the thermocline, advection (runoff, mixing and upwelling) and regeneration, either by zooplankton or bacteria. These mechanisms of nutrient supply are not evenly distributed over either time or space (Shanks & Trent, 1979). As a result, the supply of nutrients to a system is not homogenous with respect to time or space. Spacial patchiness, in relation to a phytoplankton cell, could be modelled identically to temporal patchiness since in both cases there would be a finite time between patch encounters.

It is known that different species of nutrient limited phytoplankton respond to the addition of the limiting nutrient with different uptake rates and that nutrient patchiness can be expected in aquatic ecosystems. This chapter attempts to demonstrate how patchiness, average substrate concentration and growth rate of a phytoplankter could be related. The ecological implications of such interrelationships and the insights into future experimental methodology will be discussed.

3) Model

This model was developed to predict the growth of nutrient limited phytoplankton under conditions of limiting nutrient patchiness. The model was designed to account for steady state growth kinetics as well as to provide insight into the effects of nutrient patchiness on phytoplankton growth. The model components are outlined below.

3.1) Nutrient uptake:

The amount of nutrient taken up by a cell per unit time, ρ , ($\mu g\text{-at}\cdot cell^{-1}\cdot hr^{-1}$) is given by the following equation:

$$\rho = \frac{\rho_m [S]}{K_s + [S]} \qquad (2)$$

ρ_m = maximum uptake rate per cell ($\mu g\text{-at}\cdot cell^{-1}\cdot hr^{-1}$)

$[S]$ = substrate concentration ($\mu g\text{-at}\cdot \ell^{-1}$)

K_s = half saturation constant ($\mu g\text{-at}\cdot \ell^{-1}$)

In simulations, ρ_m is assumed to be constant (Dugdale, 1977), or it is varied in response to the cell quota using the data of McCarthy and Goldman (1979).

3.2) Dependence of population growth rate on cell quota:

The growth rate of the population is determined as a function of the cell quota using the equation of Droop (1968);

$$\mu = \bar{\mu} (1 - Q_{min}/Q) \qquad (3)$$

μ = growth rate (hr^{-1})

$\bar{\mu}$ = growth rate when ($Q \to \infty$ (hr^{-1})

Q = cell quota ($\mu g\text{-at}\cdot cell^{-1}$)

Q_{min} = minimum quota needed for growth to proceed ($\mu g\text{-at}\cdot cell^{-1}$)

3.3) Determination of quota:

The change in quota per unit time ($\dot{Q}$) ($g\text{-at}\cdot cell^{-1}\cdot hr^{-1}$) is the net result of an increase in Q due to uptake and a dilution of Q as a result of growth as described by the following equation:

$$\dot{Q} = \frac{\rho_m [S]}{K_s + [S]} - \bar{\mu} (1 - Q_{min}/Q) Q \qquad (4)$$

At steady state growth, with a constant substrate concentration $[S]$, Q will approach equilibrium when $\dot{Q} = 0$ such that equation (4) reduces to:

$$\frac{\rho_m [S]}{K_s + [S]} = \bar{\mu} (Q - Q_{min})$$

or

$$Q = Q_{min} + \rho_m [S]/(K_s + [S])\bar{\mu} \qquad (5)$$

The steady state growth rate of the population is obtained by substituting equation (5) into equation (3) as follows:

$$\mu = \bar{\mu} \left\{ 1 - \frac{Q_{min}}{Q_{min} + \frac{\rho_m \cdot [S]}{\bar{\mu} \{K_s + [S]\}}} \right\}$$

or rearranging the above equation yields:

$$\mu = \frac{\mu \rho_m}{\bar{\mu} Q_{min} + \rho_m} \cdot \frac{[S]}{\frac{\bar{\mu} Q_{min} K_s}{\bar{\mu} Q_{min} + \rho_m} + [S]} \quad 1 \qquad (6)$$

Therefore at steady state, the dependence of growth (μ) on substrate [S] is described by a rectangular hyperbola. The population's maximal growth rate (μ_{max}) under any set of conditions (see equation 6) is described by:

$$\mu_{max} = \frac{\bar{\mu} \rho_m}{\bar{\mu} Q_{min} + \rho_m} \qquad (7)$$

and the half saturation constant for growth (see equation 6) by:

---

1 The equation for steady state growth as a function of external nutrient concentration was derived by Droop (1968). My terminology follows that of Dugdale (1977).

$$K_s' = \frac{K_s \bar{\mu}_{min} Q_{min}}{\bar{\mu} Q_{min} + \rho_m} \qquad (8)$$

I have chosen to call the half saturation constant for growth, K_s', to avoid confusion with the half-saturation constant for uptake (K_s). This expression for K_s' indicates that it is a variable, dependent on both K_s and ρ_m, providing $\bar{\mu}$ and Q_{min} are constant under all conditions.

Since μ_{max} and K_s' depend on three cell parameters, $\bar{\mu}$, $\frac{\rho_m}{Q_{min}}$, and K_s, two organisms can have the same dependence of μ on [S] at steady state (i.e., same μ_{max} and K'_s) and still have different growth and uptake parameters ($\bar{\mu}$, $\frac{\rho_m}{Q_m}$ and K_s). As an extreme example, a population with $\bar{\mu} = 0.2\ hr^{-1}$, $\frac{\rho_m}{Q_{min}} = 0.2\ hr^{-1}$ and $K_s = 0.2\ \mu g\text{-at}\cdot\ell^{-1}$ will show the same steady growth kinetics as a population with $\bar{\mu} = 0.105\ hr^{-1}$, $\frac{\rho_m}{Q_{min}} = 2.1\ hr^{-1}$ and $K_s = 2.1\ \mu g\text{-at}\cdot\ell^{-1}$.

The similarity in growth kinetics ends at steady state. As the nutrient available to the cell fluctuates over time, differences arise in the abilities of the two species to procure the nutrients and grow. To illustrate this point, the average growth rate of cells exposed to an environment in which the nutrients come in short pulses, separated by periods of nutrient starvation, has been calculated. The nutrient concentration during the pulse is adjusted in order to maintain some fixed concentration $[S_{av}]$ when averaged over the period of the patch, P (Fig. 22). For the simulations, the duration of the pulse, D_ρ, was held constant at 0.1 hr and the interval between pulses, T varied. Holding D_ρ constant allowed us to use the compartment model effectively. In a more complex situation with a varying pulse duration, the model would have to be modified to include internal pool(s) and feedback control of the uptake rates (Davis et al., 1978; DeManche et al., 1979).

When a population is exposed to a periodic nutrient supply (Fig. 22) the cell quota responds according to equation 5, and approaches an equilibrium with Q increasing during the pulse and decreasing during the absence of the nutrient. An average population growth rate is calculated over this cycle. At equilibrium, for a given patchy regime, the absence of a delay between Q and μ (Cunningham & Maas, 1978) does not affect the result. As might be expected, steady state populations having the same μ_{max} and K_s' but different values of $\bar{\mu}$, $\frac{\rho m}{Q}$ and K_s, show quite different growth rates under the same patchy nutrient regimes. To emphasize this behaviour a second set of parameters can be chosen such that in a steady state system (i.e., $p = 0$, when the limiting nutrient is homogeneously distributed) species A may outgrow species B but as the system becomes patchy, B would outgrow A (Fig. 23).

Simulations were run using a variable ρ_m as a function of cell quota which was calculated from the data of McCarthy and Goldman (1979). This variable, ρ_m, combined with the growth kinetic data from the same organism (Goldman & McCarthy, 1978) produced results similar in general appearance to Fig. 23A with growth rate decreasing with lower frequencies of patchiness. This demonstrates that a high V_{max} alone will not allow an organism to grow better under patchy conditions at a given average substrate concentration. This is contrary to the assertion made by McCarthy and Goldman (1979).

Some preliminary work with natural marine phytoplankton communities (Turpin & Harrison, 1979) and unialgal cultures (Ch. IV) suggest that the maximum uptake rate of a population increases with the time between nutrient pulses. To determine the possible effects of such a condition, a relationship between ρ and T is presented (equation 9) which allows enhancement of limiting nutrient uptake when the distribution of that nutrient is patchy. Although this relationship can not be validated with existing data, it allows a representation of enhanced uptake in a patchy system. The equation is:

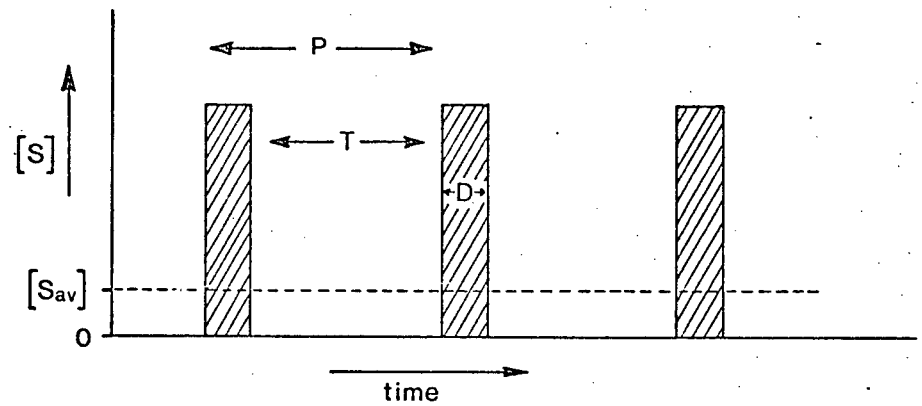


Figure 22. A graphical representation of a temporally patchy, nutrient limited environment. [S] is the substrate concentration ($\mu g\text{-at}\cdot\ell^{-1}$). P is the periodicity of the patch (hr), D_ρ the duration (hr) and T, the interval between successive pulses (hr). $[S_{av}]$ is the substrate concentration averaged over the patch period, P.

