1990

The effect of nitrogen and phosphorus supply ratios and dilution rate on phosphorus uptake and mineralization in continuous flow microcosms

Peter M. Eldridge
College of William and Mary - Virginia Institute of Marine Science

Order Number 9022042

The effect of nitrogen and phosphorus supply ratios and dilution rate on phosphorus uptake and mineralization in continuous flow microcosms

Eldridge, Peter M., Ph.D.

The College of William and Mary, 1990

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

The effect of nitrogen and phosphorus supply ratios and dilution rate on phosphorus uptake and mineralization in continuous flow microcosms

---

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree

of Doctor of Philosophy

---

by

Peter M. Eldridge

1989

Approval Sheet

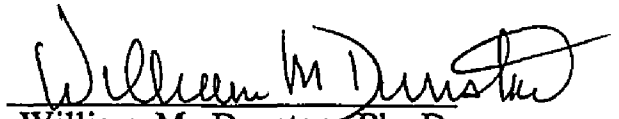
This dissertation is submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

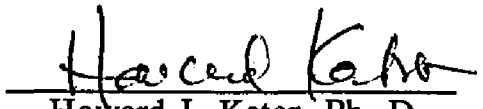
Peter M. Eldridge

Approved : December, 1989

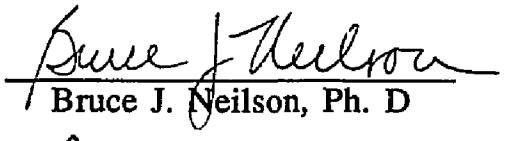
Kenneth L. Webb, Ph. D.
Committee Chairman / Advisor



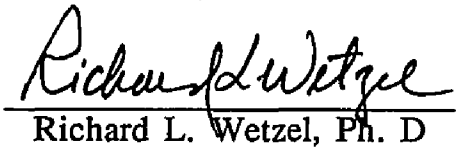
William M. Dunstan, Ph. D



Howard I. Kator, Ph. D



Bruce J. Neilson, Ph. D



Richard L. Wetzel, Ph. D

Table of Contents

ACKNOWLEGEMENTS

I am indebted to my committee chairman, Dr. Kenneth L. Webb, for his support, patience, and many hours of labor spent editing my sometimes difficult prose. A am also grateful to Dr. Webb for his financial assistance during this project.

I would like to thank the members of my committee for their constructive criticism during both the preparation of my prospectus and the writing of my dissertation. I am especially grateful to my committee members for their open-minded nature and civil regard for my feelings during the review of this dissertation.

I want to thank Dana Booth for her assistance and cooperation in filtering and processing samples from the microcosms, and Betty Salley for her help with nutrient analyses.

I owe a special thanks to my wife, Sheryl Eldridge, for her love, patience, and encouragement. Her skill in editing this dissertation is sincerely appreciated.

List of Tables

List of Figures

Abstract

Continuous flow microcosms (50L volume, salinity 18-24 ppt) were used to examine the roles of heterotrophic protozoa and bacteria as phosphorus mineralizers. Nitrogen limitation was regulated by N:P supply ratios (5:1, 16:1) and growth rate was regulated by dilution rate (0.5, 0.25 volumes day^{-1}). Rates of carbon and phosphorus uptake from dissolved inorganic pools were determined using ^{14}C and ^{32}P tracer experiments and pre- and post-fractionation incubations. Based on uptake rates and on POP and POC values of the bacteria in the <1.0 μm fraction, mass balance estimates of bacterial mineralization were determined. An isotope dilution method was used to determine phosphorus mineralization in the unfractionated sample. Heterotrophic protozoan mineralization was calculated as the difference.

Results showed that bacteria had a minor role as remineralizers of phosphorus, more often taking-up inorganic phosphorus than remineralizing it. Heterotrophic protozoa were the major remineralizers of phosphorus. Microcosm treatments with a greater supply of inorganic phosphorus over nitrogen had increased POP concentrations and increased phosphorus remineralization. The effect of dilution on phosphorus remineralization was temperature dependent. Greater remineralization occurred in the high dilution rate in the July through October experiments and less remineralization occurred in the high dilution rate treatments in the December and January experiments

The effect of nitrogen and phosphorus supply ratios and dilution rate on phosphorus uptake and mineralization in continuous flow microcosms

Chapter 1

Introduction

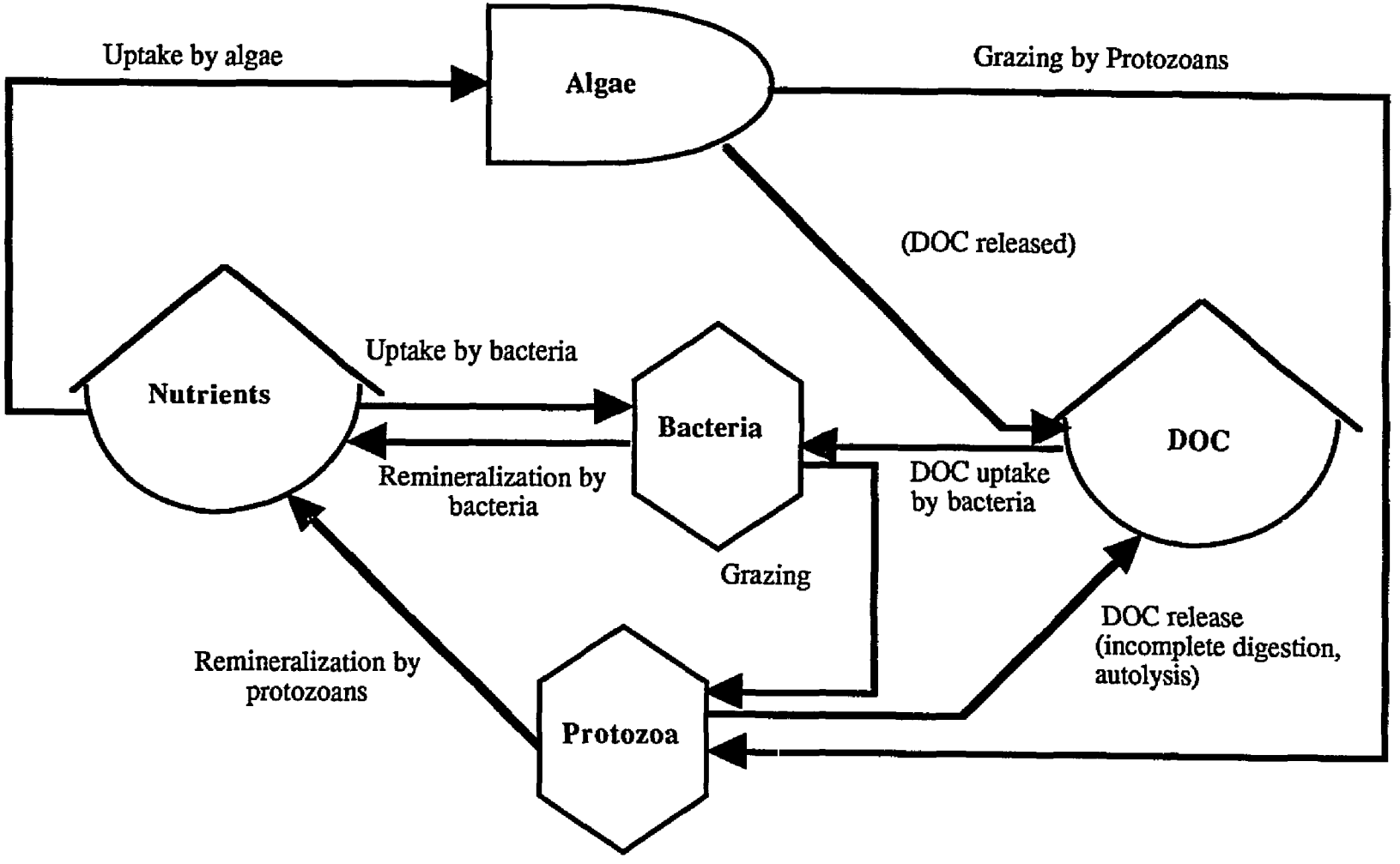
1.1 General

The nutrient status of microplankton in the York River estuary was determined in microcosm experiments between August 1985 and October 1987. This work, directed by Dr. Kenneth Webb (Virginia Institute of Marine Science), was the impetus for the dissertation research reported here. The purpose of Webb's experiments was to identify which nutrient, phosphorus (P) or nitrogen (N), limits phytoplankton primary production during each season in the lower Chesapeake Bay. This study was initiated as part of a bay-wide effort to determine the effects of nutrient enrichment and as a response to the continuing controversy over the importance of nitrogen and phosphorus limitation (D'Elia 1985). Webb's study indicated that growth rates were lower during winter than during other seasons, probably because of lower temperature and shortened day length (Webb 1988). This study also indicated that the growth rate trends based on carbon and nitrogen biomass are qualitatively the same as, but less pronounced in magnitude than, those based on chlorophyll. Phosphorus limitation was present in the winter and early spring, with the switch from phosphorus to nitrogen limitation occurring in the May-June time period. Nutrient limitation was measured as a growth response to the addition of the limiting nutrient when compared to the control. No growth response occurred with the addition of a non-limiting nutrient.

Nutrient limitation can also be expressed as a change in the molar ratio, 106:16:1, of carbon, nitrogen and phosphorus (Redfield 1958), i.e. a C:N molar ratio of 6.6 (5.7 by weight). These ratios represent normal and presumably healthy balances of elements for phytoplankton (Parsons et al. 1984). When phytoplankton growth rates are below the maximum due to limitation by N or P, the cell composition would likewise be deficient in N or P relative to carbon, e. g. the C:N ratio tends to be higher than 5.7 by weight (Goldman et al. 1979). C:N ratios may increase by as much as a factor of three (Lancelot and Billen 1985) when phytoplankton deplete the available nitrogen from the media. C:N ratios for microcosm experiments ranged from 7.5 to 14.5 when molar C:N ratios are calculated from C linearly regressed against N. This indication of nitrogen limitation thus agrees with the growth response data. Rarely, however did any of the treatments produce severely nitrogen limited plankton based on particulate C:N ratios (Webb 1987).

The 1985-1987 microcosm studies provided a data base of the production rates of carbon and nitrogen and of the relative concentrations of organisms by genus or guild during periods of inorganic nitrogen mineral limitation. This dissertation was designed to augment the data base collected in the 85-87 study. Tracer studies were used to study how mineral nutrients are transformed through the particulate phase to be remineralized or to be utilized in a higher trophic level by a predator. Measurements of these rates help one to understand the trophic level interactions which modify the nutrient status of microplankton organisms.

Figure 2-1. General model of microbial food web in microcosm experiments on the York River.



Chapter 2

Background

2.1 Microbial loop

Azam et al. (1983) introduced the term "microbial loop" for that part of the microbial ecosystem that either transports dissolved organic material (DOM) via bacteria into the food chain, or remineralizes the DOM to inorganic nutrients. The concept, however, had its roots earlier in Pomeroy's (1974) paradigm which postulates that bacteria and heterotrophic flagellates are important components in the energy flow in the ocean planktonic ecosystem, along with the traditional food chain of algae -> copepods-> fish. By repackaging bacterial biomass into cells accessible to filter-feeding zooplankton, the heterotrophic protozoa constitute a mechanism for returning the energy in DOM, previously thought lost to the detritus food chain (Caron et al. 1985), to higher level consumers. The rates of metabolism within this system have been likened by Goldman (1984) to a fast turning wheel with rapid energy flows between the various compartments. The carbon and mineral flows in the microbial loop are tightly coupled. Information on the nature of this coupling is essential for an understanding of microbial loop dynamics.

It has been suggested that bacteria may play a central role as primary consumers, with the heterotrophic flagellates performing most of the remineralization tasks and the phytoplankton supplying DOM to power the microbial loop (Johannes 1965; Azam et al. 1983; Goldman 1984; Gude 1985; Goldman et al 1987). The concept of the heterotrophic flagellate as

the primary remineralizer of nutrients contradicts the more traditional view which regards bacteria as degraders and remineralizers of phytoplankton biomass (reviewed by Ducklow et al. 1986).

There is little agreement as to the role of each component of the microbial food loop. New flows and compartments have been suggested for this loop (i. e. see Fig 2-1), making it more of a web than a loop, as the original concept has expanded. Recently it has been suggested that heterotrophic protozoans, in addition to their role as a grazer and remineralizer of nutrients, also release dissolved organic material (DOM) (Hagstrom et al. 1988). The heterotrophic flagellates and small ciliates utilize virtually all the cyanobacteria and heterotrophic bacteria production; they release about half of the ingested organic carbon due to incomplete assimilation of food particles in the feeding vacuoles and/or by senescence and mortality (Hagstrom et al. 1988).

2.2 Carbon conversion efficiency

Sherr et al. (1986) have carried out studies designed to determine whether the "microbial loop" is a source of biomass for the food chain leading up to nekton, or whether this loop respires most of the organic carbon that enters it and thus is only important in terms of remineralization of nutrients. The main requirement for the microbial loop to be a source of biomass is extremely high carbon conversion efficiency. Carbon recovery efficiency of bacteria is equivalent to 10-50% (Williams 1984). Even higher carbon conversion efficiencies may occur, however, under inorganic nutrient-rich conditions (Azam et al. 1983). Under these conditions, nutrients not available to bacteria in DOM are supplied, with a

minimum energy expenditure, from external inorganic nutrient pools (Horrigan et al. 1988). Laboratory studies suggest that the heterotrophic protozoan component of the microbial food loop has a maximum carbon assimilation efficiency of about 40% when feeding on bacteria (Williams, 1984). Thus, even if 30% of phytoplankton photosynthate is lost as extracellular material, only about 1.2 to 6% of total primary production would be recovered by the microbial loop and repackaged as heterotrophic protozoans as a source of biomass for higher trophic levels. Pomeroy suggests that the most important consideration in the examination of trophic transfers is the number of steps involved, not the efficiency at each step (Pomeroy 1988). Little carbon can be transferred to metazoans even if efficiencies are high in food chains consisting of bacteria, microflagellates, small ciliates, and large ciliates,.

The standing stock and growth potential of bacteria in the oceans is not well documented. Williams (1984) suggested that since large accumulations of microbial biomass rarely occur, microbial gross production must either be respired away by the producing organisms, be lysed, sink out of the water column, or rapidly grazed. He postulated that both grazing and bacterial respiration of assimilated carbon play an important role in keeping the concentration of cells low considering their potential high growth rate. On the other hand, observations by Morita (1986) indicated that bacteria are not being effectively grazed by heterotrophic protozoa and generally are an inactive component of the open ocean ecosystem. These bacteria may actually be starving (carbon limited) in oligotrophic oceans.

Sources of DOM available to bacteria result from sloppy feeding, excretion, and lysis of senescent cells during and following a bloom. This DOM may episodically increase bacterial and heterotrophic protozoan production by increasing the flow of carbon into the microbial loop. As discussed above the energy released as DOM by phytoplankton is rather inefficiently returned to the main food chain via the microbial loop of bacteria->flagellates-> microzooplankton. Thus, the microbial loop would seldom be a food source for microzooplankton and, in terms of energy and material transfer, cannot be as efficient (Azam et al. 1983) as direct herbivory on phytoplankton. There is significant evidence, as reviewed by Sherr et al. (1986), that heterotrophic microbial biomass in the form of herbivorous ciliates can directly be an important source of nutrition for metazoans. These organisms short circuit the microbial loop by feeding directly on phytoplankton, thereby improving throughput by reducing the number of steps in the food chain.

2.3 Mineralization

Mineralization is the result of catabolic processes and the release of unassimilated nutrients, and as such, is affected by the nutrient status and diet of the excreting organism (Thingstad and Pengerud 1985; Goldman et al. 1987). Remineralization of nitrogen (N) by bacteria requires that nutrients come from an organic substrate (e.g. amino acid, peptides, or protein) which contains N, and that the C:N ratio of the bacteria biomass ($C:N_B$), when corrected for carbon losses through respiration, be greater than the C:N ratio of the available substrate ($C:N_S$). Quantitatively this relationship can be expressed as follows (Goldman et al. 1987):

$$R = C_T \left[\left(\frac{C}{N_s} \right)^{-1} - (GGE) \left(\frac{C}{100\ N_B} \right)^{-1} \right] \qquad \text{Equation 2-1}$$

R is the rate of nitrogen excretion in μg-atoms N ($cell^{-1}\ h^{-1}$), $C_T = C_B + C_R$, C_R is respired as CO_2 in catabolic reactions, C_B the portion of carbon used in anabolic reactions leading to biosynthesis of cell material. GGE is the gross growth efficiency in percent, and both C:N ratios are by atoms. Positive values of R represent net nitrogen excretion and negative values indicate net consumption of nitrogen (Goldman et al. 1987). A similar expression can be developed for phosphorus. Thus, if bacteria are using organic matter with high C:N or C:P ratios, they will take up inorganic N or P and compete with phytoplankton. If the C:N or C:P ratio is low the bacteria are net "remineralizers" and will release DIN or DIP (or both).

Heterotrophic bacteria and phytoplankton need a balanced diet of nitrogen, phosphorus, and carbon to produce new biomass. Balanced or nutrient sufficient growth in phytoplankton occurs when the molar ratio of carbon (C), nitrogen (N), and phosphorus (P) is in the Redfield ratio 106:16:1 (King 1987), i.e. C:N=6.6 and C:P = 106. Bacteria, because of their high nucleic acid content, tend to have C:N ratios between 4.1 and 6.1 when undergoing balanced growth. Little information is available on the chemical composition of protozoa, but Fenchel (1987) suggests that their optimal chemical composition is similar to that of bacteria. When a producer and consumer are closely linked, mineralization at one level tends to reduce mineralization at the next higher level. We would, therefore, not expect heterotrophic protozoa to excrete much N or P unless its C:N or C:P ratio is higher than that of its food source (Goldman et al. 1987). The magnitude of remineralization is a function of both the growth efficiency

and the carbon to nutrient ratios of the prey and predator (Fenchel and Backburn 1979).

Elements in marine phytoplankton exist in the same relative abundance as that found in surface seawater. This concept, originally formulated by Redfield (1958), has fueled much speculation about the effect of biological processes on the geochemical distribution of key nutritive elements. It appears that the C:P and C:N ratios are not, however, constant with depth, but increase markedly with depth as a result of the more rapid P and N regeneration relative to C regeneration (P >N >C) (Martin et al. 1987). In the marine and estuarine environment, either N or P usually causes nutrient limitation of bacterial or algal growth (King 1987). Marine bacteria and phytoplankton, under laboratory induced conditions of severe nutrient limitation, have been observed to contain elemental ratios quite different than the Redfield ratios (Lancelot and Billen, 1986). In surface waters, especially in nutrient-depleted oceanic environments, it was therefore suspected that particulate Redfield ratios may seriously misrepresent elemental exchange (i.e. based on Redfield ratios). Goldman et al.(1979) showed that differences exist between the effect of nitrogen-limitation on elemental ratios in natural ocean populations and in laboratory cultures. Natural populations of plankton were found to contain ratios of elements close to Redfield ratios. Goldman et al. (1979) suggests that these populations are growing rapidly under conditions of balanced growth at low population concentrations even in the most oligotrophic of oceanic environments .

The differences between the nutrient status in laboratory cultures and in natural plankton communities under nutrient limited conditions suggests

that a mechanism exists that allows nutrient-sufficient growth of marine phytoplankton to be maintained in the presence of small absolute amounts of inorganic nutrients. This seems to be the result of a trophic interaction between predator and prey. Goldman et al. (1979) hypothesized that rapid exchanges of nitrogen between phytoplankton and their grazers occur in micro-environments which are unobservable with standard sampling technology. This recycling mechanism adds sufficient "old" nitrogen to maintain the microbial loop components in balanced or nitrogen-sufficient growth (King, 1987). Goldman et al. (1979) observed such nitrogen sufficient algae in continuous cultures with no remaining measurable dissolved nitrogen. This is consistent with the general observation that phytoplankton assimilate proportionately more nitrogen associated with heterotrophic excretion (ammonia and urea) in nutrient-depleted surface waters than in more eutrophic environments where $NO3^{-2}$ can be a larger proportion of the N input.