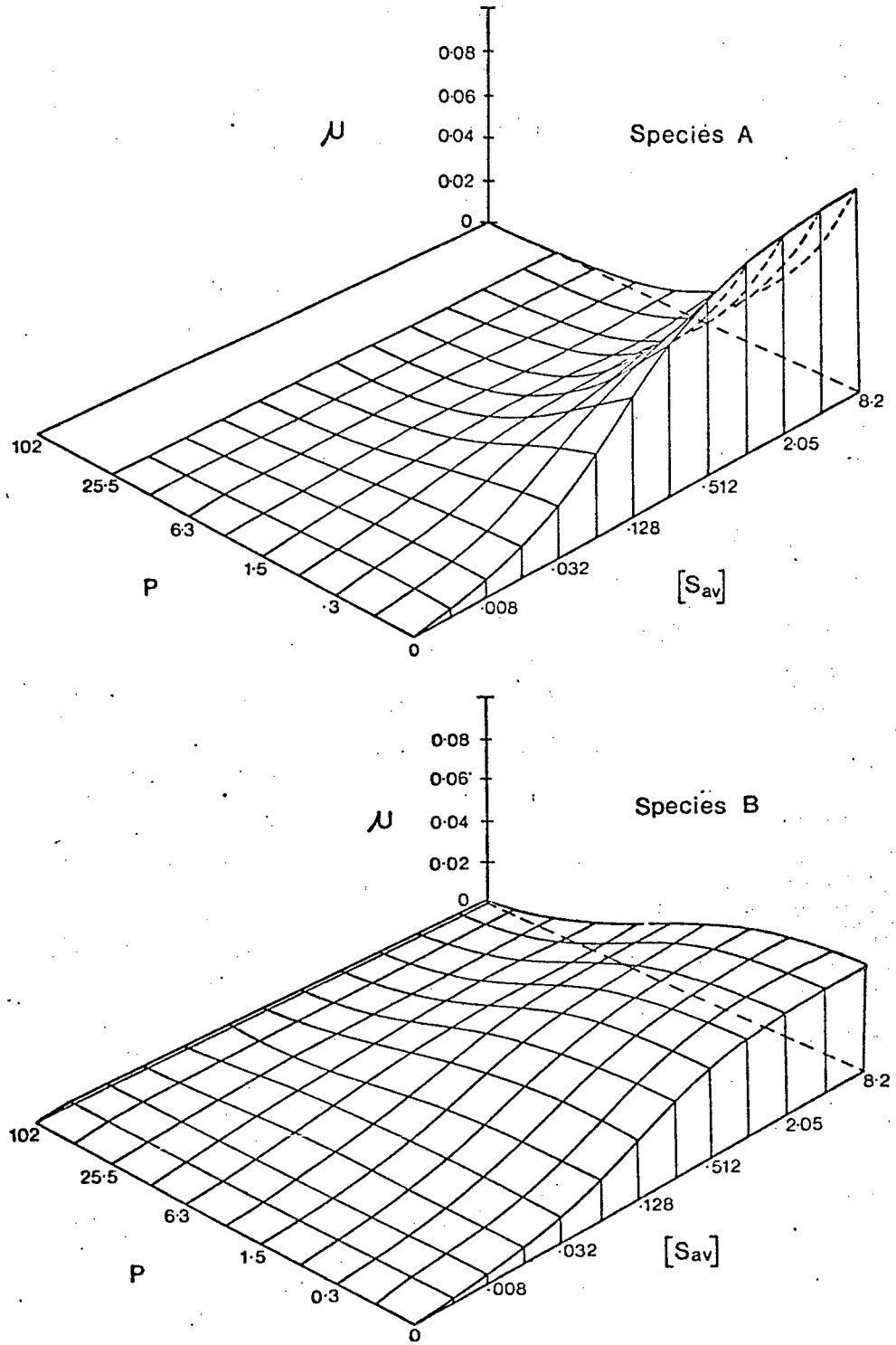


Figure 23. The growth rate, μ , (hr^{-1}) of two hypothetical species as a function of average substrate concentration, S_{av}, ($\mu g\text{-at}\cdot\ell^{-1}$) and the patch period P , (hr). The patch duration is constant at 0.1 hr. Species A has the growth parameters: $\rho_m/Q_{min} = 0.2\ hr^{-1}$; $K_s = 0.2\ \mu g\text{-at}\cdot\ell^{-1}$; and, $\bar{\mu} = 0.2\ hr^{-1}$. Species B has: $\rho_m/Q_{min} = 4.1\ hr^{-1}$; $K_s = 2.05\ \mu g\text{-at}\cdot\ell^{-1}$; and, $\bar{\mu} = 0.0512\ hr^{-1}$.

$$\frac{\rho_m}{Q_{min}} = \frac{\rho'_m}{Q_{min}} + \frac{\frac{\rho_{P\,max.}\ T}{Q_{min}}}{T_h + T} \qquad (9)$$

T_h = the interval, T, at which ρ_p is $1/2\,\rho_{p\ max}$

$\rho_{p\ max}$ = maximum increase in uptake under patchy conditions ($\mu g\text{-at}\cdot cell^{-1}\cdot hr^{-1}$)

ρ'_m = maximum uptake under homogeneous nutrient distributions ($\mu g\text{-at}\cdot cell^{-1}\cdot hr^{-1}$)

T = interval between pulses (hr)

The resulting community then grows at an enhanced rate when the limiting nutrient is patchy with respect to time even though $[S_{av}]$ is constant (Fig. 24). In other words, the effective K'_s of the community is lowered under conditions of optimal patchiness (Fig. 25) if a population can demonstrate an enhancement in uptake ability in response to patchiness.

A similar enhancement of μ as a function of patchiness can be obtained if one uses an $[S_o]$ value (a substrate concentration below which uptake is zero). An example of this simulation is given in Fig. 26.

4) Insights Into Future Experimental Methodology

The use of the chemostat is precluded in elucidating the relationship between growth rate, (μ), average substrate concentration, $[S_{av}]$, and patchiness, (P). When a patch is added to a chemostat population, the nutrient patch remains in the medium until it is assimilated or washed out. This would not allow the investigator to predetermine $[S_{av}]$ or the patch duration as these parameters would depend on the uptake rate of the culture. Also the average growth rate would be predetermined or set by the dilution rate and it would be independent of both $[S_{av}]$ and P providing they are within the limits of growth for the organism in question. The only method by which a μ vs. $[S_{av}]$ and P plot could be generated using a chemostat

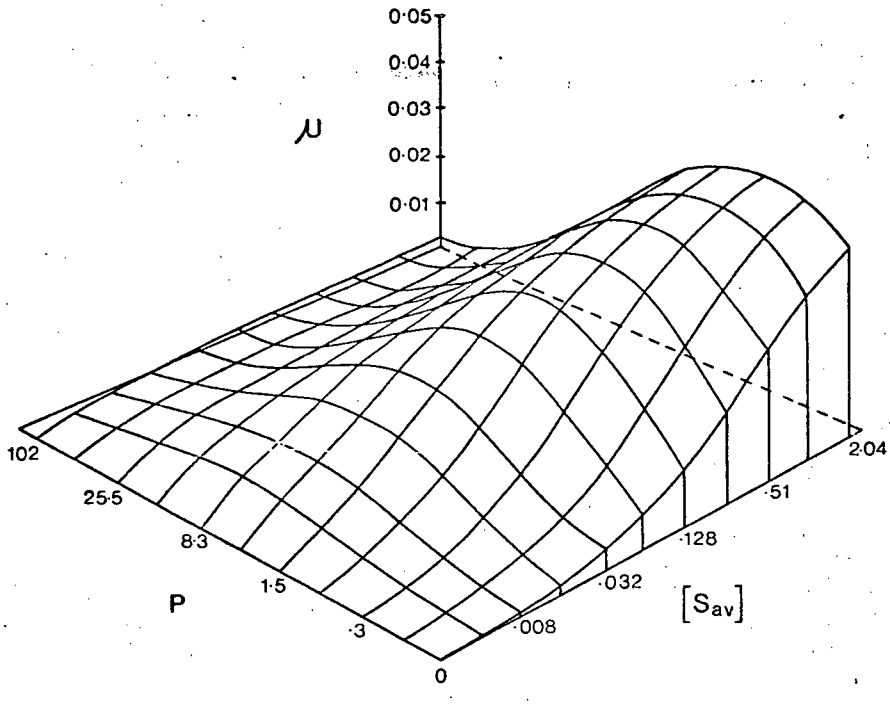


Figure 24. Growth rate of a patch-adapted population with: $\rho_{pmax}/Q_{min} = 2.5\ hr^{-1}$; $K_s = 0.8\ \mu g\text{-at}\cdot\ell^{-1}$; $\bar{\mu} = 0.1\ hr^{-1}$; $\rho'_{m}/Q_{min} = 0.1\ hr^{-1}$; and, $T_h = 2.0$ hr.