A quantitative understanding of the coupling between C, N, and P metabolism is important to our understanding of material flows in the microbial ecosystem (Fenchel 1982). Of special interest is the question of whether bacteria act mainly as net consumers of inorganic nutrients or as net remineralizers in the recycling of the "old" nitrogen. In an ecosystem context, the composition of bacterial biomass is important since it defines the quality of food available to bacterivorous organisms. Goldman et al. (1987) hypothesized, "that an imbalance in the C: N: P ratio in the food supply, coupled with fairly rigid stoichiometric requirements for nutrients by the heterotrophic protozoans during unrestricted growth, will result in conservation of the nutrient in shortest supply." It can be expected that a

heterotrophic organism must first fill its quota of a needed nutrient before it can excrete any excess nutrient beyond the maximum cell quota (Malone et al. 1986). This is shown quantitatively for bacteria in equation 2-1, where C:N ratios of dissolved substrate and bacterial biomass were compared to determine if bacteria were net regenerators of nutrients or took up nutrients.

Mineralization in a food chain consisting if DOM> bacteria > heterotrophic protozoan is dependent on the GGE of the bacteria and protozoan and the carbon to nutrient ratio of each component. The bacterial GGE will reduce throughput of material to the protozoan by about 50% even if bacterivorous grazing is 100% efficient. Thus, heterotrophic protozoa, when feeding only on bacteria, would probably not be an important remineralizer of phosphorus or nitrogen. The protists primary function would be to maintain the bacterial assemblages in a stage of "physiological youth" (Caron et al. 1985). Because heterotrophic protozoans are often omnivorous and have been shown to consume photosynthetic eukaryotes of nearly their own size, an alternative pathway for protozoan mediated remineralization of POM exists which circumvents the bacteria (Caron et al. 1985; Parslow et al. 1986). By feeding directly on photosynthetic picoplankton and nanoplankton, the heterotrophic nanoplankton could dominate remineralization processes in the microbial community. Bacterivorous, herbivorous, and omnivorous feeding are probably the major modes of nutrition for the heterotrophic protozoa, since osmotrophy has been shown to be unlikely in these organisms (Haas and Webb 1979).

2.4 Respiration coefficients

The mechanism of nutrient limitation has implications for population dynamics and carbon cycling. It has been shown that bacterial respiration increases when going from a carbon limited to a mineral nutrient limited condition (Bratbak 1987). Respiration as a process removes carbon from the internal pool of carbon. Since respiration varies both with growth rate and with type of nutrient limitation (N or P), environmental conditions determine how efficiently carbon is transferred through bacteria to the bacterial predator (Bratbak 1987) and made available to higher trophic levels in the food chain. Changes in the degree of nutrient limitation and type of limitation (N or P), as well as the availability of a reduced carbon source will effect bacterial growth efficiency.

The rapid exchanges of nitrogen and phosphorus between phytoplankton, bacteria, and their grazers in oligotrophic oceans maintain the algae in a fast growing nutrient sufficient state, but this incurs a cost to the algal standing stock and to the exploitable primary production. These maintenance costs are a result of respiration of the several stocks within the microbial loop (King 1987).

2.5 Interspecific competition

Through the utilization of inorganic nutrients, bacteria can have a profound effect on phytoplankton. Bacteria's competitive superiority over phytoplankton, in terms of uptake kinetics for DIN and DIP, is considerable (Thingstad and Pengerud 1985). In chemostat experiments Currie and Kalff (1984) showed that an advantage in uptake kinetics is reflected in an advantage in growth. Without a reduced carbon source,

however, heterotrophic bacteria become carbon limited and their efficiency as a competitor for a limiting mineral nutrient is reduced. Since one significant source of DOC is the release of photosynthetic products by phytoplankton, a homeostasis could be postulated in the ecosystem between bacteria and phytoplankton. Thingstad and Pengerud (1985) modelled the interaction between bacteria, algae, and heterotrophic protozoans using gnotobiotic chemostat cultures. When they eliminated the protozoan from these cultures, fewer conditions of dilution rate, mineral nutrient, and carbon concentration were found in which coexistence of bacteria and phytoplankton could occur.

Bratbak and Thingstad (1985) reported a destabilizing interaction between bacteria and phytoplankton by which bacteria stimulate phytoplankton to increase exudation rates. Mineral limitation seems to stimulate phytoplankton to expend a greater proportion of their production as excretion of extracellular carbon (PDOC). The PDOC, as a result of the phytoplankton nutrient status, will have a high C:N or C:P ratio. A paradoxical situation may be created in which bacterial growth on the excreted PDOC causes additional uptake of mineral nutrients, causing competition for DIP or DIN. Thus, algae stressed by the lack of mineral nutrients respond by stimulating their competitors to obtain the lacking nutrient.

Obviously, bacteria and algae do co-exist in most aquatic communities. Thingstad and Pengerud's (1985) gnotobiotic chemostat experiments showed that when bacteria are subjected both to competition with phytoplankton and to predation by heterotrophic flagellates, bacterial growth rates become saturated before algal density becomes zero. As a

result, algae are maintained in the chemostats for all values of carbon and dilution rate. For stability to be established, a three component community of phytoplankton, bacteria, and heterotrophic protozoa must exist together. As discussed earlier, the heterotrophic flagellate fills an important role as a predator and remineralizer of mineral nutrients (Gude 1985).

In situations with large inputs of allochthonous organic material, competition for mineral nutrient between algae and bacteria may result in bacterial degradation of organic matter at the expense of primary production (Parsons et al. 1981). Allochthonous terrestrial material such as cellulose and humic acid tends to have a high carbon content (high C:N and C:P ratios). Equation 2-1 indicates that under these conditions, bacteria would balance their organic carbon uptake with concomitant uptake of mineral nutrients (DIP and DIN). An important consequence of this form of interspecific competition, noted by Parsons et al. (1981), was the increased tertiary production in glucose enriched food chains. They concluded that an increase in bacteria biomass was transmitted directly to the zooplankton and up through the food chain. Interspecific competition is important, as the bacteria-algae interaction may affect the energetics of the entire food web. Bratbak and Thingstad (1985) suggest that competition for mineral nutrients is the main ecological interaction between phytoplankton and bacteria. This conclusion should be tempered by the fact that phytoplankton and bacteria can obtain phosphorus from sources other than mineral nutrient pools (e.g. DOP), and that bacteria depend on algae for food (energy), either directly or indirectly.

Bacteria and phytoplankton have cell surface enzyme systems that convert organically bound phosphorus to inorganic phosphorus.

Utilization of organic and inorganic polyphosphate pools results from alkaline phosphatase (APase) activity on phytoplankton cell surface membranes (Perry 1972; Admiraal and Veldhuis 1987). Alkaline phosphatase activity is repressed by orthophosphate; it does not play an important role in nitrogen-limited ocean waters, but may be important in phosphorus-limited freshwater and estuarine systems (Vargo and Shanley 1985; Ammerman and Azam 1985). Marine and freshwater bacteria have a cell-surface 5'-nucleotidase that rapidly hydrolyzes 5'-nucleotides and regenerates orthophosphate. Unlike alkaline phosphatase, this enzyme is not repressed by orthophosphate concentrations. Although it has been shown that remineralization of DOP by the above mechanisms does not release phytoplankton from mineral nutrient competition with bacteria, it does moderate that competition (Ammerman and Azam 1985; Andersen et al. 1986).

Chapter 3

3.1 Objectives

This study was conducted to determine how inorganic phosphorus becomes distributed to components of the microbial food web through uptake and release processes. Experimentation was carried out in a continuous flow microcosm system in which phosphorus and nitrogen input and dilution rate could be controlled.

The first objective was to evaluate the effect nutrient molar supply ratios and dilution rate have on phosphorus uptake and remineralization in the microcosm community as a whole. The second objective was to determine the relative contribution of bacteria to total phosphorus uptake and mineralization in the microcosms.

3.1.1 Rational

When microflagellates graze on prey grown under nitrogen limitation, there is considerable regeneration of total dissolved phosphorus (Andersen et al. 1986). How these pools of phosphorus are stored (Lancelot and Billings 1986) and the effect of excess inorganic phosphorus on particulate ratios and growth rate (Goldman et al. 1979) has been studied. There is, however, little information on the interaction between phosphorus loading, growth rate, and remineralization rates. Remineralization is either the result of bacterial or heterotrophic protozoan activity, where N or P is obtained in organically bound forms (organic molecules or prey

organisms). Release of inorganic N or P from heterotrophic organisms occurs when intake of the organic bound minerals exceeds requirements.

Goldman (1980) showed that the amount of variation in the phytoplankton C:P ratio decreases with increasing growth rate. During phosphorus-limitation, an increase in phosphorus cell quota was the primary cause of the decrease in C:P variation with increased growth rate.

During nitrogen-limitation, luxury uptake of phosphorus (the nutrient in excess) can be expected at low growth rates. Intracellular storage permits an uncoupling between phytoplankton production and uptake of nutrients, resulting in a low C:P cellular ratio at low growth rates (Sommer 1989). At higher growth rates, luxury consumption of phosphorus is reduced as this nutrient is used to sustain growth (Lancelot and Billens 1984; Suttle and Harrison 1988). As a result, at high growth rates, P-cell quota will be small and the C:P ratio will be near Redfield's ratio. Thus, during nitrogen-limitation, the portion of P retained per unit carbon decreases with increasing growth rate. High growth rates also imply that there is more phosphorus uptake by a cell. When increased growth is accompanied by grazing and remineralization, increased growth rate should result in increased P-remineralization rates.

Although a microcosm does not have the strict relationship between dilution rate and growth rate that is found in monotypic chemostats, an equilibrium between dilution rate and growth rate will be attained, i.e. consumption of a limiting nutrient and its renewal by the medium input and remineralization will come to a steady state. Changing the dilution rate may change the growth rate, which, in turn, may change the rate of P-remineralization under nitrogen-limited conditions.

3.2 Hypotheses

Three hypotheses were derived from the objectives.

1. Organic phosphorus remineralization is primarily the result of heterotrophic protozoan activity.
2. Increasing the supply ratios of inorganic phosphorus relative to nitrogen will result in a proportional increase in phosphorus remineralization.
3. Phosphorus remineralization increases with dilution rate.

3.2.2 Rationale

In testing similar hypotheses, experimenters have either made successive additions of axenically cultured algae, heterotrophic bacteria, and heterotrophic protozoan to chemostats and measured changes in remineralizing rates after each addition (Gude 1985; Andersen et al. 1986). Berman and Stiller (1977) and Glibert (1982) completed isotope dilution studies of size fractioned natural assemblages and reported results of uptake and remineralization from each size fraction. In the case of successive additions, it is uncertain whether the added species is responsible for the change in remineralization or stimulates one of the other components already present to change its remineralization rate. In the size fractionation isotope dilution studies, each size grouping must independently take up and remineralize an isotope tracer. Thus, exchange of energy between size fractions is either attributed to the larger size fraction or is undetermined by this technique.

To test the hypotheses listed above, isotope tracers ^{32}P and ^{14}C were used to follow material flows through plankton communities and size fractionation was used to separate bacteria from the rest of the plankton at the end of incubations.

Chapter 4

Materials and Methods

4.0 General

Microcosm experiments were conducted from a pier located on the lower York River at the Virginia Institute of Marine Science. Phosphorus concentration, phosphorus uptake and remineralization were measured for each microcosm's picoplankton and total plankton community. Carbon uptake and particulate carbon measurements were taken in parallel with the phosphorus determinations. Physical measurements, nutrient analyses, particulate organic nitrogen (PON) determination, and microscopic examinations, were performed to characterize the microcosm community.

4.1 Size groupings

Polycarbonate 1.0 μm filters were used to separate the picoplankton component of the microcosm community from the whole water. This filtration was done either to prepare a <1 μm size fraction for incubation with radioactive tracers (pre-incubation fractionation), or to separate the <1μm size fraction from the whole water sample at the end of an incubation with tracers (post-incubation fractionation).

The objective of the pre-incubation fractionation was to provide a <1μm filtrate containing organisms in good physiological condition. Using a reverse filtration technique a sample was pressure filtered from the base of the filtration apparatus, upward through the filter into the filter head.

The sample in the base was continuously stirred to keep plankton from settling. Pressure was provided by a water tower 1.5 m in height above the filter apparatus. Because the sample was not dropped through an water-air interface, turbulence and shear forces on the organisms were minimized.

Size fractionation with 1 μm nuclepore polycarbonate filters has been used extensively to partition bacterial populations from the rest of the planktonic community. This separation technique has been shown to be efficient, resulting in approximately 90% separation of the two populations (Jones et al. 1983; Ward 1984; Waterbury et al. 1986), although instances have been reported where significant numbers of small eucaryotic organisms pass into the 1 μm filtrate (Waterbury et al. 1986; Sieburth et al. 1985). Although picoplankton are organisms between 0.2 and 2.0 μm in diameter, from a practical viewpoint a 0.2 to 1.0 μm fraction is preferred, since it will include most of the bacterioplankton while excluding all but the smallest flagellates and other photoautotrophs (Li 1986).

4.2 Measurement of Material flows

4.2.1 General Description

The flux rates of carbon and phosphorus into the whole water and size fractionated samples were measured using radiotracer methods (Eppley 1968; Lean et al. 1979; Ward 1984). The energy spectrum of the ß particles from ^{14}C and ^{32}P differ enough to allow discrimination between the two labels by scintillation counting methods (Berman et al. 1977). In this experiment, dual labeling was used to determine the distribution of labels in the plankton and the bacterioplankton size fraction.

4.2.2. Distribution of tracer between size fractions

Extracellular dissolved matter released by phytoplankton (PDOM) and subsequent utilization by heterotrophic bacteria results in a distribution of the labeled ^{14}C among several pools. Although ^{14}C-HCO_3^- is supplied to the microcosm community, the labeled compound actually assimilated by a specific organism or size fraction in these experiments may not be ^{14}C-HCO_3^-. It has been shown that substantial fractions of photosynthetically fixed ^{14}C are released from phytoplankton as dissolved organic compounds, which are rapidly assimilated by bacteria (Ward 1984). Release of newly synthesized organic compounds by actively photosynthesizing phytoplankton is well documented (Bell 1980). Rapid assimilation of labelled PDOM by heterotrophic bacteria results in indirect labelling of the bacterial fraction (Larsson and Hagstrom 1979; Ward 1984). To make a quantitative measure of carbon and phosphorus uptake into the bacterial size fraction, the uptake of inorganic tracer into phytoplankton and release of labelled PDOM must be in equilibrium. The specific activity (μCi C/ μgC) must be the same in the ^{14}C-HCO_3^- taken up as in the ^{14}C-DOC released. Obviously it takes a time increment after application of ^{14}C-HCO_3^- for this equilibrium to occur.

Similarly, ratios of ^{32}P to ^{31}P must be the same in these exchanging pools to quantitatively evaluate uptake rates. Smith and Platt (1984) performed time series experiments to determine the kinetics of ^{14}C uptake and release by a diatom in nitrogen-limited chemostat cultures. Their results were consistent with a model they proposed which treats algal cells as "2 pools of carbon; an exchangeable pool which involves photosynthesis,

respiration, and excretion, and a synthetic pool that does not exchange, but accumulates carbon from the exchanging pool "(Figure 4-1).

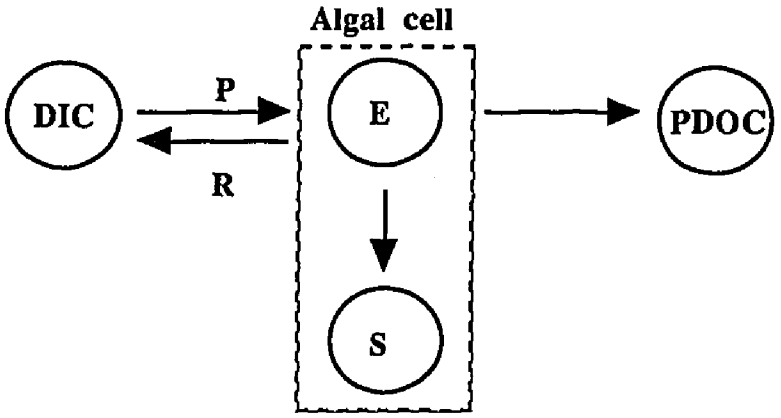


Figure 4-1. Compartmental model of algal carbon exchange. P is uptake, R is respiration, S is the synthetic pool, and E is the exchange pool from which dissolved organic carbon (PDOC) is derived (adapted from Smith and Platt 1984).

Experiments using unialgal cultures (Smith's and Platt 1984) indicated that the exchanging pool was small (mean 9% of cell carbon, SE 2.0%) and was rapidly cycled (6 hours, SE 1.3 hours), where cycling time is the 90% tracer equilibrium point. Phosphorus cycles at a faster rate than carbon (Martin et al. 1987), suggesting that ^{32}P in PDOP will reach equilibrium sooner than is the case for carbon.

Transfer of ^{14}C label into the <1μm size fractions could occur in three ways :

1. Photosynthesis by the <1μm size fraction.
2. Assimilation, by heterotrophic bacteria, of dissolved organic material that is released into the medium either by actively photosynthesizing organisms (>1μm) or as a by-product of protozoan grazing (Ward 1984; Hagstrom et al. 1988).

3. Light independent $C0_2$ fixation, in either heterotrophic or autotrophic organisms, which serves to regenerate the C_4 dicarboxylic acids essential for the continuation of cellular metabolism or primary CO2 fixation by chemoautotrophs. Romanenko et al. (1972) suggest that C4 reconstitution accounts for 6% of the total cell carbon during heterotrophic growth.

Four main pathways exist for incorporation of phosphorus in the <1μm size fraction:

1. Uptake of inorganic phosphate in photoautotrophs (<1μm).
2. Uptake of inorganic phosphate in bacteria concurrent with osmotrophy.
3. Direct assimilation of P-containing organics by bacterial.
4. Surface hydrolysis of polyphosphates and nucleotides and uptake of released phosphate by phytoplankton or bacteria.

When other uptake pathways are controlled or small, ^{14}C and ^{32}P tracers in post-incubation size fractionation allows us to determine assimilation by bacteria of organic C and P produced by phytoplankton and heterotrophic protozoa (Berman and Stiller 1977; Ward 1984).

4.2.3. Measurement of bacterial phosphorus mineralization by mass balance

It is assumed that a bacterial cell by analogy is similar to a phytoplankton cell, and may be described by a two compartment model consisting of an exchange pool and a synthetic pool (see section 4.2.2). The synthetic pool is large and has long chain structural elements. The

exchange pool is a small, rapidly exchanging pool composed of inorganic molecules and short chain organic molecules. These pools exchange carbon and phosphorus with external inorganic pools, via uptake and respiration, and they donate C and P to the synthetic pool, as shown in Figure 4-2 (Fenchel and Blackburn 1979). Table 4-1 demonstrates how an initial amount of nutrients (source) might eventually become distributed to a growing bacterium. However, based on a ^{32}P tracer alone, we cannot strictly account for the total P that enters the bacterium cell and goes into biomass and that portion of P that is released.

Goldman et al. 1987 suggested that the P:C uptake ratio can be compared to the P:C particulate ratio to determine P released by bacteria, as long as the uptake C value is adjusted for respiratory losses. The difference in the adjusted ratios indicates the portion of P in excess. Goldman et al. (1987) assumed that concentrations of ambient dissolved organic nutrient (N or P) and DOC were proportional to uptake and applied a gross growth efficiency to adjust for respiration losses. In this study, however, uptake of ^{32}P and ^{14}C into the bacterial fraction was measure over 6 hour incubations and compared to the particulate C:P ratio to estimate P released.