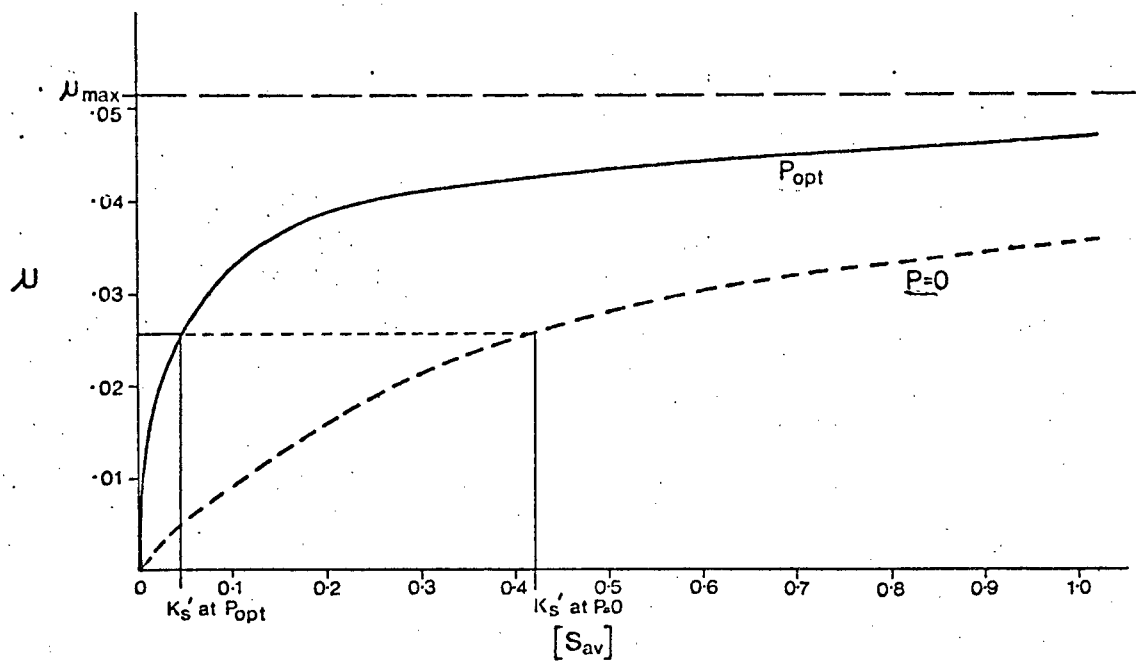


Figure 25. Growth rate (μ) as a function of average substrate concentration, $[S_{av}]$, for the population in Fig. 24. The growth response at $P = 0$ is represented as (-----) and at optimal patchiness (P_{opt}), as (———).

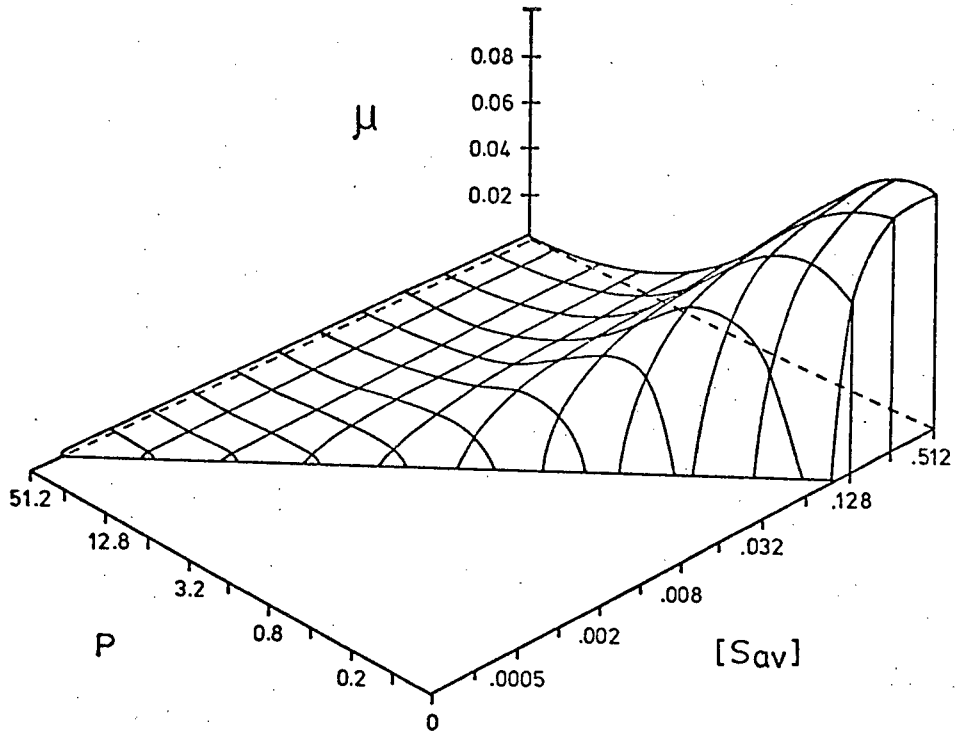


Figure 26. The growth rate (μ) of a hypothetical species with growth parameters $[S_o] = 0.1$ μg-at·ℓ$^{-1}$; $m/Q_{min} = 0.325$ hr^{-1}; $K_s = 0.1$ μg-at·ℓ$^{-1}$; and $\bar{\mu} = 0.155$ hr^{-1}.

would be to follow [S] over the period of the patch to determine $[S_{av}]$. There are two major problems with this method. The first is that $[S_{av}]$ would be a function of the culture uptake rate and the concentration of the pulse. The second problem is the complicating effect of declining substrate concentration following the creation of the patch. This varying nutrient level and varying patch duration would make the interpretation of results far more difficult. The construction of μ vs [S] and P plots, under conditions of constant patch duration (D_p), as in Figures 23 - 26, would be impossible. What is needed is a system where the experimenter can set $[S_{av}]$, P and D_p, while measuring the resulting growth rate. At present such a system does not exist for phytoplankton cultures.

5) Ecological Considerations

Two general conclusions are drawn from this study. First, the degree of patchiness in the environment can affect growth rates of different species and thus alter community structure even though there is no change in the average ambient nutrient concentration. Second, for patch-adapted populations, the apparent K_s' for growth can be lowered significantly by making the distribution of the limiting nutrient patchy with respect to time. Figure 25 shows how growth may vary with respect to average substrate concentration under homogeneous, (P = 0), and optimal patchiness, (P_{opt}). In some simulations the K_s' was over an order of magnitude lower than the K_s' for growth under homogeneous conditions assuming that there is an enhanced ρ_m as a function of patchiness.

The presence of an $[S_o]$ value, a substrate concentration below which growth does not occur, also results in an enhancement in growth as a function of patchiness at limiting substrate concentrations. Figure 26 shows the growth response of a hypothetical species with an $[S_o]$ value.

Different species with different uptake abilities could then be expected

to dominate under various patch regimes even though $[S_{av}]$ does not change. Hence, not only is the ambient nutrient concentration in the environment important in determining species composition, but its temporal and spacial distribution may be equally important.

Nutrient patchiness in nature could range over many orders of magnitude, from millimeters (Shanks & Trent, 1979) to kilometers. Response to this wide range of patchiness would be expected to be very different. Small scale patchiness would tend to generate a regime with pulses of short duration (as seen by the cell). Growth resulting from nutrients procured in these patches could occur outside the patch at some later time as a result of uncoupling of uptake and growth (Caperon, 1969; Cunningham & Maas, 1978). Large scale patchiness would tend to result in pulses of long duration with growth and an increase in biomass taking place in the patch.

Random fluctuations in the distribution of the limiting nutrient could then give rise to a form of coexistence, with species limited by a common nutrient. In a simulation model, Grenney, et al. (1973) showed that fluctuations in chemostat dilution rates and inflow concentrations could result in the coexistence of several species in a single reactor.