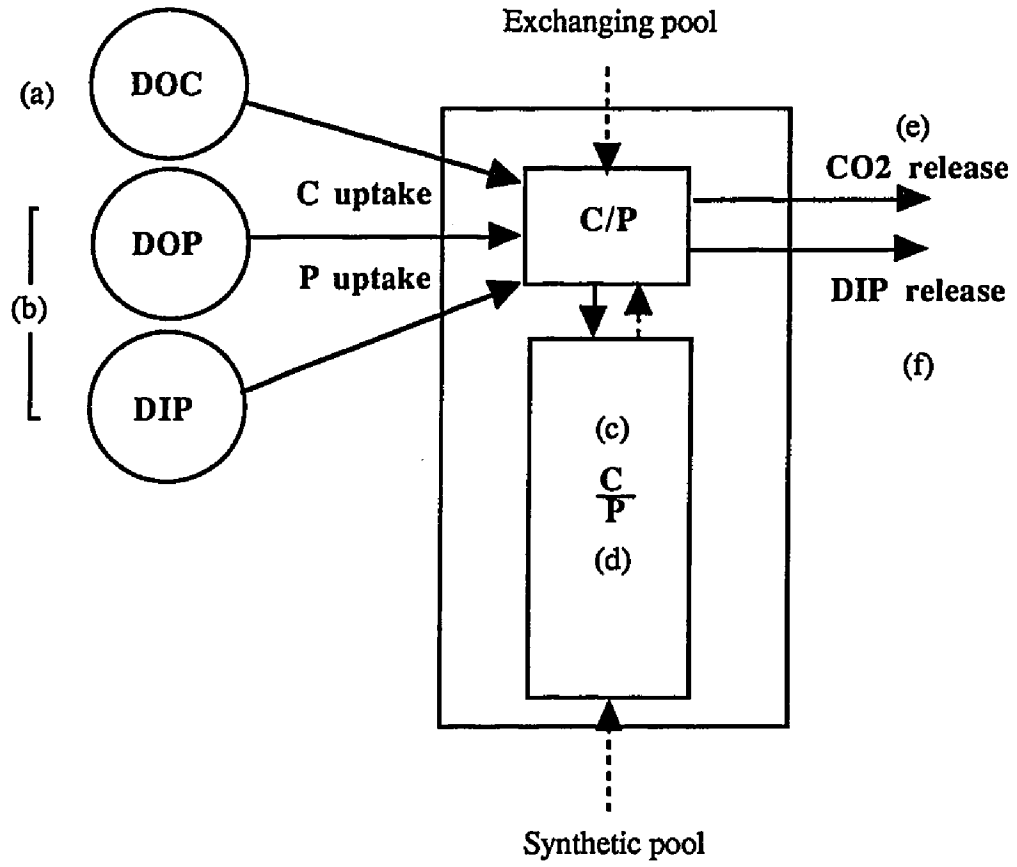
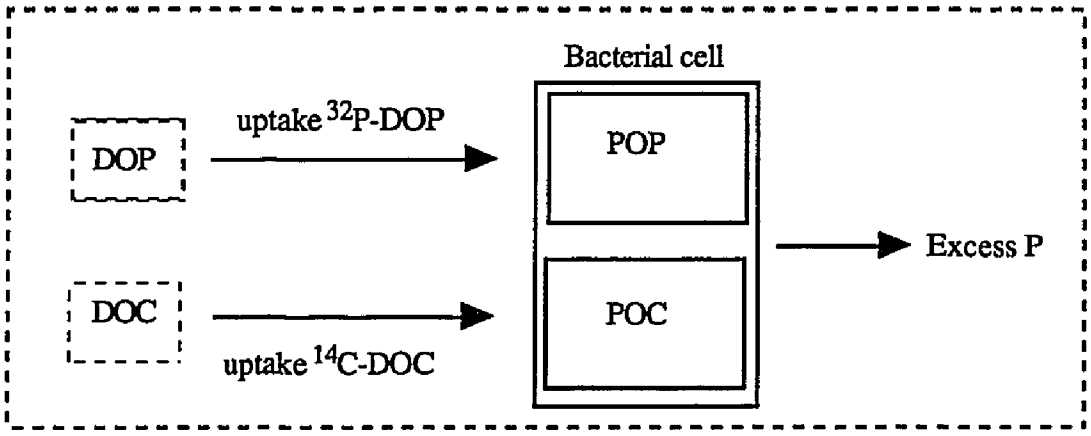


Figure 4-2. Carbon and phosphorus flows through a bacterial cell. The cell is composed of an exchange pool and a synthetic pool. The synthetic pool is large and has long chain structural elements. The exchange pool is a small, rapidly exchanging pool composed of inorganic and short chain organic molecules. This pool exchanges carbon and phosphorus with external inorganic pools, via uptake and respiration, and donates C and P to the synthetic pool. The labels in parentheses are described in Table 4-1.

Table 4-1 Mass balance of carbon and phosphorus through a bacterial cell. Letters refer to labels for pools in Figure 4-3. Either release or uptake of DIP is dependent on the uptake ratio of C to P and the particulate C to P ratio. Uptake of DIP is represented by negative values of release. Arbitrary units are used

---------- Source ----------			--- bacterial biomass ---			--- release ---	
A (DOC)	B (DOP+DIP)	ratio A/B	C (POC)	D (POP)	ratio D/E	E (CO2)	F (DIP)
1000	10	100	800	8	100	200	2
1000	9	111	800	8	100	200	1
1000	8	125	800	8	100	200	0
1000	7	143	800	8	100	200	-1
1000	6	167	800	8	100	200	-2



Equation 1

$$\frac{\text{uptake } ^{32}\text{P-DOP}}{\text{uptake } ^{14}\text{C-DOC}} - \frac{\text{POP}}{\text{POC}} = \text{Fraction of total P in excess}$$

Equation 2

$$\text{Fraction of total P in excess} \times \text{Bacteria } ^{14}\text{C uptake} = \text{P-remineralization by bacteria}$$

$$\frac{\mu gP}{\mu gC} \qquad \frac{\mu gC}{1\text{- hr}} \qquad \frac{\mu gP}{1\text{- hr}}$$

Figure 4-3 Mass balance equation for the calculation of bacterial remineralization from bacterial ^{32}P and ^{14}C uptake and particulate ratios. For remineralization to occur, P uptake must be in excess of the requirements for maintaining the cellular C:P ratio. The assumptions for this technique are shown on page 29.

Smith and Platt (1984) suggested that in long incubations the portion of the ^{14}C that passes through the particulate phase to be lost as ^{14}C-CO_2 is representative of respiration, and the portion that remains is assimilated into the synthetic pool (Figure 4-2), thus adjusting carbon uptake for respiratory losses. Only short-term ^{14}C measurements (1-3 h) reflect gross carbon production (Weger et al. 1989). There is, however, a fundamental difference between carbon and phosphorus cycling in bacteria. Whereas the bacteria cannot take up CO_2 (except for a small amount used in C4 reactions), they can take up DIP.

The ^{32}P tracer taken up as ^{32}P-DOP by the bacterial cell may be assimilated by the synthetic pool (Figure 4-2, D), or be converted by dissimilatory metabolism into inorganic phosphorus. This inorganic (P_{excess}) will reside in the exchanging pool and depending on cellular requirements will be released from the bacterial cell as DIP (Figure 4-2, F) or reused by the synthetic pool (Fenchel and Blackburn 1979). Thus, unlike CO_2 which is lost to the bacterial cell during respiration, there is a pool of inorganic phosphorus (P_{excess}) that is constantly being turned over to either meet cellular phosphorus needs or being released by remineralization (Fenchel and Blackburn 1979).

The inorganic ^{32}P partition in the particulate phase is in excess of ^{32}P assimilated, suggesting the following relationship (Wetzel, personal communication),

$$\frac{POP}{POC} = \frac{DOP_a - P_{excess}}{POC_a} \quad \text{equation 4-1}$$

where DOP_a and DOC_a are DOP and DOC assimilated.

If we assume that when bacterial mineralization of P occurs, the cell requirements for P are already met, then P_{excess} will be proportional to DIP released from the cell. The C:P ratio of the synthetic pool, is then a measure of cellular requirements and the DOC_a:DOP_a ratio is a relative measure of what is taken up (adjusted for carbon loss by respiration). By taking the difference of the reciprocals of these ratios, the fraction of total P in excess is determined (Figure 4-3, equation 1). DIP released is calculated by multiplying the fraction of P in excess by the bacterial production rate (Figure 4-3, equation 2).

This method of calculating DIP release is only valid when all phosphorus pools are assumed to be in isotopic equilibrium and that ^{32}P assimilated is derived from DOP. When DOP uptake does not meet cellular P requirements, dissolved inorganic P (DIP) will be assimilated. When this occurs, the assumption that all P assimilated is derived from DOP is not met. Thus negative values obtained from this model will probably underestimate DIP uptake.

The assumptions of this technique for the measuring bacterial mineralization are as follows:

1. That phytoplankton and bacteria are composed of a small exchange pool and a large synthetic pool.
2. That the exchange pools rapidly come to a steady state with both added tracer and tracer in the cellular dissolved release products (DOP and DIP).
3. When remineralization occurs, ^{32}P assimilated is derived from DOP.

4. That there is a measurable component of ^{32}P-DOP uptake in excess of that assimilated into the synthetic pool.
5. When bacterial mineralization of P occurs, the cell requirements for P are already met.

Phosphorus remineralization rates of bacteria were calculated using equation 4-2, so that the usual convention of C/P ratios could be preserved.

$$R = C_u \times \left(\left(\frac{C_u}{P_u}\right)^{-1} - \left(\frac{C_p}{P_p}\right)^{-1}\right) \quad \text{Equation 4-2}$$

R is remineralization when positive (+), and is an indication of phosphorus uptake . C_u is carbon uptake, P_u is phosphorus uptake, C_p is particulate carbon, and P_p is particulate phosphorus.

4.2.4. Measurement of total remineralization by isotope dilution

If water samples with intact autotrophic and heterotrophic populations are incubated for short periods of time with added ^{32}P-PO4 label, then the change in the ^{32}P : ^{31}P isotope ratio reflects both the autotrophic $^{32,31}PO_4^{-2}$ uptake and the heterotrophic $^{31,32}PO_4^{-2}$ excretion rate. Regeneration rates then can be determined using equation 4-3 (Harrison 1983):

$$R = \frac{\ln (R_o/R_t)}{\ln (S_o/S_t)\cdot t} \cdot (S_o - S_t) \qquad \text{Equation 4-3}$$

R= DIP regeneration rate (mg- at m^{-3} t^{-1}); R_o, R_t= specific activity of DIP (DPM/mg-at) at times 0 and t; S_o, S_t = DIP concentration at times 0

and t. This equation assumes no recycling of the tracer ^{32}P, and a constant regeneration rate over the incubation period. However, when DIP levels are either near detection limits or when there is no significant difference between ambient DIP levels (S_0 and S_t) at the beginning and end of an incubation, results of equation 4-2 indicate that no remineralization has occurred (multiplication by zero). This result is obvious incorrect when there is a difference in R_0 and R_t. An analysis of variance (ANOVA) was used to test for significant differences between treatment means of initial and final activities (R_0 and R_t), and substrate concentration (S_0 and S_t). No differences in S_0 and S_t could be found between the beginning and end of incubations ($P >> 0.05$), even though R_o and R_t were significantly different ($P < 0.05$). This condition can be expected when uptake and remineralization are equivalent, a situation which may exist in a continuous flow microcosm at or near steady state and probably in the real world as well. Glibert (1982) suggested a modification by which the average substrate concentration (S) can be used to calculate remineralization. Whole water remineralization was calculated by equation 4-4 :

$$R = \frac{\ln \left(\frac{Rt}{Ro} \right)}{t} \times S \qquad \text{Equation 4-4}$$

The assimilation rate of DIP was determined by equation 4-5 :

$$P = \frac{DPM_t - DPM_0}{R \times t} \qquad \text{Equation 4-5}$$

where P= DIP uptake rate (mg-at m^{-3} t^{-1}); DPM_t, DPM_0 = radioactivity of the particulate matter at times 0 and t; and R= specific activity of DIP (DPM/mg-at) corrected for isotope-dilution. R is the average of the initial

and final activity. As discussed earlier, this method requires that the photosynthesizing and remineralizing organisms reside in the size fraction being measured. In this study, isotope dilution measurements were made on the unfractionated samples. Estimated bacterial remineralization was calculated by equation 4-2, and heterotrophic protozoan remineralization was calculated as the difference between total and bacterial remineralization.

4.3 Experimental Platform

Studies for this dissertation were conducted using small scale continuous flow experimental systems. The continuous culture technique is useful in studying both steady state growth and interspecific competition for limiting nutrients in model ecosystems. I choose a biostimulation approach, using microcosms, to study processes associated with nutrient enrichment. With this method, changes in nutrient type and concentration of the estuarine microplankton community are simulated (Webb et al. 1987; Sommer 1988).

A schematic of the chemostat microcosm is presented in Figure 4-4. The operation of this system in an experiment involves pumping nutrients and filtered York River water for dilution into the growth chamber at controlled rates. A constant volume is maintained in the growth chamber by means of an overflow port (rate of input equals overflow). A dilution rate (input rate divided by volume) may thus be calculated. All microcosm temperatures were maintained within 0.5 °C of each other. The microorganisms were exposed to natural sun light screened to approximately 50% of incident. Small additional shading resulted from the

container walls and the water bath. For the microcosm experiments, YRW was filtered by passing it sequentially through spun cartridges of 5 and 1μm porosity and then membrane cartridges of 1 μm and 0.22μm porosity. Stock solutions of nitrogen, as NH_4Cl, and phosphorus, as NaH_2PO_4, were made up with filtered YRW and provided continuously to the microcosms in molar ratios (N:P) given in Table 4-2. The microcosm containers were translucent fiberglass cylinders (37 cm diameter, 50 cm high) with two overflow holes to maintain a 50 liter volume. Experiments were started by filling the microcosms with filtered YRW (<50 μm pore size Polyversol Gelman filter). After startup, Cole-Parmer Master Flex™ peristaltic pumps supplied the microcosms with filtered dilution water and nutrients. An initial nutrient addition was made to the microcosm experiments at startup to bring nutrient concentrations to treatment levels. Aeration and mixing was provided to each microcosm through 15 cm airstones at a rate of approximately 1.8 liters per minute.

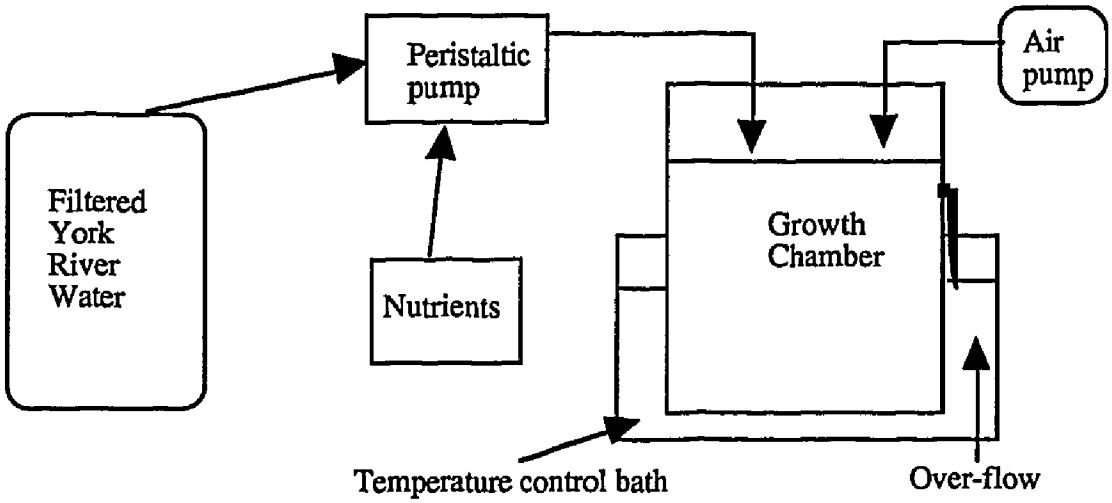


Figure 4-4. Flow diagram with principle components of the flow-through microcosm system.

4.4. Experimental design

4.4.1 Treatments

The independent variables were N:P ratio and dilution rate, and the dependent variables were biomass and estimated phosphorus remineralization by bacteria and heterotrophic protozoans.

These experiments consisted of 2 treatments and a control; nutrients (N and P) in molar ratios shown in Table 4-2. Each of these treatments is conducted at 2 dilution rates, 1/4 and 1/2 volume replacement per day, for a total of 6 tests per experimental unit. At least two experiments per time period (August, October, Dec-Jan) were completed.

Table 4-2 Summary of microcosm experiments 1988-1989.

Supply ratios of nutrients (3)		**µM N**	**µM P**
Control	-	-	-
N:P	= 5:1	25	5.0
N:P	=16:1	40	2.5
Dilution rates (2)		**Flow rate (l Day^{-1})**	
1/4 volume per day	(Low)	12.5	
1/2 volume per day	(High)	25.0	

4.4.2. Procedures

When maximum biomass was reached, after 4 to 12 days, radio-isotope dilution and size fractionation experiments were performed. The point of maximum biomass was estimated from plots of daily chlorophyll concentration. The treatments and dilution rates were randomized among microcosm containers before the start of each new experimental unit.

Three different types of incubations, with ^{14}C labelled $NaHCO_3$ and ^{32}P labelled NaH_2PO_4,were done in these experiments. As discussed (Section 4.1), size fractionation was used to perform two types of incubations, a pre-incubation fractionation in which the bacteria size fraction (<1μm) was separated from the whole water before incubation, and a post-incubation fractionation in which the bacteria size fraction was separated from the whole water at the end of the incubation. An isotope dilution experiment was done in a third whole water incubation. Radioisotope stocks (^{14}C-Na HCO_3) were prepared as described by Ray (1986), and isotope stocks of ^{32}P-$NaHPO_4$ were prepared as described by Berman et al. (1977). Samples were incubated in 100 ml bottles that were suspended in the microcosm tanks with their tops just at the water surface. Incubations ended by filtering samples onto pre-wetted 25 mm diameter GF/F Whatman filters in conditions minimal light. Filters were rinsed twice with filtered York River water (YRW) and placed in glass scintillation vials containing 10 ml of Aquasol scintillation fluor (New England Nuclear). ^{14}C and ^{32}P samples were counted on a Beckman LS 5000 TD scintillation counter .

Using about two liter samples from each microcosm, size fractionation and isotope dilution experiments were carried out (Figure 4-5). For the

preparation of the pre-incubation fractions, one liter of sample was filtered through a 1.0 μm 47 mm diameter Nuclepore™ filter. The fractionated and unfractionated samples were then distributed into the 100 ml bottles. Incubation bottles were washed with Micro™ detergent, rinsed with tap water, soaked overnight in 10% HCL, and rinsed again with distilled water. ^{14}C-Na HCO_3 and ^{32}P-$NaHPO_4$ were added to the bottles immediately before incubation. These samples were incubated for a 6 hours time period which encompassed noon (Eastern Standard Time) using 3 replicate light treatments and 2 replicate dark experiments. At the end of the incubation samples for post-incubation fractionation were filtered by vacuum (less than 100 mm Hg) in 20 ml aliquots, through 1.0 μm 25 mm diameter nuclepore filters. The filtrate was then refiltered on to GF/F filters as shown in Figure 4-5. Pre-incubation fractionated samples were filtered through GF/F filters at the end of incubations. One ml samples were collected from the whole water incubations after tracer addition and from each filtrate at the end of the incubation. These samples were counted in scintillation vials with Aquasol. DIP samples were collected at the beginning and end of each incubation period.

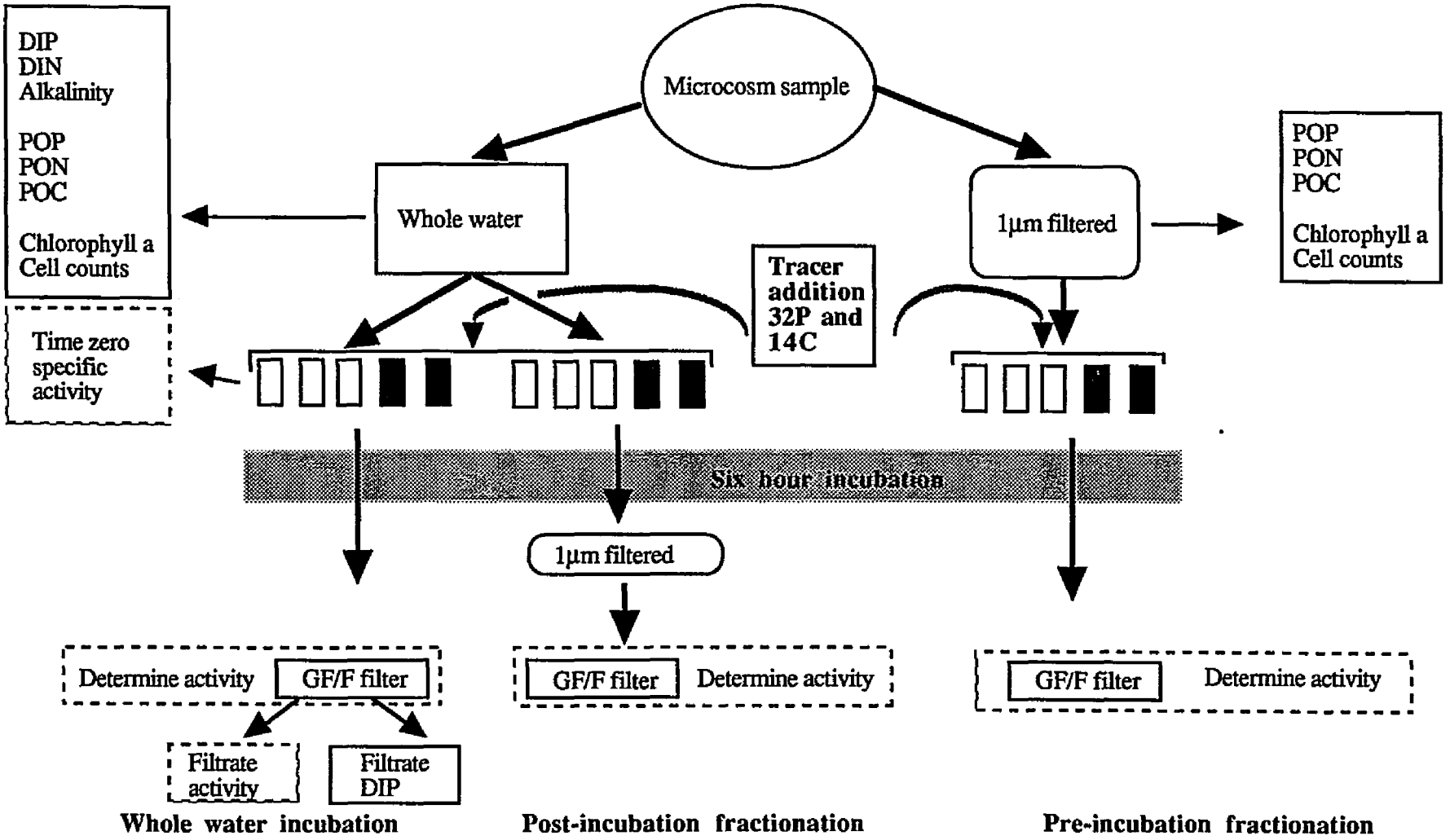


Figure 4-5. Procedures for processing light bottle ☐ and dark bottle ■ incubations for pre-incubation fractionation, for whole water, and for post incubation fractionation. Activity is DPM of 14C and 32P. Dashed boxes indicated measurements of activity were made.

Samples for CHN, PIP, and DIP analysis and for epi-fluorescent microscope examination were collected from each size fraction using methods referenced in Table 4-3. Flows and stocks within the microcosm community that were measured in this experiment are shown in Figure 4-6 Procedures for these samplings are described in Appendix A.

Table 4-3. Particulate and dissolved analysis for carbon, nitrogen and phosphorus. All samples for particulate analysis were collected on GF/F filters. Samples for DIP and DIN were filtered through GF/F filters during collection and filtrates were analyzed. Procedures for these analyses are described in Appendix A.

Particulate Analysis	Instrument	Reference
POC	Elemental Analyzer	Perkin-Elmer 1978
PON	Elemental Analyzer	Perkin-Elmer 1978
POP	Auto-Analyzer	Technicon Industrial 1976
Chlorophyll (a)	fluorometer	Webb and Hayward, unpublished
Dissolved Analysis		
Alkalinity	pH meter	Parsons et al. 1984
Orthophosphate	Auto-Analyzer	Technicon Industrial 1976
Nitrate	Auto-Analyzer	Technicon Industrial 1972
Nitrite	Auto-Analyzer	Technicon Industrial 1972
Ammonium	Auto-Analyzer	U.S. EPA 1974

Three types of controls were used in these experiments, a filter blank, a filter and sample with no tracer, and a filter and dilution water with tracer. Counts of these samples were no different than background. The choice of Whatman GF/F filters used in the ^{32}P and ^{14}C uptake experiments was predicated on the need to use this type of filters in the analysis of particulate carbon, nitrogen and phosphorus. To calculate bacterial remineralization, uptake and particulate ratios of phosphorus and carbon need to be compared. For the comparison to be valid, particulate retention on the filters used for the uptake experiments and for particulate

analysis had to be the same. GF/F filters are routinely used to measure bacterial uptake in ^{3}H-thymidine uptake experiments (Koepfler 1989), in $^{13}NH_4^+$uptake experiments (Fuhrman et al. 1988) and in ^{14}C uptake experiments (Geider 1988). The use of these filters is suggested when small picoplankton cells are present (Hilmer and Bates 1989). Compared to 0.22 polycarbonate or 0.2 cellulose nitrate filters, GF/F filters retain more activity and their results are less variable in tracer experiments (Hilmer and Bates 1989; Goldman and Dennett 1985; Li et al. 1983). In our experiments, filtrates of GF/F filters were periodically checked for bacteria with DAPI stained preparations for epifluorescent microscopic examination. Only a few bacteria per field were found in these filtrate samples compared typically to over 1000 cells per field found in a whole water samples from the microcosms.

The assumption that the <1.0 μm fraction was composed primarily of bacteria, and the >1.0 μm fraction was composed of eucaryote cells, was verified by microscopic examination.

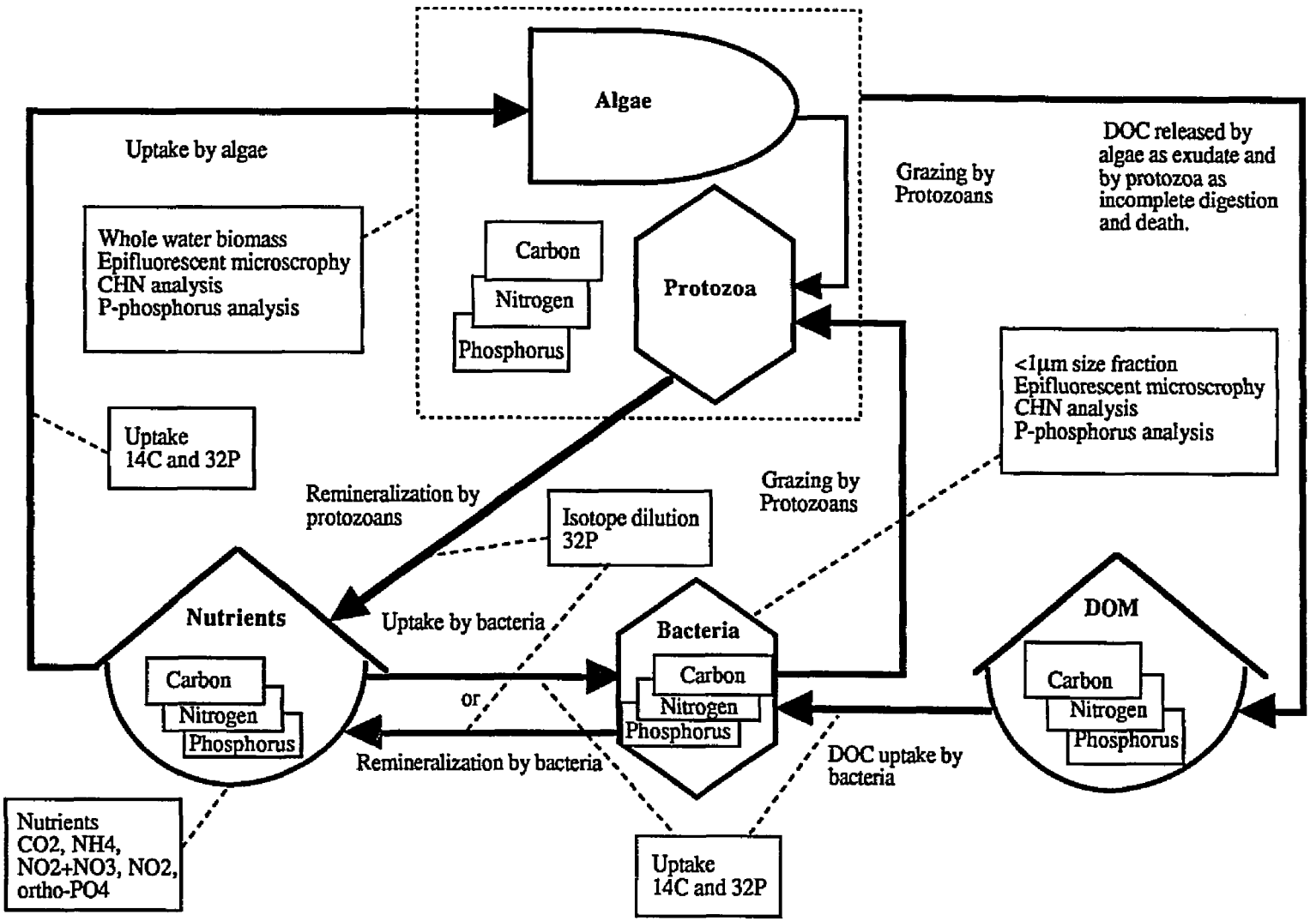


Figure 4-6 General model of microbial food web in microcosm experiments on the York River. Lines and boxes denote measured net flows and stocks of carbon , nitrogen and phosphorus. The type of analysis used to measure biomass and flow are shown in boxes attached with dashed lines.

4.5 Statistical methods

Several types of statistical analyzes were undertaken for this dissertation. Biomass measurements were expressed as ± a standard error of differences between replicates. P-remineralization was expressed as ± a standard error of replicate measurements. Trends were explored with either model 1 or model 2 regression analysis. Ratios of particulate carbon, nitrogen, phosphorus, and chlorophyll were represented as the slope of a model 2 regression (Laws and Archie 1981). Model 1 regression was used to determine the relationship between the elemental nitrogen or phosphorus pools in successive nutrient pools (dissolved inorganic --> particulate). Relationships between nitrogen, phosphorus and carbon in the particulate and dissolved pools were examined with correlation analysis (r). To determine if biomass and nutrient analysis met the requirements for the use in linear models, residuals were plotted and compared to Zar (1984, Figure 17.12) to determine if a log transformation should be applied to the samples.

Multisample hypotheses were tested with one or two factor fixed effects analysis of variance (ANOVA). Two factor ANOVA was used to test the effect of nutrient treatment (5:1, 16:1 N:P treatments and controls) and dilution on mean P- remineralization. When interactions between the treatments were significant, each factor was tested separately in one factor ANOVA. The analysis of variance is robust with respect to the assumption of the underlying populations' normality. The validity of the analysis is affected only slightly by considerable deviations from normality (Zar 1984).

Chapter 5

Results

5.1 Trends in nutrients and biomass

Chlorophyll (averaged over all treatments for each experiment) ranged between 14 to 20 μg l^{-1}, except for an autumnal increase to nearly 100 μg l^{-1} in the October 21 experiment (Table 5-1). This high chlorophyll biomass was reflected in high values of particulate carbon, nitrogen, and phosphorus as well as biovolume (Table 5-1). Carbon, nitrogen, phosphorus and biovolume concentrations, although lower in the summer than during the October maximum, were greater than concentration of these parameters in the December-January experiments. As a result of increased chlorophyll per cell, an average biomass concentration of 16.2 μg chl l^{-1} during the December experiment was similar to that recorded in the summer experiments (Table 5-1).

Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) in York River water increased from July though October, after which the DIP concentrations decreased during the annual switch from nitrogen- to phosphorus-limitation occurred (Webb 1987). Nitrogen availability increased between October and December from 0.70 to 10.79 μmolar (Table 5-2). The high biomass in the October 21 experiment occurred when the N:P ratio was 12. Nitrogen-limitation resulted in lower average biomass in the summer when the naturally occurring DIN:DIP river supply ratios ranged from 2.2 to 3.8.

Table 5-1. Concentration of Chlorophyll ($\mu g\text{-}l^{-1}$), p-carbon, p-nitrogen, p-phosphorus (μM) and biovolume ($\mu m^3\text{-}\mu l^{-1}$) in whole water samples and bacterial size fraction.

Whole	Chl	Carbon	Nitrogen	Phosphorus	Cell volume
29-Jul	19.4	253.0	28.0	0.92	30417
5-Aug	11.6	156.0	19.3	1.20	19071
11-Aug	14.0	136.5	19.5	1.17	35428
21-Oct	99.2	416.2	35.3	1.66	74468
28-Oct	30.2	91.7	14.7	1.64	29138
12-Dec	16.2	70.3	10.7	0.47	1733
13-Jan	16.6	60.2	9.9	0.60	3497
Bact. fract.	Chl	Carbon	Nitrogen	Phosphorus	Cell volume
29-Jul	0.4	52.4	8.18	0.37	650
5-Aug	0.6	35.2	4.09	0.22	506
11-Aug	0.5	18.8	3.00	0.17	501
21-Oct	0.9	28.9	3.36	0.24	806
28-Oct	0.5	21.0	1.94	0.17	534
12-Dec	0.4	12.2	2.01	0.11	180
13-Jan	0.3	8.7	2.06	0.13	146

Table 5-2 Nutrient concentrations and physical data from York River waters during experiments. Light (Langleys min^{-1}) was measured as mean daily solar radiation.

Date	Light	Temp.°C	Salinity	DIN µM	DIP µM	Ratio N:P
Jul 29	0.43	28.0	21.0	0.357	0.16	2.21
Aug 5	0.42	29.5	24.5	2.43	0.64	3.76
Aug 11	0.43	28.5	22.0	3.07	0.90	3.40
Oct 21	0.24	15.0	23.5	10.1	0.84	12.0
Oct 28	0.16	15.0	23.5	13.0	1.03	12.6
Dec 12	0.14	10.2	23.0	10.8	0.55	19.7
Jan 13 *	0.10	6.5	20.0	1.83	0.09	20.3

* York River sample was taken on January 16, 100 m north of the microcosms

5.1.1 Changes in population composition and biomass after microcosm inoculation

The growth phase is the period from the inoculation of the microcosm system to the time the microcosm reaches maximum biomass. Many of the microcosms probably did not reach maximum biomass; others reached maximum biomass, then declined in biomass. Time series chlorophyll measurements of the microcosms from inoculation through the final samplings are shown in Appendix B. Chlorophyll and biovolume, as measured by epifluorescence microscopy, did not always show the same trends in biomass during the growth phase of the microcosms (Figures 5-1 and 5-2). In July, regressions of chlorophyll and biovolume were not significant. However, all six December treatments were significant and had R^2 of .68 to .97. Diatoms, the major component of the phototrophic nanoplankton, became abundant as the microcosm approached steady state. Coccoid cyanobacteria generally decreased in abundance after the start of each experiment. No clear trends in heterotrophic nanoplankton were found in these microcosm experiments (Figure 5-1 A and B; Figure 5-2 A and B). In the summer experiments, biomass showed an initial increase between day 1 and day 2 in all treatments, which was followed by a divergence in response to treatment effects (Appendix B-1). In the December and January experiments, there was a 2 to 3 day delay after inoculation before biomass increased in the microcosms (Figure 5-2 A and B; Appendix B-2).

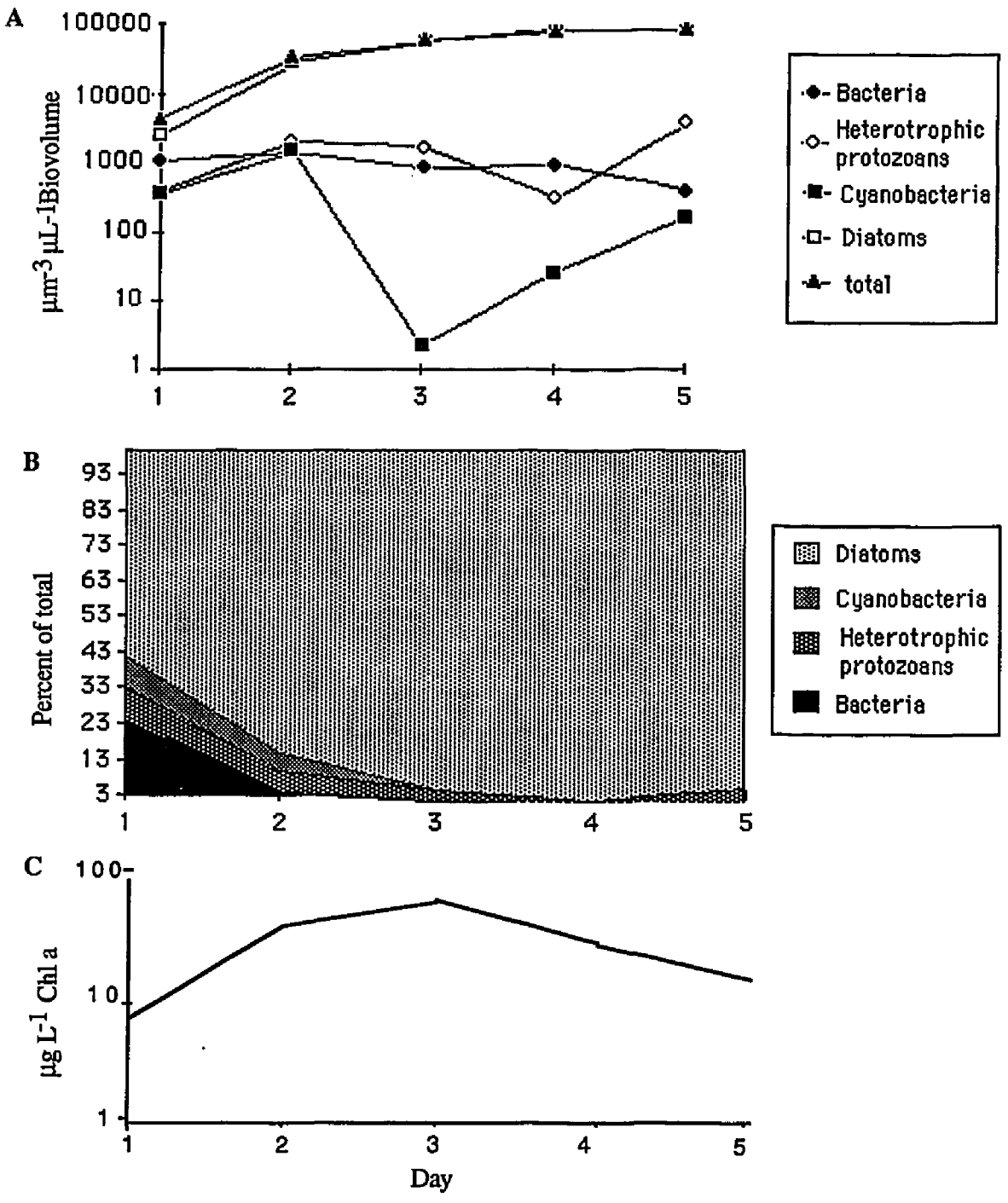


Figure 5-1 Shows July 29 experiment N:P ratio of 16:1, low dilution rate. A time series of measurements from the initation until tracer studies were done. Shows A, epi-fluorescent microscopy counts converted to biovolume, B. percentage composition by volume and, C. chlorophyll concentrations.