There are other factors that have not been considered in the model but they are likely to be associated with communities that are adapted to a patchy environment. Of great importance for a patch-adapted species is the ability to store excess nutrients which would be used when exogenous nutrient supplies are lacking. This ability could be manifested in the form of large intracellular inorganic pools. Species with large pools would take up large amounts of nutrient before the pool filled and uptake decreased to an internally controlled rate, termed V_i, by Davis, et al. (1978). Organisms with small internal pools would not be able to sustain the rapid initial uptake due to more rapid pool filling (DeManche

et al., 1979)

Cell size may also be related to an organism's ability to survive in a patchy nutrient limited environment. A moving cell would increase its chances of encountering a patch. If it possessed 'chemosensory'-motility coupling (Spero, pers. comm.), it might further increase its chances of being able to stay in the patch. Such a situation would increase the duration of the nutrient exposure, hence further optimize uptake and growth.

Other considerations such as phased cell division in a population and the resulting phased nutrient uptake would need to be considered in a complete simulation model. The effects of light and the resulting diel periodicity would also affect uptake of various nutrients and possibly modify trends in the community structure controlled primarily by nutrients.

This work has provided a conceptual framework for the evaluation of the importance of nutrient patchiness in determining phytoplankton growth and community structure. The problem at this time is the lack of information on the time and space scales of the patches being discussed and the nutrient concentrations within them. Future research should approach this problem from the biological, chemical and physical viewpoints in an attempt to further understand this potentially important phenomenon.

Chapter VI

A CONCEPTUAL APPROACH TO NUTRIENT BASED PHYTOPLANKTON ECOLOGY

Since Dugdale (1967) postulated the importance of nutrient concentration in determining phytoplankton community structure, many models have been proposed to account for the effect of nutrients on phytoplankton growth, competition and succession. Few models deal with an integration of factors such as dilution rates[1], nutrient ratios, light and temperature. Those that do (Kremmer & Nixon, 1978), follow a highly mechanical approach. In this chapter, I attempt to combine a number of simple nutrient based growth models, to integrate the effects of nutrient ratios, nutrient fluxes, temperature and light, and to present a simple conceptual approach to nutrient based phytoplankton ecology.

The importance of dilution rates in determining the outcome of chemostat competition experiments is well documented for both bacteria (Harder et al., 1977) and phytoplankton (Michelson et al., 1979; Harrison & Davis, 1979). A schematic representation of the importance of the dilution rate, or the specific flux of nitrogen through a nitrogen limited system in determining the general phytoplankton community structure is presented in Fig. 27 (Turpin & Harrison, 1979). At high specific nutrient fluxes fast growing centric diatoms dominate while at low fluxes, μ-flagellates dominate. This scheme agrees with laboratory experiments (Michelson et al., 1979; Harrison & Davis, 1979), and field observations in which the outcome of

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[1] This is equivalent to the specific flux of the limiting nutrient (i.e. $\frac{ds/dt}{[S]} = \text{time}^{-1}$) at steady-state.

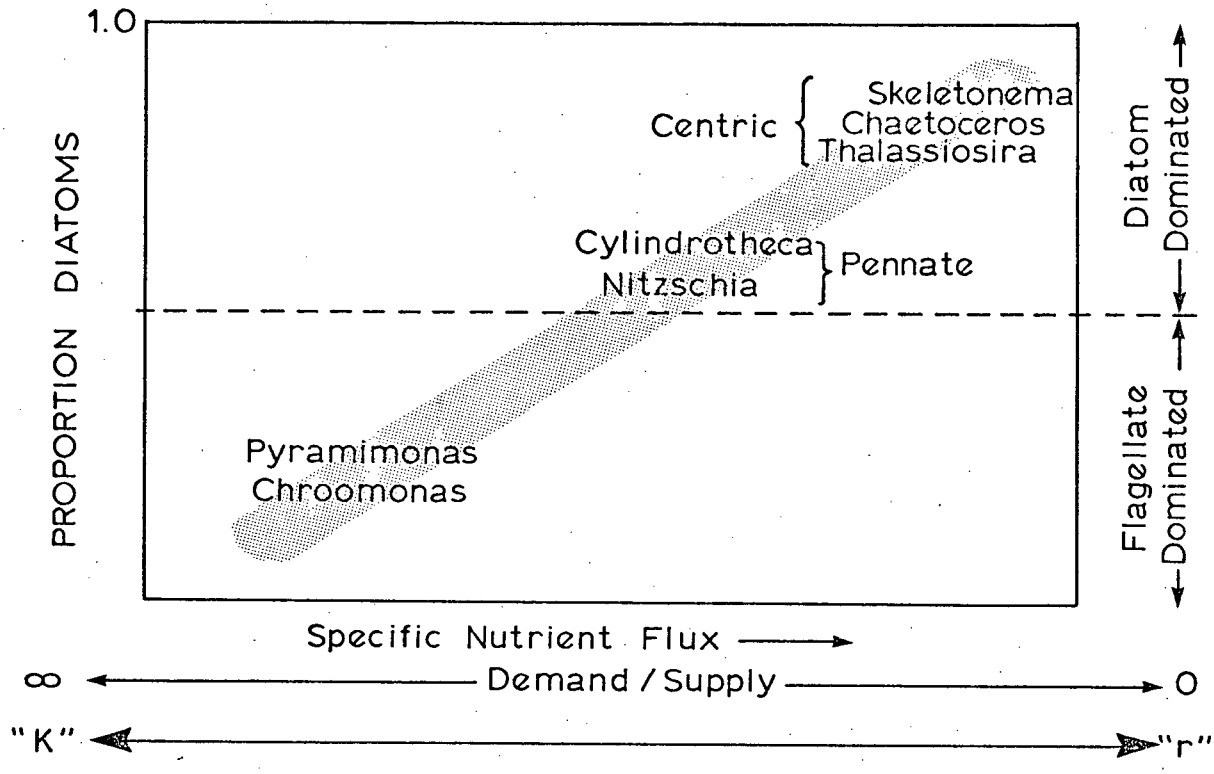

Figure 27. The relationship between specific nutrient flux, D/S ratio, and "r" and "K" competition strategy and the resulting phytoplankton community structure. The competition strategy is represented as a continuum between "r" and "K" strategists.

competition was related to dilution rate or specific nutrient flux.

Recent work showed the importance of nutrient ratios, independent of the absolute magnitude of a given flux (Titman, 1976; Tilman, 1977). Such work has confirmed resource-based competition theory, stipulating that under ideal conditions (identical mortality rates or temporal and spacial homogeneity) coexistence of two competing species can take place only when both species are limited by different resources. The only reason that the outcome of competition was flux independent in the preceding work was because the growth rate (μ), vs. substrate, [S], curves for the chosen species did not intersect (Titman, 1976; Tilman, 1977).

Using species whose μ vs. [S] curves intersect, it is possible to demonstrate the interaction of both the specific flux or dilution rate and the nutrient ratio. This was accomplished by estimating Monod (1942) growth parameters for four hypothetical species competing for two different potentially limiting nutrients, X and Y (Table IV). The outcome of competition between the four hypothetical species is given as a function of both the dilution rate and the nutrient ratios in Fig. 28. The boundaries mediated by changes in dilution rate were determined by the intersection of μ vs. [S] curves (Harder et al., 1977) (see Introduction). The boundaries mediated by resource ratios were determined as described by Titman (1976) (see Introduction). The result is that both the dilution rate and the ratio of limiting nutrients interact in such a way that substantial changes to the community occur, ranging from complete dominance by one species to coexistence of a number of combinations of two species. If the μ vs. [S] curves of the species in question did not cross, then the resource ratio would be the only factor determining the outcome of competition.

In the natural environment the specific nutrient flux and the ratio of the nutrient fluxes may interact also. In a system where nutrient fluxes were

Table IV. Growth kinetic parameters for the hypothetical species in Figure 28. μ_{max} is the maximal growth rate, K_x is the half-saturation constant for growth limited by resource X and K_y is the half-saturation constant for growth limited by resource Y.

	PARAMETERS			
	μ_{max}	K_x	K_y	K_x/K_y
SPECIES A	1.0	4.0	0.1	40
B	0.85	3.0	0.05	60
C	0.88	2.0	0.5	4
D	1.0	2.5	0.25	10

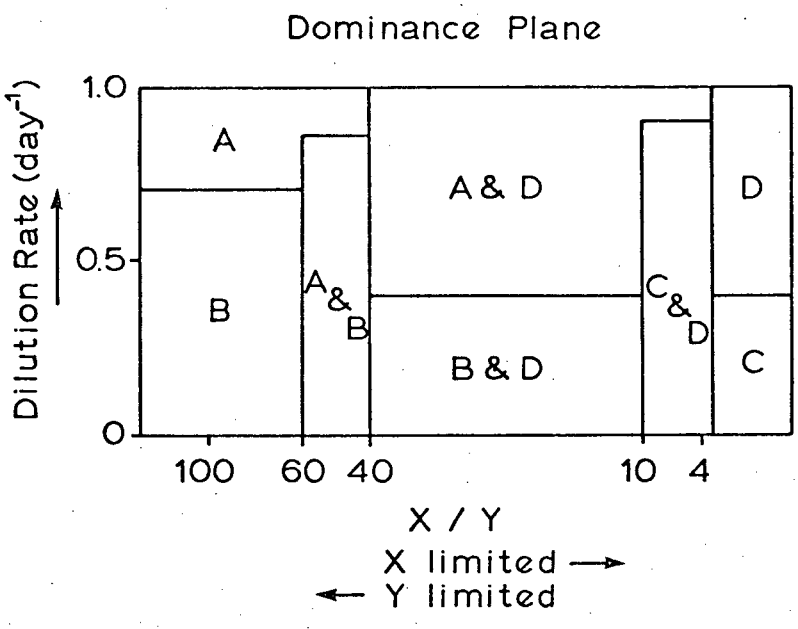

Figure 28. A dominance plane representing the outcome of competition, for the species in Table IV, as a function of dilution rate (specific nutrient flux) and resource ratio. Representation of two species in the same area indicate stable coexistence with both species limited by a different resource.

determined mainly by physical processes such as transport across a thermocline, increases in mixing rates would tend to increase all nutrient fluxes simultaneously, resulting in little change in ratios. Conversely, when nutrient fluxes are controlled predominantly by biological phenomena, the nutrient ratios may change due to differential regeneration. Since both physical and biological factors play an important role in the nutrient dynamics of natural systems, both the magnitude and ratio of fluxes can be expected to vary quite dramatically with space or time.

In an attempt to integrate the possible effects of temperature and light on the structure of a nutrient-based system, a demand/supply (D/S) continuum was imposed, such that at high flux rates, S was large and D/S approached zero. When little nutrient was added to the system (low flux), S was small and D/S approached infinity (Fig. 27). A similar approach has been taken by Kilham and Kilham (in prep.). The result of changing temperature and light can then be viewed through its effect on the D/S continuum as a result of changes in demand (D).

An increase in water temperature within a given range would increase the growth potential of the community (Eppley, 1972), and therefore increase the demand (D) for the limiting resource and hence increase D/S. This increase in temperature should result in a change in community structure similar to that implemented through decreasing the specific nutrient flux, which also increases D/S by decreasing S. Consequently, a low temperature centric diatom community would be expected to shift through a pennate community to a μ-flagellate dominated assemblage in response to a temperature increase and a concomitant increase in D/S. Such a result has been demonstrated by Goldman & Ryther (1976) where an increase in temperature drove nitrogen-limited phytoplankton assemblages from centric through pennate diatoms to flagellates.

An increase in light that is below the saturating level would also tend

to increase the growth potential of the community resulting in an increase in the potential demand (D) for the limiting resource and consequently, community structure should tend to shift to that governed by higher D/S ratios. Some evidence also supports this simplified approach (Harrison & Davis, 1979). They found that when the light intensity was decreased for a culture growing at a low dilution rate, it resulted in natural assemblages of phytoplankton that were similar to those in cultures that were growing at a higher light and dilution rate.

Temperature and light effects can be conceptualized through their interaction with the D/S continuum and to some extent may mimic specific flux changes. Changes in temperature and light are obviously more complex than indicated by our D/S continuum, especially at the extremes of the temperature and light ranges for a given species. The example of changes in light and temperature mimicing specific flux changes may be restricted to small changes of light and temperature occurring at intermediate values within a species range.

There is a continuum of "r" and "K" competition strategy (Pianka, 1970) corresponding to the D/S continuum. An "r" selected organism is selected on the basis of high growth rates, whereas a "K" selected organism is selected on its ability to compete for the limiting resource. When D/S is low, selection occurs for fast growing "r" selected organisms, such as the centric diatoms. When D/S is high, competition for the limiting resource is high and the "K" strategist, the flagellates, succeed.

In conclusion, this simple approach allows for the integration of some of the major factors, such as nutrient flux, nutrient ratio, light and temperature, that affect nutrient-based phytoplankton ecology. Nutrient flux governs the supply, (S), of the limiting nutrient while changes in sub-optimum temperature and light affect the demand, (D), for the nutrient. Large changes in these parameters appear to affect between group (e.g. diatoms vs.

flagellates) dominance, which is also the case for large changes in nutrient ratios. On the other hand, patchiness of the limiting nutrient or frequency of addition of the limiting nutrient has been termed a fine-tuning variable since this parameter appears to select for certain species within the phytoplankton group that was selected by the major selection factors.

SUMMARY

The fluctuations in the free intracellular amino acid pools of ammonia limited *Gymnodinium simplex* and *Skeletonema costatum*, in response to an ammonia perturbation, are best explained if the enzyme glutamine synthetase (EC. 6.3.1.2) acts as the primary ammonium assimilating enzyme. Changes in the levels of all measurable amino acids in *G. simplex* following the perturbation indicated that the amino group was rapidly shunted between the constituents of the amino acid pool.

The temporal patchiness of a limiting nutrient (ammonium) affected the outcome of phytoplankton competition. When the limiting nutrient was homogeneously distributed with time, members of the genus *Chaetoceros* dominated, while under patchy conditions (daily ammonia addition), *Skeletonema* dominated. It was shown that each resulting assemblage was best able to optimize uptake under its particular patchy regime. Optimization of a patchy environment took place by an increase in the maximal uptake rate (V_{max}), while optimization of a homogeneous environment appeared to take place by an increased substrate affinity (i.e., low K_s).

Limiting nutrient patchiness (ammonia) was shown to affect the mean cell diameter that was selected in phytoplankton competition experiments. Low frequency patchiness selected large cells while high frequency patchiness and homogeneous distribution of the limiting nutrient selected small cells.

Limiting nutrient patchiness induced periodicities in carbon assimilation and *in vivo* fluorescence in unialgal cultures of *S. costatum* and *C. gracile*. Observed changes in nutrient uptake ability under varying limiting nutrient patchiness regimes suggested that a given population may adapt its nutrient uptake characteristics to optimize the temporal distribution of the limiting resource. This intraspecific variability in nutrient uptake is minor when compared to interspecific differences.

A theoretical framework is developed to explore the effects of limiting nutrient patchiness on phytoplankton growth. Two general conclusions can be drawn from this study. First, the degree of patchiness of the limiting nutrient in the environment can affect individual growth rates and thus alter community structure even though there was no change in the average ambient nutrient concentration. Second, for patch-adapted populations, the apparent K_s' for growth may possibly be lowered by making the limiting nutrient patchy with respect to time.

Gross changes in nutrient-based phytoplankton community structure were mediated by the specific flux of the limiting nutrient. Low specific fluxes resulted in flagellate dominated assemblages, while high specific fluxes resulted in diatom dominated assemblages. The effects of temperature and light on community structure can be conceptualized through their effect on a Demand/Supply continuum and hence mimic specific flux changes.

Phytoplankton respond to fluctuations in nutrient supply at all levels of biological organization (biochemical, physiological and ecological). Nutrient pulses are rapidly taken up and assimilated. Differences in the ability of species to procure nutrients in this manner result in changes in competitive advantage as a function of the patchiness of the resource. Nutrient supply rates to nutrient limited systems are not homogeneous with respect to time it appears that the area of fluctuating nutrient conditions is one that merits further study.

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Appendix I

CHEMOSTAT THEORY

Inflow medium is pumped into the culture reactor at a constant flow rate (f). Upon addition, this medium is mixed rapidly and homogeneously throughout the culture. The addition of new medium forces an equal volume of culture out of the reactor. Consequently the culture volume stays constant. The dilution rate of the culture (D) is defined as the flow rate, divided by the culture volume, i.e.:

$$D = f/v$$

where f, the flow rate, has units of $ml \cdot time^{-1}$, and the culture volume, v, has units of ml. Hence the dilution rate, D, has units of $time^{-1}$.

The change in cell numbers in a chemostat is a function of the growth rate of the population, μ (hr^{-1}), defined as $\frac{1}{x} \cdot \frac{dx}{dt}$, and the dilution rate, D. The equation for net growth is:

$$\frac{dx}{dt} = (\mu - D)x \qquad (1)$$

where $x = cell \cdot \ell^{-1}$ at time t.

When the culture is at steady state, such that $\frac{dx}{dt} = 0$, then from equation 1 $\mu = D$.

By integrating and rearranging equation 1, we obtain the solution for the specific growth rate of a population in a chemostat as:

$$\mu = D + \frac{1}{t} \ell n \; x/x_o \qquad (2)$$

where $x_o = cell \cdot \ell^{-1}$ at time o

$x = cell \cdot \ell^{-1}$ at time t

If the culture is at steady state, equation 2 reduces to $\mu = D$. For a more detailed description of chemostat theory see Herbert et al. (1956).

Appendix II

DETERMINATION OF NUTRIENT UPTAKE KINETIC PARAMETERS: A COMPARISON OF METHODS

1) Summary

The marine chrysophyte, *Pseudopedinella pyriformis* N. Carter, was grown in ammonium limited continuous culture. This steady state population was used to carry out a comparison of three methods presently used to determine the nutrient uptake kinetic parameters, V_{max} and K_s. The first two methods involved a multiple flask incubation where different concentrations of substrate were added to each flask and therefore the culture past history was constant for each uptake determination. These two methods were similar except the incubation time was variable (method 1), or short and constant (method 2). The third method, the perturbation method, involved monitoring the uptake of one large addition of the substrate to a culture. Hence, in this method, the past history varied during the experiment.