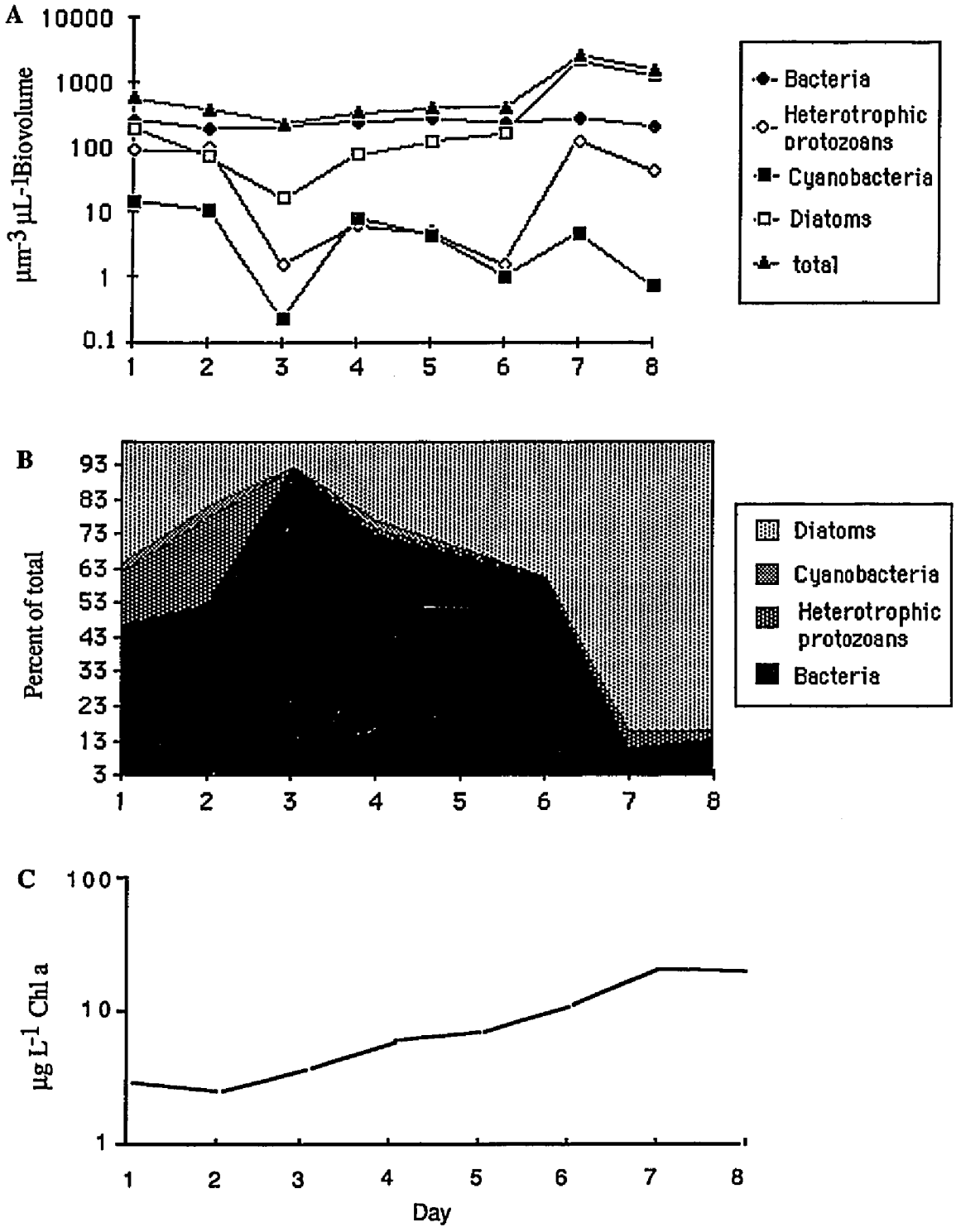


Figure 5-2 Shows December 8 experiment N:P ratio of 16:1, low dilution rate. A time series of measurements from the initation until tracer studies were done. Shows A, epi-fluorescent microscopy counts converted to biovolume, B. percentage composition by volume and, C. chlorophyll a concentrations.

5.1.2. Treatment effects on biomass parameters

The effect of dilution rate and nutrient treatment on carbon, chlorophyll, and nitrogen biomass is shown for three representative experiments in Figure 5-3 and 5-4. All three parameters showed similar trends in the summer experiment, represented here by the July 29 experiment. Trends in the three parameters were similar in the Dec-Jan experiments, although they were different from the summer trends (trends described below). These are represented in Figure 5-3 by the Dec 12 experiment. Within the October 21 experiment (Figure 5-4), notably different trends in the three parameters were found, with carbon generally following winter trends and nitrogen and chlorophyll following summer trends. In the October experiment, only the controls showed consistent patterns in the three parameters. Analysis of Variance (ANOVA) based on carbon biomass was used to explore these trends.

Greater carbon biomass was found in the high dilution rate (1/2 volumes per day) than in the low dilution rate (1/4 volumes per day) during the three summer experiments (ANOVA, $F=5.372$, $df=17$, $p=0.04$). In the December and January experiments this trend was reversed, and greater carbon biomass was found in the low dilution rate treatments (ANOVA, $F= 4.836$, $df=11$, $p = 0.07$) (Figure 5-3 B). Greater carbon biomass was found in the nutrient treatments than in the controls (ANOVA, $F= 6.029$, $df=17$, $p=0.01$) during the summer and early autumn experiments. There was no difference in carbon biomass between the 5:1 and 16:1 nutrient treatments in any of these experiments (ANOVA, $df=27$, $F=.212$, p= N.S.). In the December and January experiments no differences were found in carbon biomass between nutrient treatments and the controls (ANOVA, $F=2.265$, $df=11$, p=N.S.) (Fig. 5-3B).

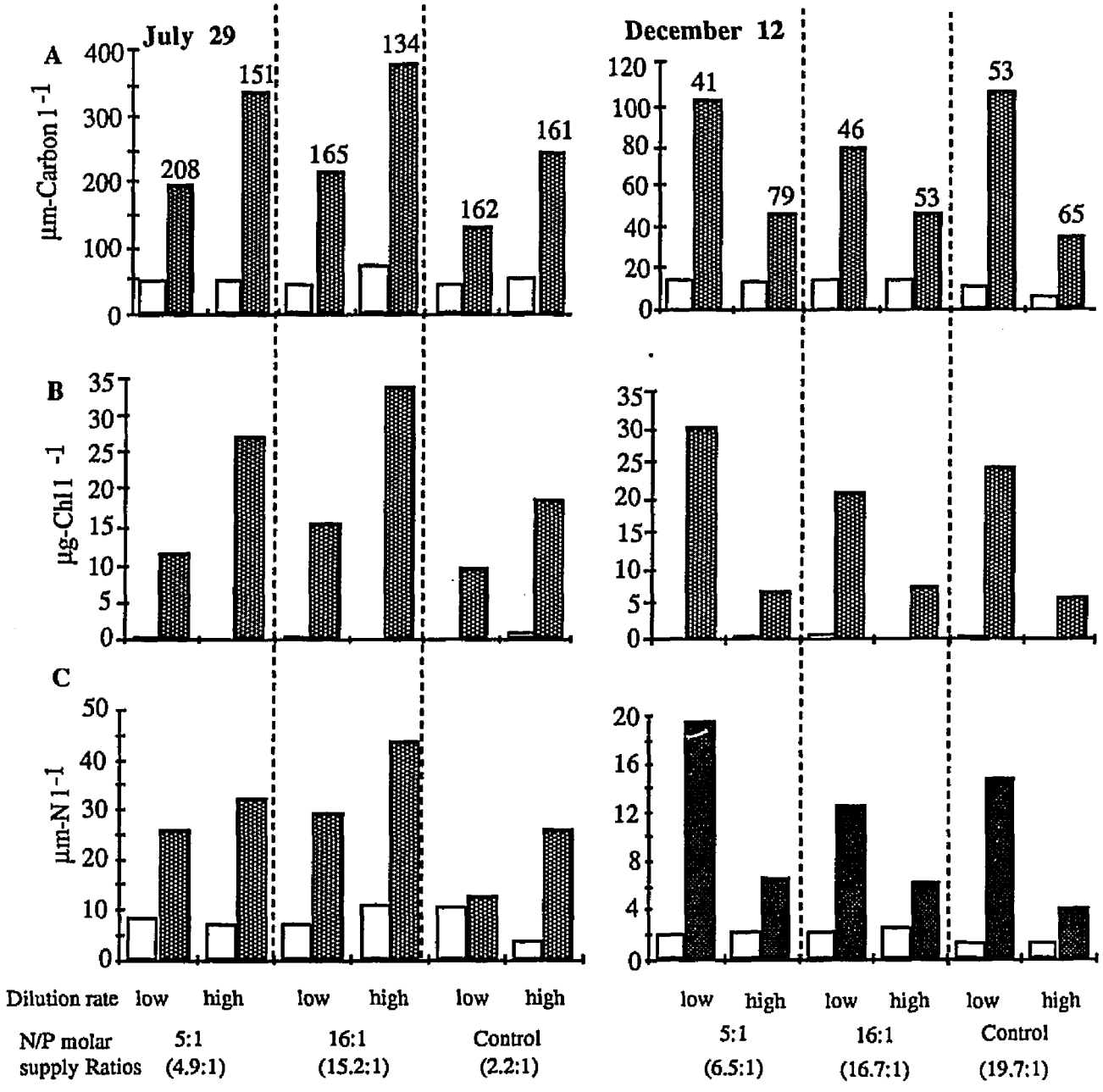


Figure 5-3. A. Carbon, and B. chlorophyll (a) biomass, C. nitrogen biomass for a <1µm size fraction ☐ and whole water ▩ sample. The standard error of differences between replicates was 0.015 and 2.26 µg chl per l respectively for the <1µm size fraction and the whole water samples. Standard error of differences for carbon <1µm size fraction and whole water samples was 0.46 and 2.47 µmol per l. Data from two microcosm experiments completed during the period of maximun nitrogen limitation in July and near the end of the period of annual nitrogen limitation in December. Numbers over chlorophyll bars are C:Chl ratios. Actual supply ratios including DIN and DIP additions from YRW are in parentheses.

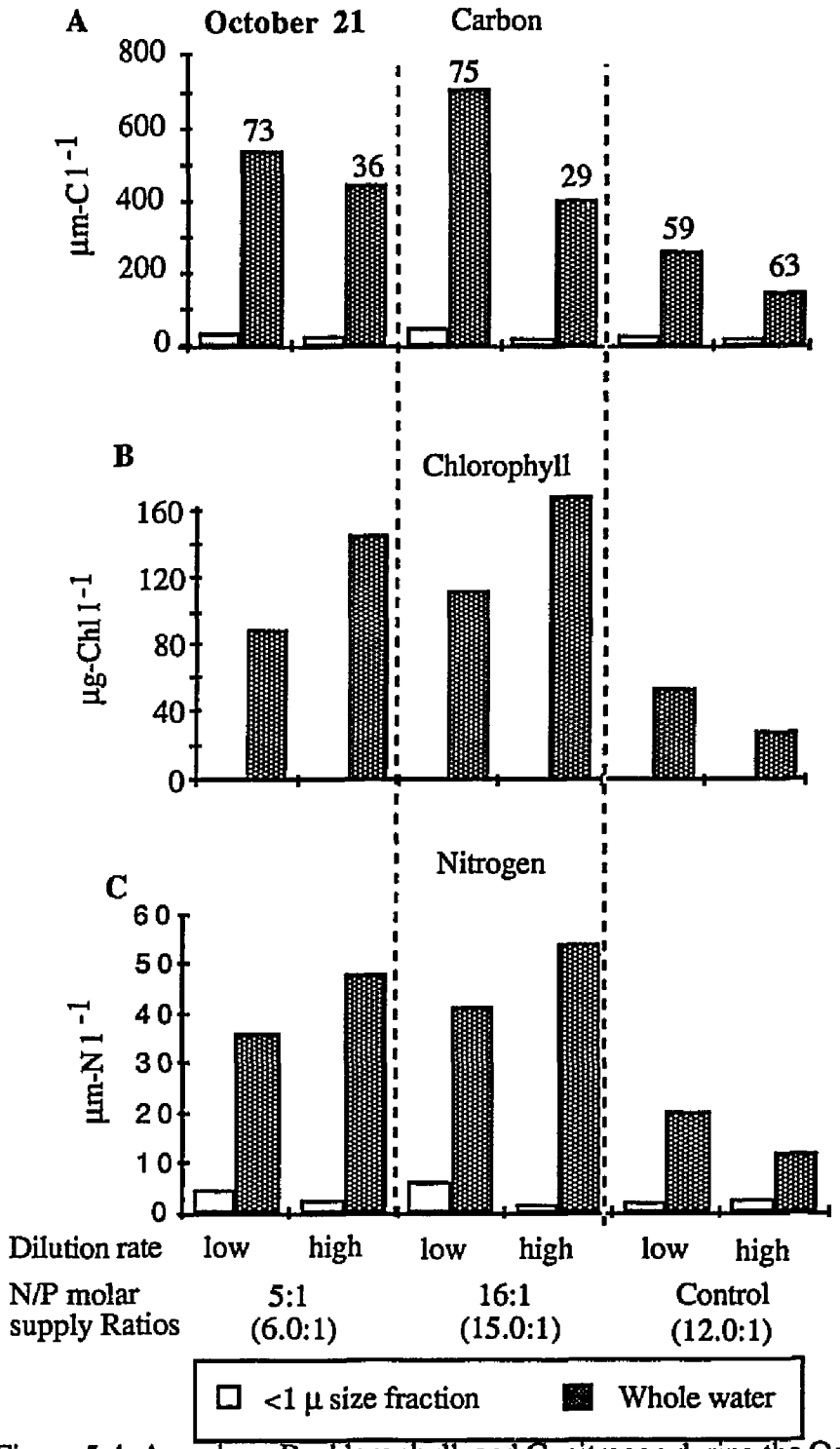


Figure 5-4 A. carbon, B. chlorophyll, and C. nitrogen during the October experiment. Dilution rate trends in chlorophyll and nitrogen are similar, having greater biomass in the high dilution rate treatments. Carbon shows the reverse of this trend, having greater biomass in the low dilution rate treatment. Actual supply ratios of nutrients including DIN and DIP from YRW are in parentheses.

In these experiments, particulate carbon, nitrogen and phosphorus concentration data were correlated with chlorophyll (r=0.752 for C, r= 0.787 for N, r= 0.649 for P, df= 40, p>0.05). However, ratios of these elements were not constant, and often the effects of nutrient treatment and dilution rate were reflected in them. Carbon and chlorophyll are the most commonly measured parameters of biomass and can be used in ratios to indicate the photosynthetic potential of the particulate matter (Steele and Baid 1961;1962). There was a substantial decrease in the carbon to chlorophyll ratio from July through January in the microcosm experiments as light and day-length decreased (Table 5-3).

Table 5-3. Slopes from model 2 regressions of C : Chl combining experiments from three time periods during 1988. All slopes were significant. The numbers in parentheses are 95% confidence limits of the mean slope (ratio).

Dilution rate	Low			High		
	R^2	ratio		R^2	ratio	
Jul - Aug	.732	131	(51-171)	.618	143	(33-171)
October	.926	89	(52-120)	.941	30	(19-39)
Dec - Jan	.764	27	(6 -42)	.895	19	(9-26)

Nutrient treatments had no significant effect on C:Chl ratio in any experiments (ANOVA, df=39, F=0.846, p=N.S.). The C:Chl ratio was significantly effected by dilution rate in the October and December experiments, but not in the summer or January experiments (Table 5-4).

Table 5-4. ANOVA 1 way analysis of variance Dilution vs. C : Chl ratio.

July and August experiments

Source:	DF	Sum Squares:	Mean Square:	F-test:
Between	1	578	578	.245
Within	16	37820.444	2363.778	p = .6277
Total	17	38398.444		

October experiments

Source:	DF	Sum Squares:	Mean Square:	F-test:
Between	2	16332.667	8166.333	3.365
Within	9	21839	2426.556	p = .081
Total	11	38171.667		

December experiment

Source:	DF	Sum Squares:	Mean Square:	F-test:
Between	1	962.667	962.667	22.563
Within	4	170.667	42.667	p = .009
Total	5	1133.333		

January Experiment

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between	1	416.667	416.667	.049
Within	4	33738.667	8434.667	p = .835
Total	5	34155.333		

In the October experiment, there was an alteration of high and low C:Chl ratios in the two dilution treatments (Figure 5-4 A and B). In this experiment carbon was higher in the low dilution rate, and chlorophyll was low in the high dilution rate treatments. ANOVA of C:Chl ratio (Table 5-4) indicates, that their are also significant differences in the ratio in the two dilution rates in December. The ratios this time is lower in the low dilution rate than in the high dilution rate. This is just the opposite of the trend noted in the October experiment (Figure 5-3; 5-4). In all the other experiments the carbon and chlorophyll seem to follow the same trends.

5.1.3. The size fractions

Size fractionation was done to separate the heterotrophic bacteria from other components of the microcosm community. Little chlorophyll was found in the <1.0 μm size fraction (Figure 5-3; Figure 5-4). The chlorophyll present in the <1 μm size fraction was caused by cyanobacteria that had penetrated the 1 μm polycarbonate filters. A 14% reduction in Hpico cell concentration occurred as a result of size fractionation during these experiments ($p<0.0001$, paired T-test, df=40). The mean whole water Hpico count was 2.6×10^6, SE 2.2×10^5, and the mean pre-fractionation Hpico count was 2.2×10^6, SE 2.0×10^5. This compares reasonably well with the bacterial loss rate attributable to size fractionation found by other experimenters (Jones et al. 1983; Ward 1984; Waterbury et al. 1986). No heterotrophic protozoans were found in the smaller size fraction in any of the experiments. There was a significant increase in cell counts of Hpico taken from before and after the 6 hour incubations ($p= 0.0002$, paired T-test, df=40, mean increase = 22%). By this measurement, the average growth rate (μ) of bacteria was about 0.8 divisions per day. If a reduction in phytoplankton exudate production in the dark causes bacteria to be substrate limited at night, this estimate would be high.

The bacterial carbon was a maximum percentage of total biomass in the August 5 and October 28 experiments and the minimum percentage of total biomass in October 21 experiment when phototrophic biomass was high (Table 5-5). Bacterial PON, POC and POP were all

Table 5-5. Bacterial carbon, nitrogen, and phosphorus as a percent of total biomass elemental biomass.

	POC	PON	POP
July 29	20.7	29.3	40.1
August 5	22.6	21.2	17.9
August 11	13.5	15.4	14.5
October 21	6.9	9.5	14.3
October 28	22.9	13.2	10.4
December 12	17.4	18.8	22.7
January 13	14.5	20.9	21.6

correlated (r=0.873 for C to N, 0.644 for C to P, and 0.737 for the N to P, df=40, $p<0.05$). In the July experiment, PON and POP were a larger percent of the total than in other experiments (Table 5-5). Bacterial carbon was an average of 15% (mean bacterial carbon µmole l^{-1} 25.3, SE=2.606; mean total carbon µmole l^{-1} 169 SE=22.56) of the total biomass during these experiments and consumed about 12% of the primary production in the form of PDOC (Table 5-6).

On the average 18% of the microcosm phosphorus uptake went into the bacterial size fraction. As discussed in section 4.2.3 DIP, uptake by bacteria was probably underestimated. But even this measure showed that more DIP was taken up by bacteria than PDOP. The relative removal rate of DIP and PDOP by bacteria was in a ratio of 3:2 (Table 5-6).

Table 5-6 Uptake of phosphorus and carbon into whole water and bacterial size fraction in (µM hr^{-1}).

Type	Mean	% of total	Std. Dev	Range	C.V.
Carbon					
Whole	11.8	-	7.89	38.89	66
Bact. total	1.4	11.8	1.87	11.59	133
Phosphorus					
Whole	0.28	-	0.020	.111	72
Bact.total	0.005	17.8	0.004	0.018	82
Bact. DIP	0.003	10.7	0.006	0.033	188

5.2 Phosphorus remineralization

Exchange of DIP between bacteria and the water (eq 4-1) can either be positive (remineralization) or negative (uptake). For net bacterial remineralization to occur, the particulate C:P ratio must be greater than the uptake C:P ratio. Generally, however, the particulate C:P ratio was less than the C:P bacterial uptake ratio, suggesting a net uptake of inorganic phosphorus by bacteria instead of remineralization (Figure. 5-5A). The difference between C:P uptake and particulate ratios was smaller when bacterial remineralization of phosphorus occurred (mean difference =79.17, SE=23.848, n=11) than when uptake of phosphorus occurred (mean difference 631.72, SD=197.02 n=31). Because of this, the calculated bacterial P-remineralization was small (Table 5-7).