Results indicate for nutrient limited cultures that the parameters, V_{max} and K_s, are best estimated by employing a short, constant incubation time at varying substrate concentrations (method 2). It appears that this method determines the initial maximum uptake rate, relatively free of feedback regulation, when incubation time is very short. The short incubation time is necessary because the measured V_{max} decreases with increasing incubation time. Method 3 provides valuable information on a third uptake parameter, V_i, the approximate rate of assimilation of the limiting nutrient, that is not obtained using either of the other methods.

2) Introduction

Nutrient uptake by marine phytoplankton can be related to the ambient concentration of the nutrient by a rectangular hyperbola, similar to the Michaelis-Menten equation for enzyme kinetics where, $V = V_{max} \cdot [S]/(K_s + [S])$

and V is the uptake velocity (hr^{-1}), V_{max} the maximal velocity, [S] the concentration of limiting nutrient and K_s the half-saturation constant representing the value of [S] where $V = V_{max}/2$. The determination of the nutrient uptake kinetic parameters, V_{max} and K_s , have been useful in explaining competition for the limiting nutrients in the marine system (Dugdale, 1967; Eppley et al., 1969; Tilman and Kilham, 1976; also see Ch. 1).

Frequently nutrient uptake is determined indirectly by measuring the decreasing concentration of the limiting nutrient in the culture medium. Direct measurements of nutrient uptake rates are also made by using isotopes such as ^{15}N, ^{32}P, and ^{30}Si. Nutrient incorporation into the cell is then determined after a suitable incubation time.

In the methods which indirectly measure uptake rate by the disappearance of the nutrient from the medium, there are several possible approaches. The first determinations of V_{max} and K_s were conducted on batch cultures which had just run out of nutrients, however, if the culture was without nutrients for too long a period, the subsequently determined uptake was "non-linear" (Eppley et al., 1969; Eppley & Thomas, 1969). The general protocol for this method was to set up a series of 5 to 10 flasks to which different concentrations of the limiting nutrient were added. The experiment was initiated by adding a sub-sample of a few hundred milliliters of the culture to the flasks. The cultures were incubated for different times. The criterion for terminating the experiment was when the limiting nutrient concentration was thought to be reduced to approximately half the concentration of the original addition or after a depletion of 2 $\mu g\text{-at}\cdot\ell^{-1}$ at higher substrate levels. The uptake rate for the incubation period was then associated with the mean substrate concentration in the flasks. Therefore, in this method, past history was constant (i.e., each flask had the same inoculum) and time of incubation and substrate addition varied.

The second approach was identical to the first, except that the time of incubation of the cultures exposed to different substrate concentrations was constant and relatively short. Rhee (1978) used a constant incubation time of 20 min for *Scenedesmus* in an attempt to alleviate past history effects of nitrate uptake during the experiment.

Another approach to measuring nutrient uptake rates was developed by Caperon and Meyer (1972b). This is termed the perturbation method. These workers grew the experimental culture in a chemostat and upon reaching steady state, the culture was perturbed by adding a relatively large addition of the limiting nutrient (e.g., 10 $\mu g\text{-at} \cdot \ell^{-1}$). Continual sampling and analyses with an autoanalyzer provided a time series of disappearance of the limiting nutrient from the culture until steady state was regained or the limiting nutrient was completely taken up. The changing uptake rate was then related to the average nutrient concentration over the sample period. This approach was also used by Conway et al. (1976). In this latter work, silicate or ammonium-limited cultures exhibited a surge in the uptake rate, termed V_s, or V'_{max} by Goldman and McCarthy (1978), immediately after the addition of the limiting nutrient. This method thus incorporated a variable past-history effect into the parameter determination. The population at the end of the experiment will have been exposed to high nutrient concentrations for a longer time than it was at the beginning of the experiment. The interval over which the uptake rate is calculated is constant and a function of the autoanzlyzer-sampling speed.

Since it is unclear how the choice of either of these three methods affects the values of V_{max} and K_s determination, a systematic comparison of these methods was undertaken in this study. Each method is then discussed in relation to the other two and recommendations are made as to the suitability of the various methods.

3) Methods and Materials

3.1) Chemostat system and analyses:

Pseudopedinella pyriformis N. Carter was obtained from the Northeast Pacific Culture Collection, Department of Oceanography, The University of British Columbia, Vancouver, Canada. The culture was grown at 18°C in a 6-liter borosilicate flat-bottomed boiling flask and under continuous light with an irradiance of 150 $\mu Ein \cdot m^{-2} \cdot s^{-1}$. The dilution rate was 0.5 d^{-1}. The ammonia-limited inflow medium was artificial seawater (Davis et al., 1973) enriched with f/20 vitamins and trace metals (Guillard & Ryther, 1962). The concentrations of the macronutrients, ammonia, silicate and phosphate were 10, 45 and 5.5 $\mu g\text{-at} \cdot \ell^{-1}$, respectively.

The methods for nutrient analysis, cell counts and fluorescence were described previously (Davis et al., 1973). When no trend was observed in the effluent nutrient concentrations, cell numbers of fluorescence for several days, the culture was assumed to be at steady state and the following experiments were initiated.

3.2) Uptake experiments:

Since a large 6-liter chemostat was used, 200 ml could be removed from the chemostat without appreciably changing (4%) the dilution rate. The 200 ml subsample that was removed was replaced by pumping in new medium by the time the next experiment was performed. A small amount of the limiting nutrient (ammonia) was added to the subsample and the culture was immediately incubated under previous growth conditions. Samples were taken every 3 min starting 2 min after the nutrient addition. Ammonia disappearance was followed until depletion occurred. After that time the flask was rinsed and filled with another 200 ml subsample from the chemostat to which another concentration of the limiting nutrient was added and the same continuous sampling repeated. These series of uptake experiments were performed over a limiting substrate concentration range from 0.5 to 20 $\mu g\text{-at} \cdot \ell^{-1}$. These time series data allow

the calculation of uptake rates using three methods. Method 1 determined the uptake rate over the time it took for the substrate to reach a concentration of ∿1/2 its original concentration. Method 2 used constant time intervals of either 2, 5, 17 or 30 min for all uptake calculations. Method 3 used the instantaneous rate of disappearance of the nutrient and related it to the average substrate concentration over that interval.

The initial substrate concentration (i.e., at T = 0) could not be accurately determined by sampling immediately after the substrate was added to the culture and thoroughly mixed. Therefore, the same substrate additions were made to filtered chemostat effluent and measurement of the concentration of the limiting nutrient was made and taken to represent the concentration at T = 0. The uptake rates that were calculated were related to the nutrient concentration at the middle of the time interval over which the uptake rate was calculated. Uptake rates for Method 1 were also calculated by taking the initial substrate concentration as the first measurement taken after the substrate addition rather than the true initial substrate concentrations at T = 0.

3.3) Uptake rate calculations:

Uptake rates were calculated from measurements made during the variable substrate addition procedure described above. Uptake rates were calculated as described by Conway et al. (1976). The particulate values of nitrogen used in the calculations was determined at steady state from a mass balance where the disappearance of the limiting nutrient was assumed to be equal to the increase in particulate nitrogen in the culture. The nutrient uptake kinetic paramenters, V_{max} and K_s, were determined by a direct hyperbola fit and statistical analysis was made using the program of Cleland (1967).

4) Results

4.1) Variable incubation time and variable substrate concentration, Method 1:

The results from this method are given in Figure 29. When the uptake rate was calculated using the true T = 0 concentration (see Methods) the V vs. [S] curve generated was not in the form of a rectangular hyperbola. If the first measured concentration was used as the initial substrate concentration a rectangular hyperbola could fit the data. In this case V_{max} was 0.27 ± 0.04 (s.e.) hr^{-1} and K_s was 0.14 ± 0.12 (s.e.) $\mu g\text{-at}/\ell$

4.2) Constant incubation time and variable substrate concentration, Method 2:

In Table V, the estimates of the uptake parameters V_{max} and K_s are given as a function of the incubation time used to determine them. These results indicated that the shorter the incubation time, the larger the estimate of the maximum uptake rate obtained. Figure 30 shows the relationship of V_{max} with the incubation time used for its determination. No observable trend was exhibited in the half-saturation constant for uptake (K_s) as a function of incubation time.

4.3) Perturbation technique, Method 3:

The uptake parameter estimates are given in Table VI as a function of the concentration of the perturbation. The V_{max} from these experiments were all remarkably similar, ranging from 0.24 ± 0.02 to 0.27 ± 0.04 hr^{-1}. The half-saturation constants, however, showed a great deal of variability depending on the magnitude of the initial perturbation. The larger the perturbation used, the greater the K_s estimate. A graphic representation of this relationship is given in Fig. 31.

5) Discussion

It is apparent from the results of this study that the method chosen to determine uptake kinetic parameters greatly affects the value of the estimate obtained. Therefore, comparison of kinetic estimates for various phytoplankton

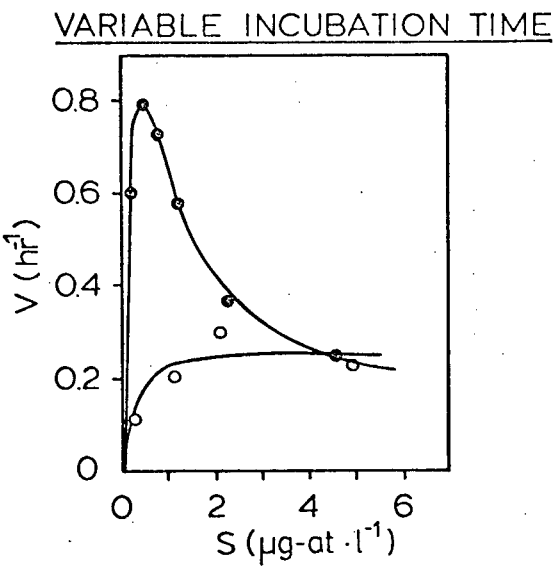

Figure 29. Ammonia uptake (hr^{-1}) as a function of substrate concentration for *P. pyriformis* grown in an ammonia limited chemostat at 0.5 d^{-1}. The incubation time over which the uptake rate was calculated was the time at which the substrate concentration had dropped to half of the original concentration. Uptake rates were calculated using the true t = 0 substrate concentration, ●, and the first measured substrate concentration, 0.

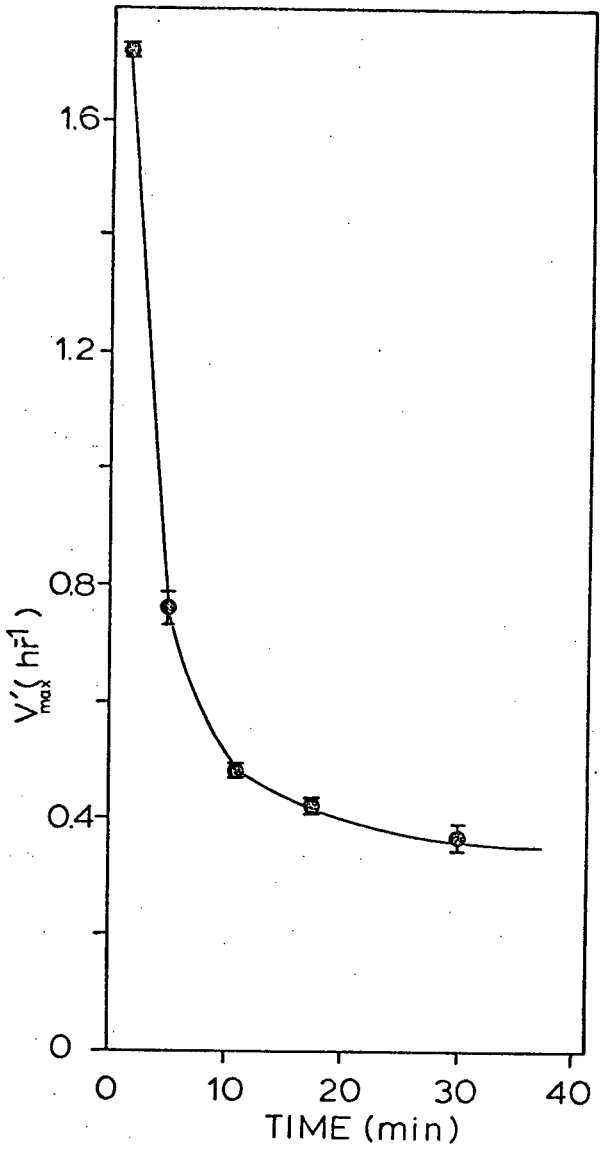

Figure 30. Determination of V'_{max}, using Method 2 (constant incubation time at all substrate concentrations) as a function of incubation time. Bars represent one standard error.

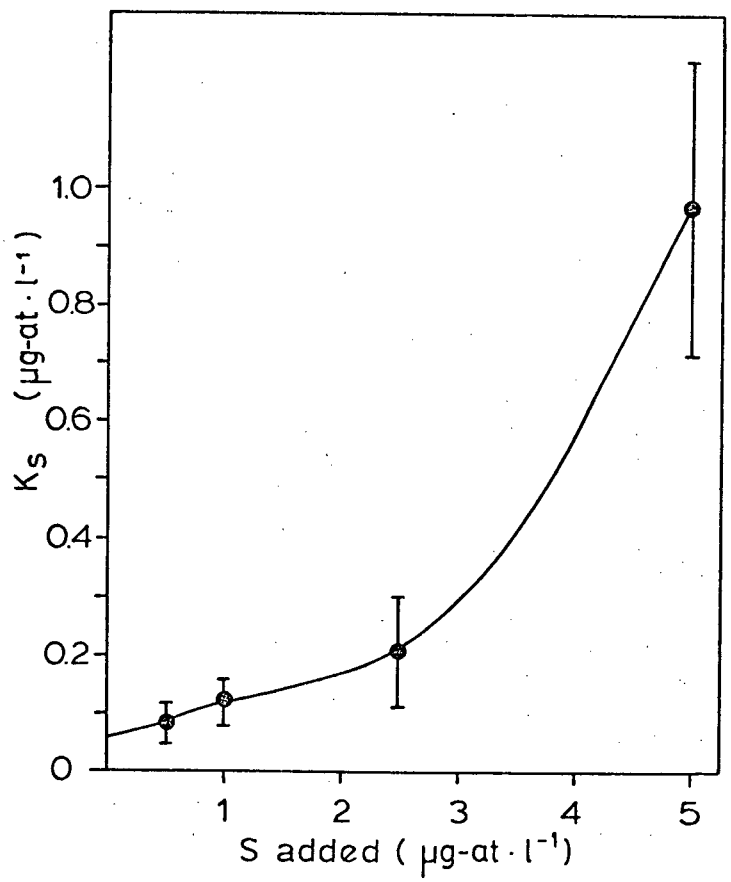

Figure 31. The half-saturation constant (K_s) as determined by the perturbation technique for different initial substrate additions. Bars represent one standard error.

Table V. K_s and V_{max} (V_{max}') values as determined for different incubation times using method 2 in which incubation time was constant at all substrate concentrations.

Incubation Time (min)	K_s ± s.e. ($\mu g\text{-at} \cdot \ell^{-1}$)	$V_{max}(V_{max}')$ ± s.e. (hr^{-1})
2	0.32 ± .09	1.72 ± .07
5	0.05 ± .07	0.76 ± .03
11	0.04 ± .02	0.47 ± .01
17	0.18 ± .04	0.42 ± .01
30	0.45 ± .13	0.37 ± .02

Table VI. K_s and V_{max} (V_i) values as determined by the perturbation technique (method 3) at different perturbation concentrations.

Perturbation ($\mu g\text{-at} \cdot \ell^{-1}$)	K_s ±s.e.	$V_{max}(V_i)$ ±s.e.
5.0	0.97 ±.26	0.24 ±.02
2.5	0.21 ±.09	0.27 ±.02
1.0	0.15 ±.05	0.24 ±.04
0.5	0.08 ±.03	0.27 ±.04

species in the literature should be made with caution since the actual method used to determine the parameters may bias the results. Recent work by Burmaster and Chisholm (1979) have compared the estimate of parameters obtained through the direct incorporation method (isotope uptake) and the disappearance method. Providing a true $t = 0$ substrate value was not obtained in the disappearance method and absorption was controlled for in the incorporation method, little difference in the two methods was apparent. Consequently, the conclusion of the study outlined in this appendix should be applicable to studies employing the isotope incorporation method.

Of the three methods used, Method 2, the constant time, variable substrate method, gave the highest estimate of V_{max} especially over short incubation periods. This is a result of the non-linearity in nutrient uptake rate with time exhibited by this and other organisms (Conway et al., 1976; Conway & Harrison, 1977) in response to a perturbation. Figure 32 shows the disappearance of ammonia with time for *P. pyriformis* for a $\sim$3 $\mu g\text{-at} \cdot \ell^{-1}$ perturbation. The initial rapid disappearance followed by a slower, long-term uptake response is the phenomenon responsible for much of the variability in V_{max} estimates, depending on the incubation time used. If short incubation times are employed, the initial rapid uptake phenomenon is weighted more heavily in the estimate, and consequently high maximal uptake rates are determined. If longer incubation times are used, the slower long-term uptake response (V_i) (Conway et al., 1976) is weighted more heavily in the estimate, resulting in a lower estimate of V_{max}. This result is not unique for ammonia uptake. Similar decreases in uptake rate with increasing incubation time have been observed for ^{14}C uptake (Marra, 1978a).