Values of bacterial P-remineralization were nearly always less than 10% of the total P-remineralization. Because metazoans were excluded, the only other heterotrophic organisms, capable of remineralizing phosphorus were the heterotrophic protozoans. They were presumably responsible for more than 90% of the total phosphorus remineralization. Bacterial remineralization or uptake was highly correlated with Hnano remineralization ($r=0.982$, $df=40$, $p<0.001$) during all experiments. In August 5 and October 21, greater remineralization was correlated with the higher dilution rate ($r=0.335$, $df=22$, $p < 0.05$). In December when temperatures were lower, greater remineralization occurred in the lower dilution rate treatments (Table 5-7). Thus, the effect of dilution rate on remineralization was similar to the effect dilution had on biomass; both parameters, biomass and remineralization, was greater in the high dilution rate in the summer and autumn and was greater in the low dilution rate

treatment in the winter. When considering the effect of dilution only, greater remineralization was merely the result of greater biomass. Since the relationship between dilution rate and biomass was different in the summer and winter experiments, the effect of dilution over all experiments (ANOVA, Table 5-8) was not significant.

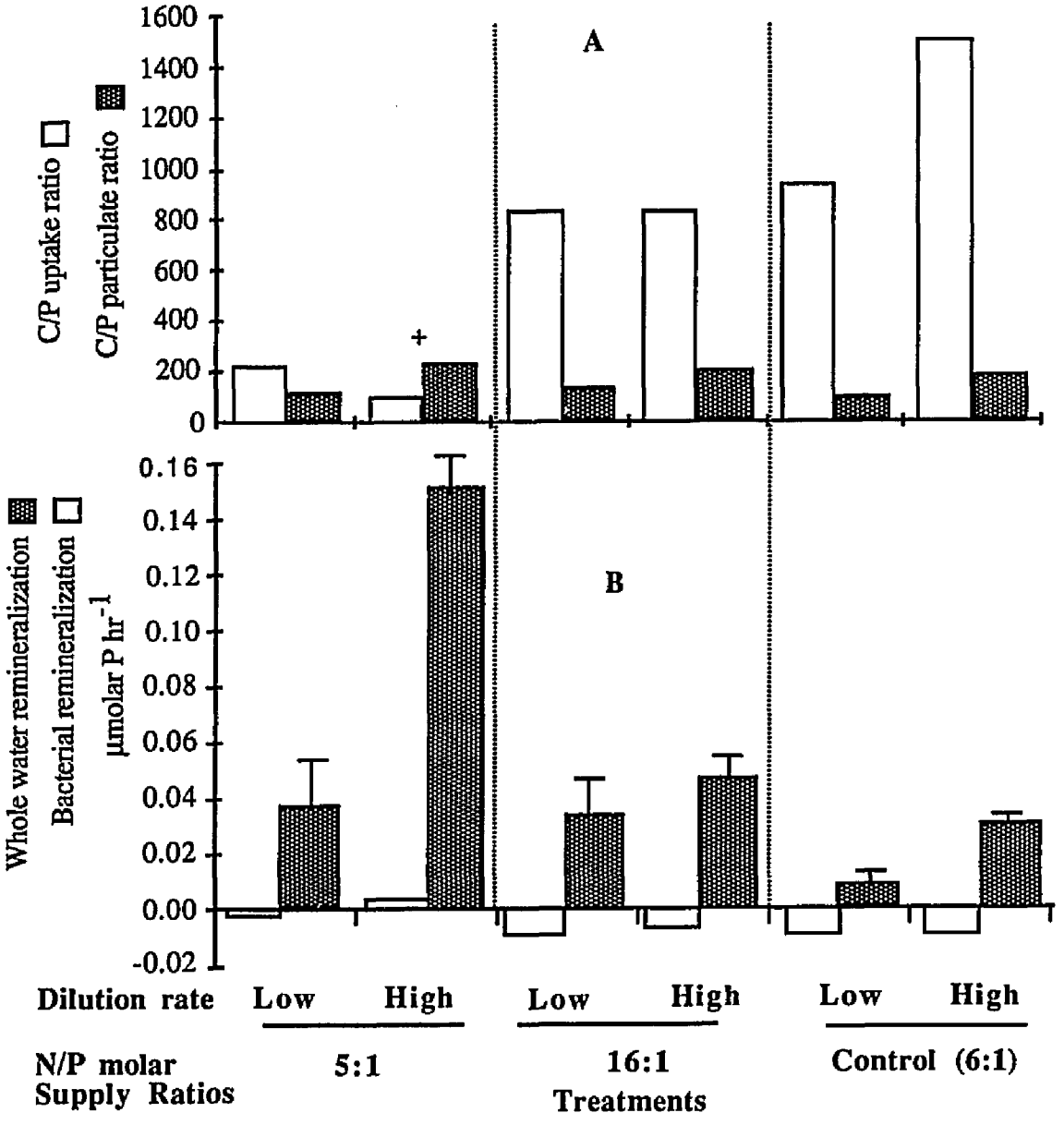


Figure 5-5 A. C:P uptake ratio □compared to the C/P particulate ratio ▩ in the < 1 µm size fraction during the August experiment. A plus (+) indicates remineralization in the <1 µm size fraction. B. Phosphorus remineralization of < 1 µm size fraction □ and whole water ▩ sample. Negative remineralization for bacteria is equivalent to uptake of DIP. Error bars are 95% confidence limits of data.

Table 5-7 Remineralization rates of phosphorus (nM hr^{-1}) for Hpico, and total. Shows data from 4 microcosm run between July 29, 1988 and January 13, 1989. Numbers in parentheses are YRW DIN:DIP ratios.

Dilution rate	Low			High		
Treatment	Hpico	total	% total	Hpico	total	% total
29-Jul						
5 :1	5.52	75.67	7	14.15	110.6	12
16:1	-7.92	38.70	-17	-6.77	39.7	-15
C (2.2:1)	0.52	49.72	1	-7.12	37.7	-16
5-Aug						
5:1	-3.0	38.1	-7	4.2	150.1	3
16:1	-10.2	34.9	-23	-7.5	48.5	-13
C (3.6:1)	-11.7	8.5	-58	-10.3	32.0	-24
21-Oct						
5:1	-2.1	25.8	-7	-0.2	44.7	-0.4
16:1	-2.9	61.9	-4	-8.8	82.8	-10
C (12.7:1)	-3.9	48.2	-8	-2.6	63.2	-4
12-Dec						
5:1	4.0	62.9	6	7.2	33.2	22
16:1	0.3	31.9	1	0.6	21.9	3
C (19.7:1)	-2.2	29.4	-7	-1.9	12.7	-13

Both bacterial and Hnano P-remineralization were negatively correlated with treatment N:P supply ratio (r=-0.578 for bacteria and r=-0.346 for Hnano df=40, $p<0.05$). The amount of P-remineralized was related to both the N:P ratio and the concentration of P supplied (N:P ratio 5:1> 16:1 > control) (Table 5-8; 5-9). The large variations in N:P ratio in the control did not contribute much to P-remineralization because the concentration of P supplied was low relative to that of the treatments. When the controls were not included, the difference between remineralization in the two N:P ratios (5:1 >16:1) was still significant (ANOVA, df=27, F=5.449, p=.03).

Table 5-8 ANOVA, a 2-factor (dilution rate and nutrient treatment) Analysis of Variance on total phosphorus remineralization, includes all experiments.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
dil (A)	1	.001	.001	.735	.3969
Treatment (B)	2	.012	.006	6.469	.004
AB	2	.001	2.806E-4	.313	.733
Error	36	.032	.001		

There were no missing cells found.

Table 5-9 The treatment vs. dilution rate incident table of total remineralization ($\mu M\ hr^{-1}$, includes all experiments. Numbers of samples are at the top of each box and means are at the bottom.

	Treatment:	5:1	16:1	C	Totals:
dilution	Group 1	7 .061	7 .038	7 .03	21 .043
	Group 2	7 .079	7 .043	7 .031	21 .051
Totals:		14 .07	14 .041	14 .03	42 .047

Nutrient treatments in the December and January experiments had no effect on biomass, however, the effect of nutrient treatment on remineralization was significant. ANOVA indicated significant differences in the 5:1 treatment, the 16:1 treatment, and the control ($F=5.115$, $DF=11$, $p=0.05$). Greatest to smallest P-remineralization was in the order of greatest to smallest P-supplied to the microcosm (N:P, 5:1>16:1>control).

As might be expected from ANOVA results already presented, the N:P supply ratio, including all experiments, was negatively correlated with remineralization ($r=-0.424$, $df=40$, $p=0.01$) and was independent of biomass. This indicated that increasing the phosphorus supply relative to nitrogen resulted in increased P-remineralization, and that this P-remineralization was due to supply ratios and was not just the result of increased biomass. This contrasts considerably with the effect dilution rate had on P-remineralization rates. Increased dilution rate resulted in an increased biomass from July through October. The resulting larger microbial community had a greater total P-remineralization rate. While increasing the ratio of phosphorus relative to nitrogen had a direct effect on the phosphorus remineralization rate; it did not change the size of the microbial community. This was true even in the December and January experiments when the York River is considered to be weakly phosphorus limited (Webb 1987).

Factors that increased total remineralization also affected bacterial phosphorus remineralization or, uptake in the same way, either increasing remineralization or more often, decreasing the amount of uptake. That is, supply ratios of nutrients had a significant effect on bacterial P-remineralization and dilution rate had no effect on P remineralization

(Table 5-10). The mean P-remineralization rates were negative in all but the 5:1 N:P treatment (the treatment with the most phosphorus) (Table 5-11).

Table 5-10 ANOVA, a 2-factor (dilution and nutrient treatment) Analysis of Variance on bacterial phosphorus remineralization, includes all experiments.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
dil (A)	1	4.255E-4	4.255E-4	.581	.451
Treatment (B)	2	.006	.003	4.042	.0261
AB	2	.001	4.524E-4	.617	.545
Error	36	.026	.001		

Table 5-11 The treatment vs. dilution rate incident table of bacterial remineralization ($\mu M\ hr^{-1}$, includes all experiments. The upper number in each box indicates the number of groups tested, and the lower number is the mean of the group.

	Treatment:	5:1	16:1	C	Totals:
dil	Group 1	7 1.014E-4	7 -.005	7 -.037	21 -.014
	Group 2	7 .001	7 -.006	7 -.017	21 -.007
Totals:		14 .001	14 -.006	14 -.027	42 -.011

5.3 Nutrient cycling

5.3.1. Dissolved inorganic P and N transformation to particulate P and N

The relationship between inorganic N and P nutrients and particulate organic N and P was explored using linear regression analysis. In both cases, an increase in molar supply ratio of the nutrient resulted in a significant increase in the particulate organic pools (Figure 5-6 A and C). An increased supply of phosphorus over nitrogen was translated into

increased POP; conversely, an increase in nitrogen over phosphorus was translated into increased PON. The relationship between POP and phosphorus supply was important, since POP was the source of the remineralized phosphorus measured in this experiment. Not all N or P supplied to the microcosms was utilized by the microcosm community. Substantial pools of dissolved inorganic N (mean = 36% Std. Dev. 42% of the DIN concentration supplied to the microcosms) and P (mean = 32% Std. Dev. 31% of the DIP concentration supplied to the microcosms) were found in the microcosms. The residual DIN and DIP were small during the summer experiments and large in the October 28 through the January 13 experiments. When these residual nutrients were subtracted from the nutrients supplied to the microcosms (nutrients from the river + nutrients added), a better R^2 was obtained (Figure. 5-6 B and D). In the case of phosphorus this change in R^2 was minimal (.445 to .455), but in the case of nitrogen, there was a large change in the R^2 (.137 to .752). The period of experimentation was usually nitrogen-limited and since all additions of N and P to the microcosms were below the Redfield N:P ratio, nitrogen limitation should have resulted in a minimum of dissolved inorganic nitrogen.

Unlike total POP, bacterial POP was not correlated with N:P supply ratios (r=0.032, df=40, p=N.S.). This was additional evidence that bacteria were not responsible for much of the remineralization, and were not a major food source for the remineralizing organism. In either case, we might expect the bacterial POP to be correlated with remineralization.

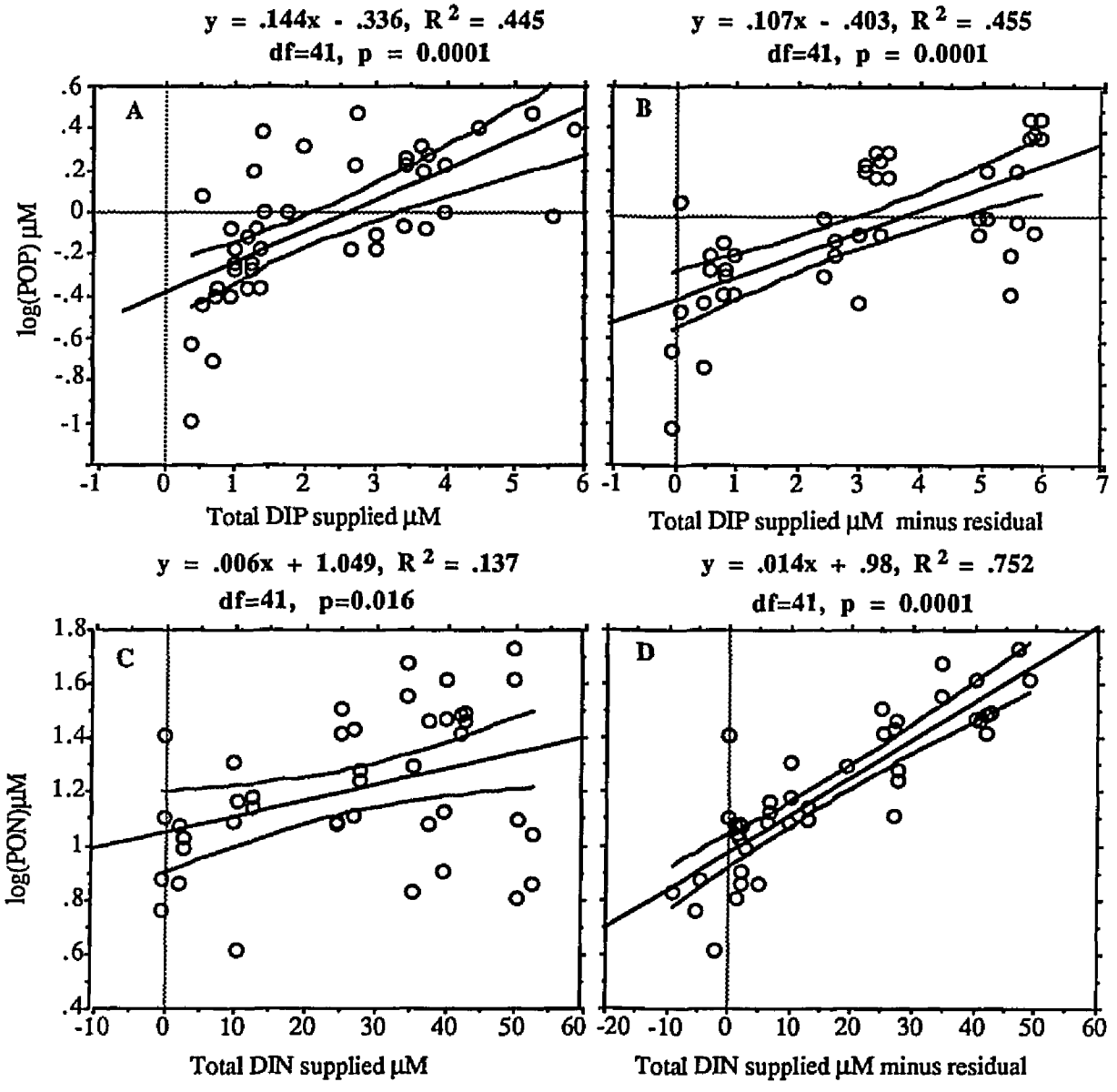


Figure 5-6 A & B show the relationship between DIP supply and POP. C & D show the relationship between DIN supply and PON. A and C are the total supply of DIP or DIN to the microcosms and B and D exclude residual DIP or DIN not converted to biomass. 95% confidence limits of the regression line are shown.

5.3.2. POP remineralization

A relationship between POP concentration and the P- remineralization rate was found with regression analysis. Dilution rate, supply ratios, and differing physical conditions under which these microcosm experiments were run interacted to reduce the strength of this relationship, causing a low $R^2 = 0.215$ for this data (Figure 5-7). The relationship was, however, significant ($p=0.002$), showing that increased particulate organic phosphorus generally resulted in increased remineralization.

5.3.3 The effect of supply ratios and dilution on carbon, nitrogen, and phosphorus ratios.

The ratios in which nitrogen and phosphorus were supplied significantly affected the N:P particulate ratio. There was, however, no relationship between dilution rate and the N:P particulate ratio (Table 5-12). The particulate N:P ratios for each treatment were considerably higher than the supply ratios of nutrients in the whole water samples. The highest N:P particulate ratio was found in the control (Table 5-13). The average supply ratios of N:P, which include the York River water and the N and P additions, were 5.46 (S.D.=0.72), 15.0 (S.D.=1.33), and 8.3. (S.D.=1.91), respectively for the 5:1 treatment, 16:1 treatment, and the control.

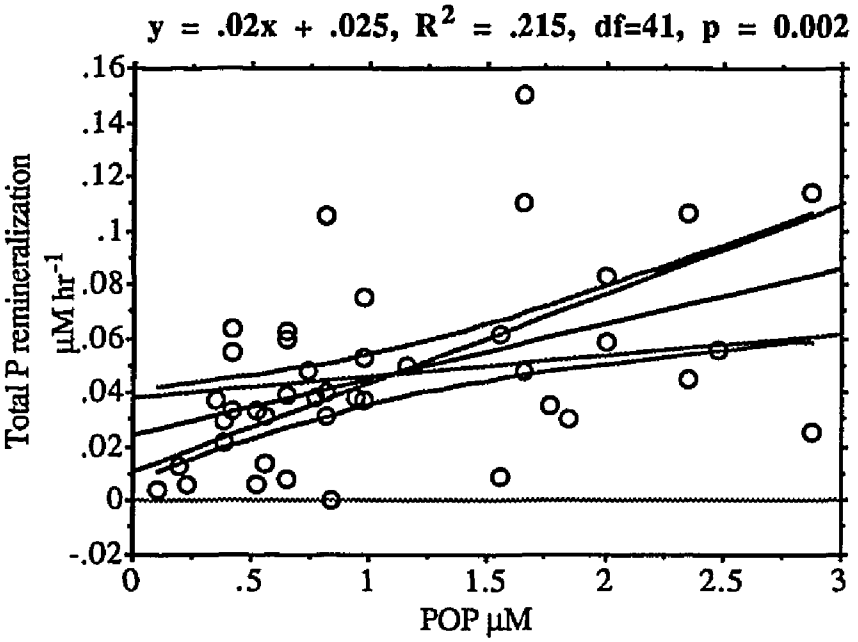


Figure 5-7 Shows the relationship between POP supply and remineralization. All data collected in seven experiments, from July 1988 to January 1989, are used in this analysis. The regression equation and the R^2 appear at the top of the graph. The regression line is plotted with a 95% confidence limit for the slope of the regression line, and 95% confidence bands for the true mean

Table 5-12 ANOVA for a 2-factor Analysis of Variance of treatment and dilution rate on whole water N:P ratio.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Treatment (A)	2	1486.463	743.232	3.114	.0566
dil (B)	1	270.561	270.561	1.134	.2941
AB	2	423.035	211.517	.886	.421
Error	36	8591.786	238.661		

Table 5-13 Means of whole water particulate N:P ratio by treatment and dilution rate, all dates.

	dil:	Low	High	Totals:
Treatment	5:1	7 16.2	7 16.5	14 16.3
	16:1	7 22.1	7 23.0	14 22.5
	Control	7 23.8	7 37.9	14 30.9
Totals:		21 20.7	21 25.8	42 23.3

The bacterial particulate N:P ratio, in contrast to the whole water samples, was affected primarily by dilution, rather than by the nutrient treatments (Table 5-14). The bacterial N:P ratio was greater in the high dilution rate treatment and the mean values (except for the 5:1 nutrient treatment) were lower than found in the whole water samples (Table 5-15). The N:P ratios of the bacterial biomass contained less variation than that the whole water samples (C.V. of whole water = 69.7, C.V. of bacteria = 45.7).

Table 5-14 ANOVA for a 2-factor Analysis of Variance of treatment and dilution rate on bacteria N:P ratio.

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Treatment (A)	2	11.945	5.972	.096	.9091
dil (B)	1	249.174	249.174	3.986	.0535
AB	2	132.897	66.449	1.063	.356
Error	36	2250.206	62.506		

The mean N:P bacterial ratio of all treatments was close to the Redfield ratio of 16:1, with the low dilution rate having a mean value of 15 and the high having a mean value of 20 (Table 5-15). The smallest difference in the bacterial N:P particulate ratios between the high and low dilution rate was found in the treatment with the Redfield supply ratio of 16: 1.

Table 5-15 Means of bacteria (<1μm size fraction) N:P ratio by treatment and dilution rate, all dates.

	dil:	Low Dil	High Dil	Totals:
Treatment	5:1	7 14.4	7 19.3	14 16.8
	16:1	7 17.4	7 18.0	14 17.7
	Control	7 13.5	7 22.8	14 18.1
Totals:		21 15.1	21 20.0	42 17.6

Data from the July experiment (Table 5-16) illustrates some of the general results suggested by Analysis of Variance (Table 5-12; Table 5-14). In the whole water treatments (total biomass), differences in the N:P ratio were related to nutrient supply. The 16:1 treatment had a mean particulate

N:P ratio of 51 and the 5:1 ratio had a mean N:P particulate ratio of 23, indicating that there were differences in the particulate N:P ratio related to nutrient treatment. In the bacterial size fraction, the 16:1 treatment had a mean particulate N:P ratio of 22.3 and the 5:1 ratio had a mean particulate N:P ratio of 20.5, indicating that there were no differences in the particulate N:P ratio related to nutrient treatment. The higher N:P supply ratio (16:1), resulted in a higher particulate N:P ratio in the whole water samples when compared to the 5:1 treatment.

In the bacterial size fraction, predictable low to high N:P ratios were found in the low and high dilution rates. In the whole water treatment there was no obvious effect of dilution rate on N:P particulate ratios, although the ratios were quite variable.

Table 5-16 Particulate ratios of carbon, nitrogen, and phosphorus for each treatment and corresponding growth rate (hr^{-1}) during the July experiment.

July		C:N	C:P	N:P	μ
Total biomass					
5:1	low	7.8	209.9	26.9	.023
5:1	high	10.5	207.3	19.7	.016
16:1	low	7.3	279.9	38.1	.045
16:1	high	9.2	586.6	64.0	.034
C (2.2:1)	low	10.3	113.0	11.0	.111
C (2.2:1)	high	9.6	697.4	73.0	.055
Bacterial biomass					
5:1	low	5.7	71.6	12.6	.010
5:1	high	6.7	212.7	32.0	.020
16:1	low	5.4	79.0	14.6	.018
16:1	high	6.7	176.5	26.5	.021
C	low	11.3	265.9	23.6	.030
C	high	5.5	265.6	48.3	.045

The relationship between treatments and C:N or C:P ratios (total biomass or in the bacterial biomass) were not as obvious as the relationships described above for the effect of treatments on the N:P

particulate ratios (Table 5-16). The C:P ratio (total biomass) was higher in the 16:1 treatment (mean=433) than it was in the 5:1 treatment (mean= 208), suggesting a relationship between P-supply and C:P particulate ratios. Bacterial biomass C:N and C:P ratios were generally higher in the high dilution rate (Table 5-16).

Seasonal trends in C:N, C:P, and N:P ratios were explored with Model 2 regression (Table 5-17). Regressions of total particulate C, N, and P against each other were often not significant, even when experiments were grouped by season. The C:P ratio was only significant in the winter experiments and in the composite of all experiments. The composite measure was much higher than the winter measure, suggesting that the C:P ratio decreased from summer to winter (Table 5-17). This is surprising, since the York river is weakly P limited in the winter.

The relationships between C, N, and P in the bacteria stock were more often significant than were these relationships in the whole water sample (Table 6-18). The bacterial C:N ratio remained constant and near Redfield ratios for all experiments (Table 5-18). The bacterial N:P ratio increased with dilution rate in the summer, was constant over both dilution rates in October, and was high in the low dilution rate in the winter. The coefficients of variation for bacterial and whole C:P were 0.47 and 0.74, respectively (N=42). This indicated that bacterial C:P ratios did not respond as radically to changes in nutrient loading or dilution rate as did the whole water C:P ratios.

Table 5-17. Particulate C, N, and P ratios as slope of Model 2 regression in whole water samples for all experiments. Numbers in parentheses are R^2.

	C:N	C:P	N:P
Dilution			
Combined			
Low	16.4 (.701)	209 (.181)	12.8 (.277)
High	9.1 (0.92)	175 (.258)	19.3 (.376)
Summer			
Low	6.4 (.948)	N.S.	N.S.
High	10.6 (.802)	N.S.	N.S.
October			
Low	21.6 (.792)	N.S.	N.S.
High	8.3 (.949)	N.S.	N.S.
Winter			
Low	5.1 (.837)	N.S.	N.S.
High	5.3 (.606)	55.7 (.988)	10.4 (.631)

Table 5-18. Particulate C, N, and P ratios as slope of Model 2 regression in bacteria size fraction for all experiments. Numbers in parentheses are R^2.

Combined	C:N	C:P	N:P
Dilution			
Low	6.1 (.695)	88 (.553)	14.6 (.812)
High	6.7 (.795)	262 (.693)	38.8 (.659)
Summer			
Low	5.0 (.725)	69.1 (.542)	13.8 (.802)
High	6.8 (.861)	255.2 (.749)	37.7 (.595)
October			
Low	6.3 (.592)	N.S.	20.3 (.857)
High	N.S.	N.S.	19.7 (.605)
Winter			
Low	N.S.	N.S.	44.4 (.671)
High	7.5 (.737)	N.S.	N.S.

5.4 Dilution rate and Growth rate

Dilution rate is equivalent to growth rate in a chemostat at steady state, but not in a flow through microcosm system (Gold 1977), where processes of competition and grazing are important factors in controlling growth. Growth rate in the microcosm may be controlled by dilution rate only if the community of organisms acts similar to a monotypic species. To determine growth rate independently of dilution rate, carbon productivity was divided by carbon biomass for both the <1μm size fraction post-incubation and whole water samples (Harris 1986). This calculation resulted in the units of reciprocal time (hr^{-1}). The usual convention of measuring growth rate as day^{-1} was not used because measurements were made in 6 hour incubations during daylight.

In the July experiment, growth rate was found to be greater in the low dilution rate than in the high dilution rate (Figure 5-8A). At this time, the Pnano:Hnano ratios (by biovolume) were significantly greater ($P<0.01$, T test) in the higher dilution rate than in the low dilution rate. This suggested that the Hnano grazers were unable to grow against the higher dilution rate, and, consequently, were washed out of the microcosms. In the low dilution rate, the Hnano grazers were able to grow against the dilution rate and therefore caused a reduction in Pnano biomass (Figure 5-8). Although there were a variety of Hnano in both dilution rate treatments, a heterotrophic dinoflagellate was responsible for the differences between treatments. This heterotrophic dinoflagellate was present only in the low dilution rate experiments in July (Table 5-19). These flagellates are voracious feeders of Pnano (Suttle et al. 1986). In an earlier experiment they were observed feeding on diatoms. They attached

a palium (stick-like connection) to the diatom, then secreted a digestive sac around it. The digested remains of the diatom, empty frustrals, were plentiful in that culture.

Table 5-19 Heterotrophic nanoplankton as cells ml^{-1} and as a percentage of total heterotrophic nanoplankton biovolume during the July 29 experiment.

N:P	Treatment	HFlag)	%	HDino	%	Ciliates	%
5:1	low	3476	9	1738	91.4	0	0
5:1	high	1738	100	0	0.0	0	0
16:1	low	7300	27	608	47.3	304	26
16:1	high	2390	100	0	0.0	0	0
C	low	3259	41	217	59.0	0	0
C	high	456	100	0	0.0	0	0

Grazing in the low dilution rate by the heterotrophic dinoflagellates did not translate into a high remineralization rate. Remineralization was actually greater in the high dilution rate treatments that had more biomass (Table 5-7). Smaller heterotrophic flagellates were found in both low and high dilution rate treatments (Table 5-19) and evidently are the component of the Hnano responsible for phosphorus remineralization.

Table 5-20 Heterotrophic nanoplankton as cells ml^{-1} and as a percentage of total heterotrophic nanoplankton biovolume during the October 21 experiment.

N:P	Treatment	H Flag	%	HDino	%	Ciliates	%
5:1	low	1217	100	0	0	0	0
5:1	high	61	1	304	82	61	18
16:1	low	0	0	152	65	76	35
16:1	high	152	2	304	65	0	0
C	low	760	100	0	0	0	0
C	high	456	100	0	0	0	0

Only in the October experiment was dilution rate related to growth rate of the total biomass. Bacterial biomass then was only 7% of the total, as opposed to about 20% of the total in the other experiments. The total

nanoplankton biomass was much greater in the October experiment, than during any other experiment. The major component of the biomass at this time was a diatom, *Chaetocerus sp.*, which dominated the microcosm community, causing it to reacted like a single species, dilution rate becoming nearly equivalent to growth rate (Figure 5-8B). There was no obvious trend in the Pnano:Hnano ratios for the microcosms in this experiment (Table 5-20).

The bacteria responded to the higher dilution rate in the July and October experiment with an increased growth rate, suggesting that predation on the bacteria was not substantially different in the high and low dilution rate, i.e., their predator was able to grow against the higher dilution rate (Figure 5-9 A and B). The October control was the single exception to this trend (Figure 5-9 B). Figure 5-10 compares the growth rate of bacteria in the low and high dilution rate for the July through October 21 experiments. The dilution rate in all experiments was 0.25 and 0.5 volumes per day corresponding to .01 and .02 volumes per hour, and the mean growth rate was .03 and .045 per day. The difference in each case is approximately .02 per hour. If the difference can be attributed to grazing, then grazing on the bacteria was about the same in both dilution rates, and was equivalent to 2% of the bacterial biomass per hour.

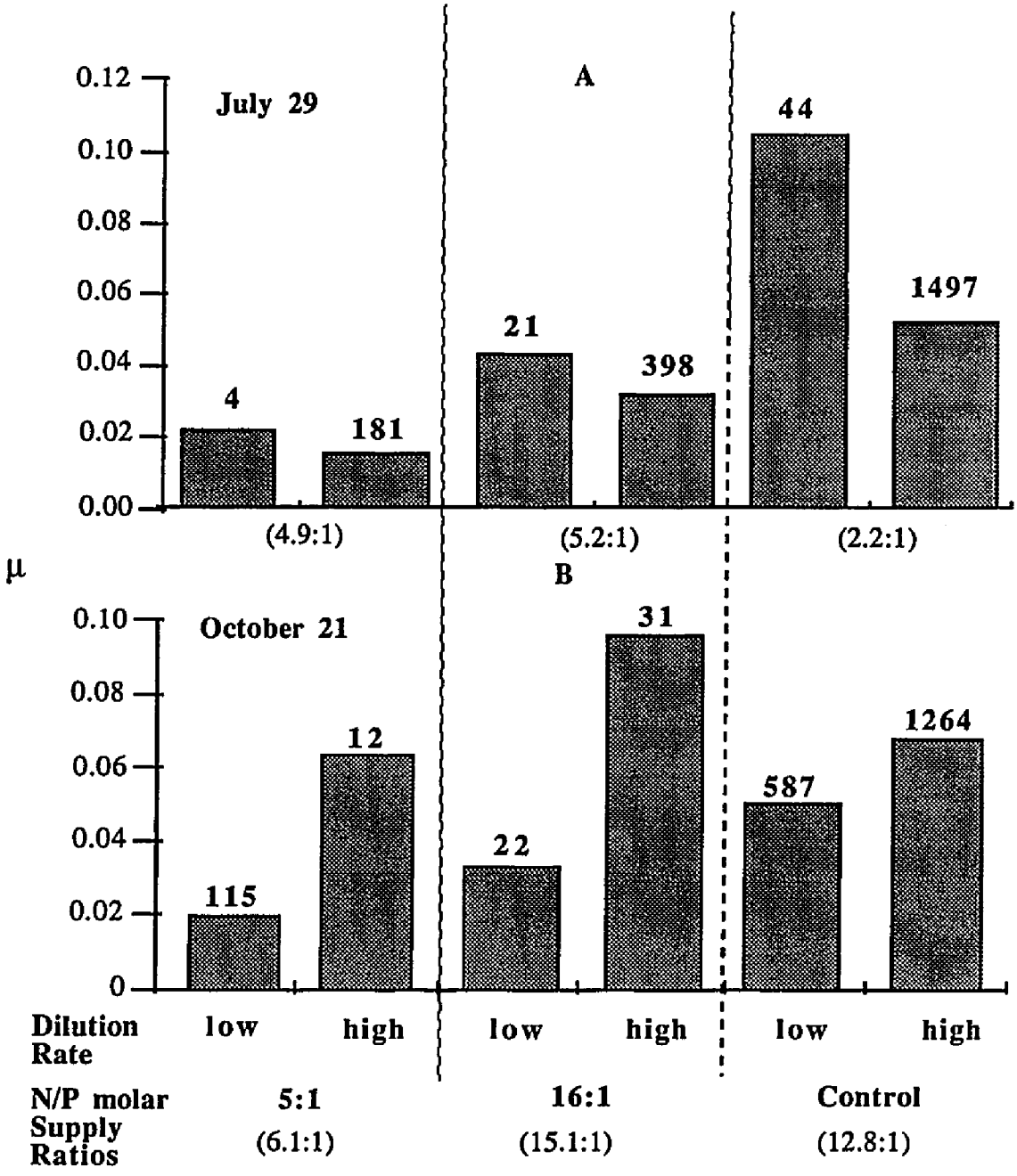


Figure 5-8 Carbon based growth rate μ (hr^{-1}) for the whole water samples. Growth rate was calculated as production per unit carbon biomass. Numbers over the growth rate bars are Pnano: Hnano ratios. **A.** July 29 experiment, when growth rate was higher in the low dilution rate treatment **B.** October 21 experiment, when growth rate and dilution rate were similar.

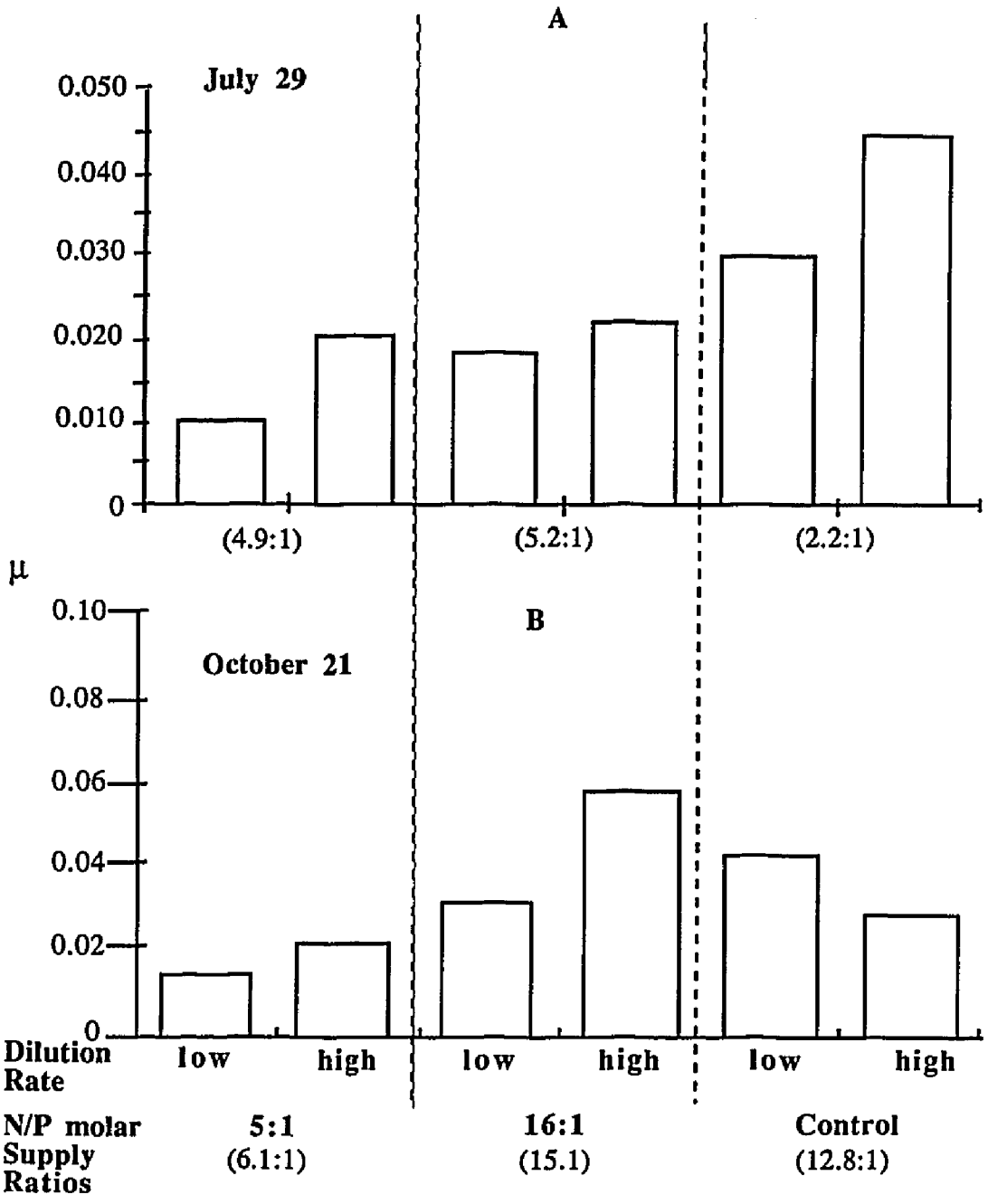


Figure 5-9 Carbon based growth rate μ (hr^{-1}) for the bacteria. Growth rate was calculated as production per unit carbon biomass. **A.** July 29 experiment, when total growth rate was higher in the low dilution rate treatment. Bacterial growth rate correlated with dilution rate even though total growth rate did not. **B.** October 21 experiment, when growth rate and dilution rate were similar.

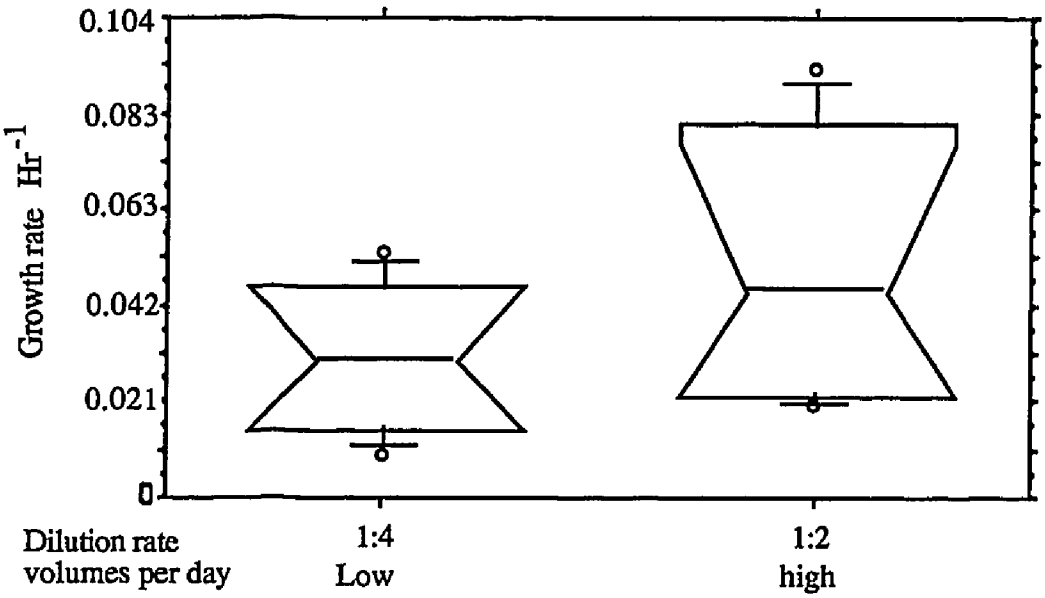


Figure 5-10 Knoched box plot of Bacterial growth rate at low and high dilution rates in July through October 21 experiments. Shows 10, 25, 50, 75, and 90 percentiles of data.