The decreased uptake rate following a perturbation has been rationalized previously (Conway et al., 1976; Conway & Harrison, 1977). These authors felt that the initial rapid uptake (hereafter referred to as V_{max}' after McCarthy

& Goldman, 1979) represented the true uptake potential of the steady state population, whereas V_i represented an assimilation phase. An additional explanation is that the initial rapid uptake, V_{max}', may be a cell surface adsorption phenomenon and V_i may be the membrane transport component. This study was not extensive enough to resolve this possibility. Consequently, this study will refer to disappearance of the nutrient from the medium as uptake.

In spite of the fact that knowledge is lacking about the exact process observed when nutrient uptake parameters are determined, the population's ability to remove ammonium from the environment is still being measured. Therefore, determining the maximum nutrient procurement (uptake ability) of a population requires use of an extremely short incubation time in order to minimize the effects of feedback regulation of the uptake rate. The shortest uptake interval used in this study was 2 min. This was the fastest time in which the nutrient could be added, mixed and the cells filtered. If a shorter time interval were used the measured uptake response may have been even greater, as it has not yet been possible to show the linearity of V_{max}' over that initial 2 min. As a result the 2-min V_{max}' estimate is probably an under-estimate of the true maximum uptake rate. In order to acknowledge the dependence of the V_{max}' estimate on the time of incubation we suggest that future designations include the time over which the uptake response was measured (i.e., V_{max}' (2 min)).

Use of the perturbation technique, as originally used by Caperon and Meyer (1972b), measures the V_i component, as defined by Conway et al. (1976) and is a great under-estimate of the initial rapid uptake phenomenon, V_{max}'. The V_i component, however, is of great ecological significance if the population is exposed to fluctuations in nutrient levels of long duration (low frequency patchiness).

The K_s estimates from the perturbation technique show a great deal of variability depending on the initial concentration of the addition (Fig.30). The curve through these data represents a visual extrapolation to a K_s value at a 0 $\mu g\text{-at}\cdot\ell^{-1}$ perturbation, in other words, the K_s of the unperturbed culture. This figure shows that the affinity for the limiting nutrient rapidly decreases after the population has been exposed to a nutrient pulse. The low level perturbations are taken up rapidly and do not affect the population's nutrient-limited state. On the other hand, large perturbations which are taken up over a period of hours by a nutrient-limited population, represents a substantial modification in the population's nutrient physiology. For example, uptake of a 5 $\mu g\text{-at}\cdot\ell^{-1}$ of ammonium represents a 50% increase in population nitrogen quota; (the populations used in this study had a particulate nitrogen value of 10 $\mu g\text{-at}\cdot\ell^{-1}$).

The variable incubation time results were calculated in two ways. The first method used the true $T = 0$ nutrient value. The uptake velocity was then calculated over the time interval required for the ambient nutrient concentration to drop to half the original concentration. This method resulted in data that did not fit a rectangular hyperbola (Fig. 29). The reason for this different curve is due to varying contributions of the rapid uptake phenomenon. For the low concentration uptake determinations, the initial rapid uptake phenomenon accounts for most of the nutrient disappearance because the uptake interval is short. Therefore, at low concentrations we measure high uptake velocities. At high concentrations the initial rapid uptake accounts for only a small portion of the total uptake. This is because the incubation time is long and there is a large contribution to total uptake by the V_i component. This results in the lower uptake estimates at high concentrations.

If the first measured nutrient concentration is used as the initial substrate concentration and uptake velocities are calculated, then the

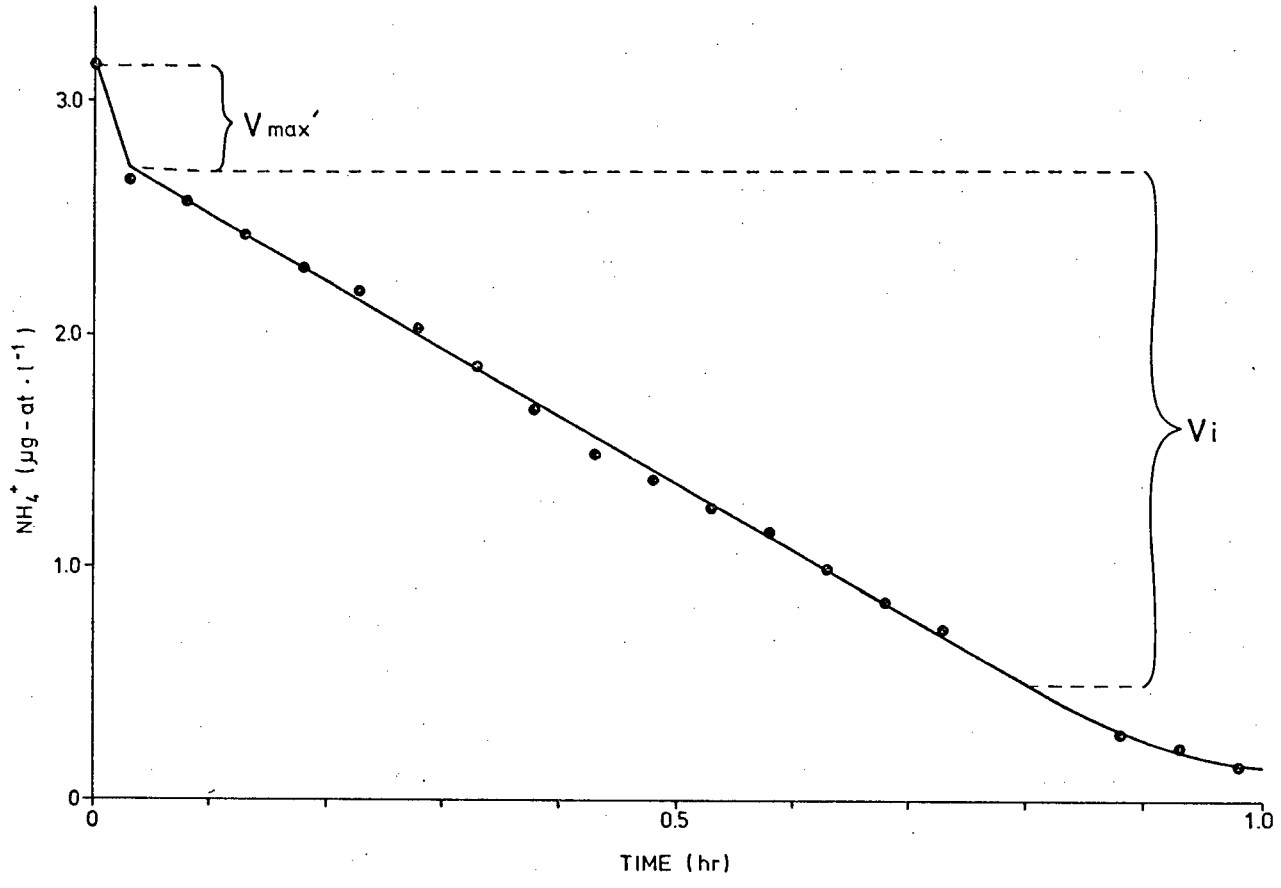

Figure 32. Disappearance of ammonium with time, showing the V_{max}' and V_i components.

data fit a rectangular hyperbola (Fig. 29). The V_{max}', as obtained by this method, was identical to the V_i of the perturbation method. The half-saturation constant as determined by this method was 0.14 ±0.12 μg-at·ℓ$^{-1}$ ammonium. The major problem with this method is that each uptake determination represents a population with a different past history or nutrient time exposure. This could result in different contributions of feedback effects, by the nutrient taken up, on the subsequent uptake rate.

It appears that the best method for determining the maximum uptake rate is to use a constant incubation time at varying substrate concentrations (method 2). The reporting of data should include a notation as to the incubation time used. The perturbation technique (method 3) and the variable time incubation method (method 1) both give an estimate of the V_i of the population.

The determination of the half-saturation constant is not straightforward. Using the perturbation technique at varying initial nutrient levels and extrapolating to a 0 μg-at·ℓ$^{-1}$ perturbation appears to be one method (Fig.31). Unpublished work (Harrison, pers. comm.) suggests that this may not be universal for all phytoplankton species. In fact, he found that the size of the perturbation had no effect on the K_s value determined by this method. Using a constant incubation time gives K_s estimates that overlap at the 95% confidence limits, regardless of the incubation times used. The variable incubation time also gives an estimate encompassed by the 95% confidence intervals of the constant incubation time method. Therefore, the K_s data are not of sufficient resolution to resolve any trends that may exist.

It is apparent from the results of this study that caution must be exercised when comparing uptake kinetic values which were determined using different methods. The point must be made that data in this study pertain

only to a nitrogen limited population. When limitation is not severe, uptake appears to be linear over time (Eppley & Thomas, 1969; Eppley et al., 1969). The degree of uptake complexity would then appear to be a function of the degree of nitrogen limitation, with the contribution of the V_{max}' component increasing with increasing nutrient deficiency (McCarthy & Goldman, 1979) and decreasing incubation time.