5.4.1 Growth rate and C, N, and P ratios

We have already seen that different stocks react to dilution rate in different ways. In these experiments, grazing seemed to be responsible for much of these differences. Since grazing affected growth rate, it created a whole spectrum of growth rates instead of two, coinciding with the two dilution rates. We might expect that the measurement of the elemental ratios of carbon, nitrogen and phosphorus scaled to growth rate would show some patterns (Goldman 1980). C:N, C:P, and N:P scaled to growth rate generally decreased to Redfield ratios with increased growth rate. This was true for the whole water and for the bacteria size fraction (Figure 5-11; 5-12). At low growth rates, large variations in the elemental composition of bacteria and phytoplankton were observed. At high growth

rates bacteria and whole water particulates in the microcosms took on C, N, and P ratios near the Redfield proportions. In the bacteria scatter plots of growth rate, the C:N and C:P ratios seemed to reach an asymptote at ratios somewhat below Redfield ratios (Figure 5-12). The trend of increasing bacterial C:N and C:P, ratios with increased dilution rate and consequently growth rate seemed to be confined to growth rates of less than .04 divisions per hour, with these ratios declining towards or below the Redfield ratio at the higher growth rates. The mean bacterial C:N ratio in the 0 to .04 growth rate range was 8.8 (range =15.8) and in the .04 and greater range was 7.5 (range = 14.1)(t-test, df=17, p=.10). The mean C:P ratio in the 0 to .04 bacteria growth rate range was 148 (range = 248) and in the .04 and greater range was 113 (range = 224) (t-test, df=17, p=0.04).

The uptake ratios of C:P for the whole water samples were homogeneous over all growth rates, having no apparent trend with increasing growth rate (Figure 5-13 A). A similar lack of trend in the relationship between growth rate and phosphorus remineralization rate was found in the whole water samples (Figure 5-14 A). In the bacterial size fraction, the C:P uptake ratio increased with growth rates up to .04 divisions per hour, then was homogeneous at greater than .04 growth rates (Figure 5-13 B). Bacteria uptake of inorganic P also increased with growth rate (Figure 5-14 B). Bacteria remineralization was confined to the 0 to 0.03 divisions per hour (μ) range with a concurrent increase in uptake of inorganic P with growth rate at > 0.03 μ range. The relationship between bacterial P-remineralization (or uptake) and growth rate remained linear for all growth rates measured.

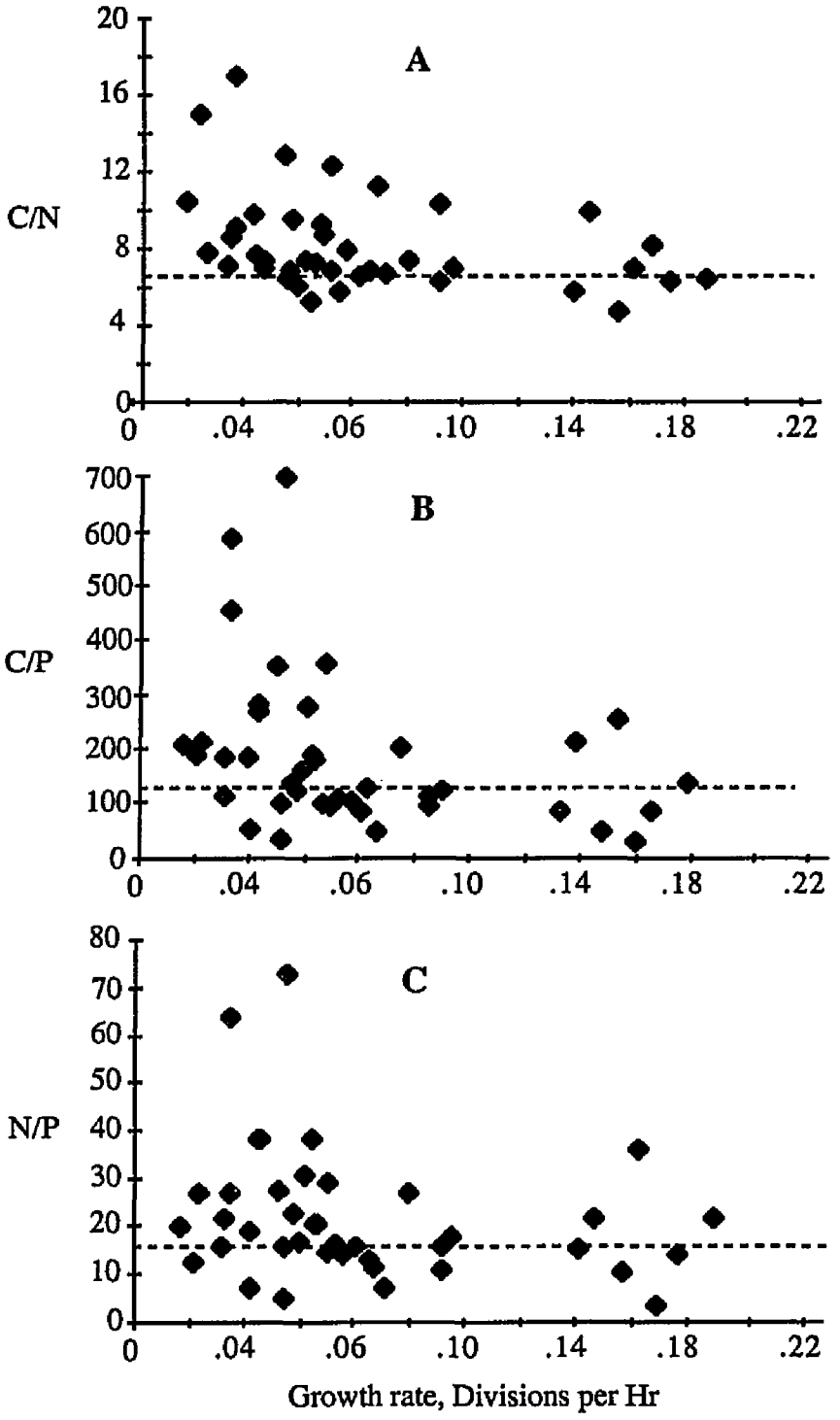


Figure 5-11 Whole water sample of A. C:N, B. C:P, and C. N:P molar ratio plotted against growth rate. Growth rate was calculated as production pre unit carbon biomass. Dashed lines show Redfield values for each ratio.

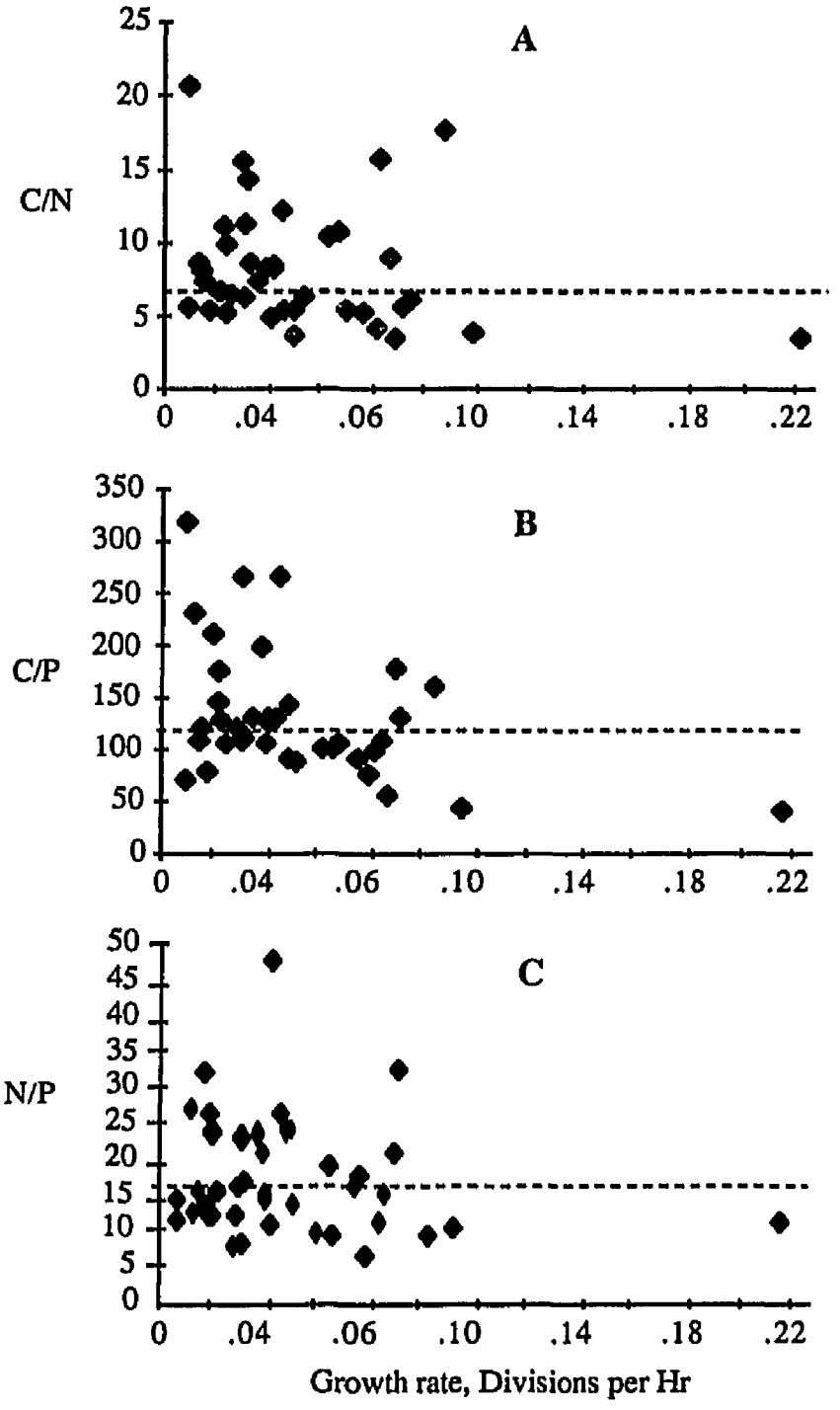


Figure 5-12 Bacterial size fraction of A. C:N, B. C:P, and C. N:P molar ratio plotted against growth rate. Growth rate was calculated as production per unit carbon biomass. Dashed lines show Redfield values for each ratio.

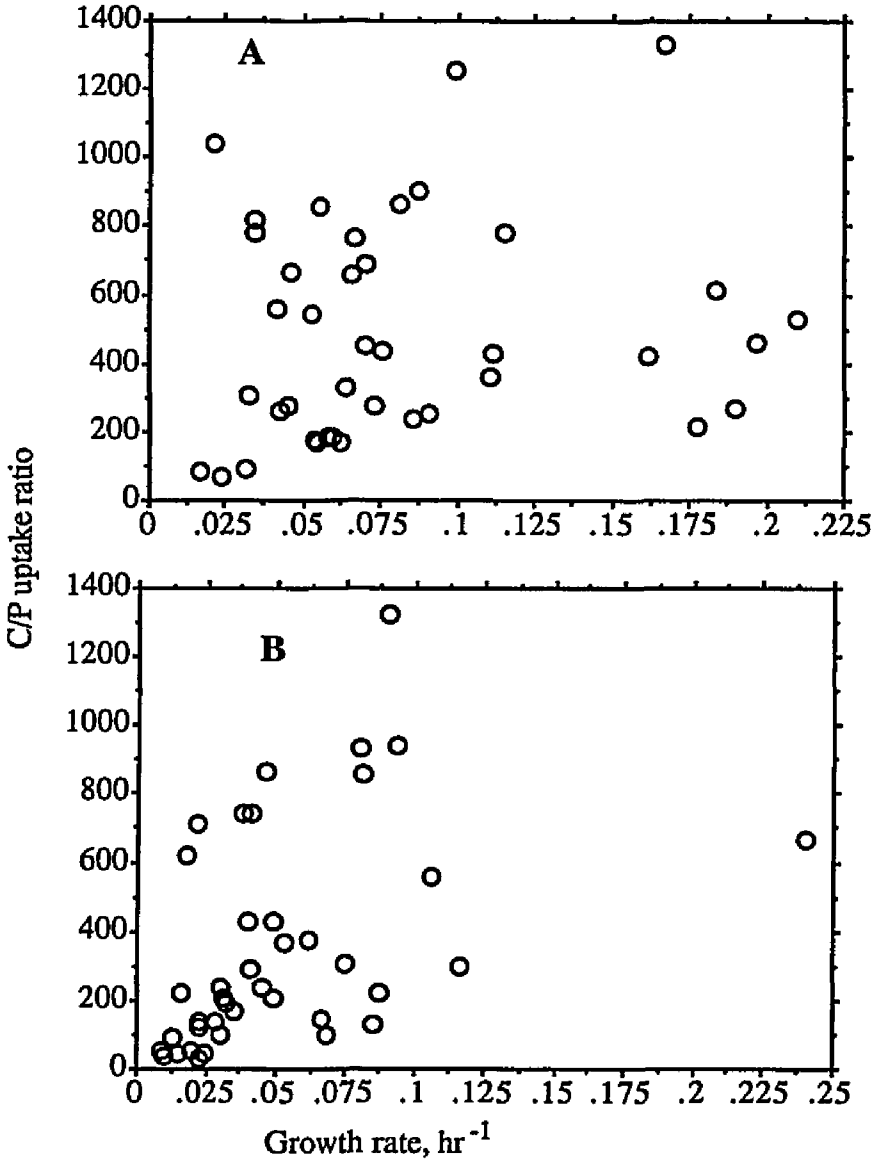
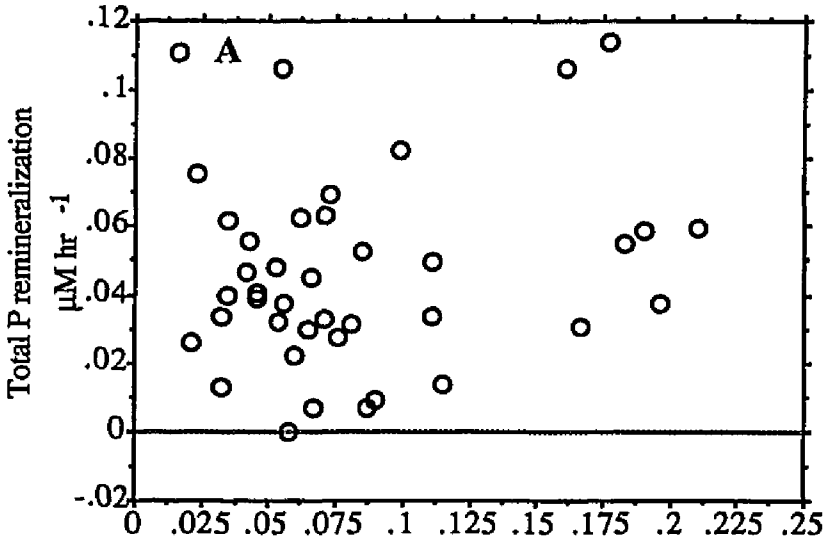
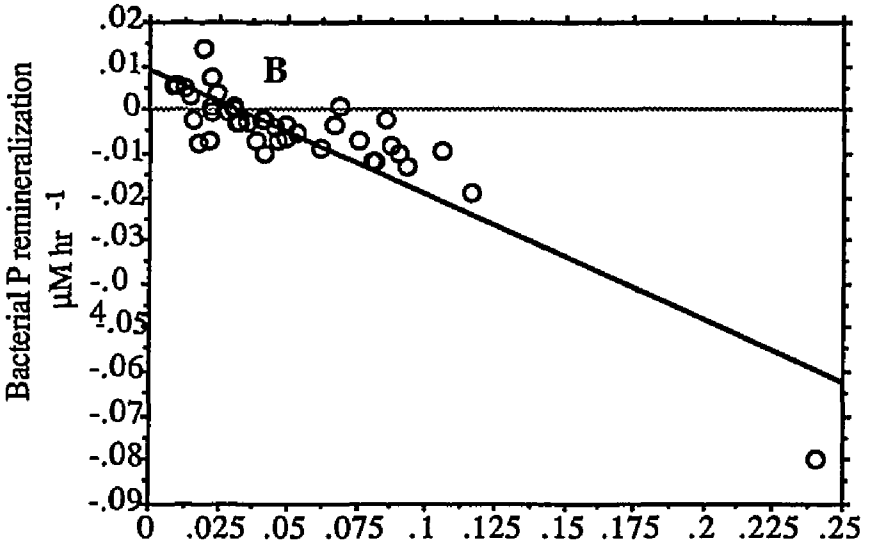


Figure 5-13 A. Whole water C:P uptake molar ratio plotted against growth rate. B. Bacteria C:P uptake molar ratio plotted against growth rate. Growth rate was calculated as production per unit carbon biomass.



y = -.2875x + .0095, R^2 = .777, df = 39, p = 0.0001



Growth rate, hr^{-1}

Figure 5-14 A The relationship between growth rate and phosphorus remineralization. B The relationship between growth rate in the <1µm bacterial size fraction and bacterial P uptake (negative remineralization).

Chapter 6

Discussion

6.0 Hypothesis revisited

The following hypotheses were posed in Chapter 2.

1. Heterotrophic protozoans are predominantly responsible for P remineralization.
2. At steady state, the supply ratios of nitrogen and phosphorus affect the rate of phosphorus remineralization.
3. The rate of phosphorus remineralization increases with dilution rate.

6.1 Hypothesis 1, heterotrophic protozoans as remineralizers of phosphorus

Data presented in section 5.2 indicate that the heterotrophic protozoans were the predominate remineralizers of phosphorus in the microcosm experiments, irrespective of dilution rate or temperature. When bacterial remineralization did occur, it was usually less than 10 percent of the total remineralization. The method used to determine bacterial remineralization of DOP is based on several assumptions (Section 4.2.3). Probably the weakest of these assumptions is that phytoplankton and bacterial exchange pools come to a steady state with added tracer in 6 hour incubations. Smith and Platt (1984) showed that the time required for a *Thalassiosira pseudonana* monoculture to reach this steady state was variable and averaged 6 hours. It is not known how soon these pools come to a steady

state in a microcosm system with several species and at least two trophic levels.

There is, however, other indirect evidence that bacteria account for only a small percentage of phosphorus cycling. The bacterial component of the POC and POP was small (17 percent, SE 2.2 for POC; and 20 percent, SE 3.7 for POP). The low bacterial POC and POP indicated that the bacteria were carbon limited. The bacterial C:P ratios were low compared to the total plankton C:P ratio (mean bacterial C:P = 128, C.V. 47; mean plankton C:P = 187, C.V. = 74). This value was similar to the ratio of 127.8 that Goldman et al. (1987) obtained in cultures of bacteria grown on arginine with both nitrogen and phosphorus in excess. To utilize DOM efficiently, bacteria will need another source of phosphorus. As discussed earlier, their ability to efficiently use inorganic nutrients can keep them from becoming nutrient limited. If DOM produced from phytoplankton has a C:P similar ratio to that of the phytoplankton, and if the bacteria use this DOM, they will need to take up inorganic P to make up for the deficiency in phosphorus.

A literature review by Lancelot and Billen (1985) suggests that the DOM released by phytoplankton is composed mainly of glycollate and polysaccharides, both of which do not contain phosphorus or nitrogen. The glycollate production is directly linked to light dependent photorespiration, and can be either excreted by the cell or metabolized. Glycollate production and release, since they are light dependent, occur during the day (Raven and Beardall 1981). At night a greater proportion of the PDOM will result from autolysis or incomplete assimilation during grazing. The C:P ratio in PDOM will vary in response to the glycollate

production, becoming greater than the phytoplankton particulate ratios during the day, and being similar to the particulate ratio at night. To mass balance uptake of carbon in DOM, bacteria would need to take proportionally more inorganic nutrients relative to carbon during the day than at night. The model of bacteria mineralization employed in this experiment also suggests that bacteria assimilate DIP during the day, competing with the phytoplankton for this resource.

Protozoan remineralization results were calculated by subtracting bacterial remineralization from total remineralization. However, if we could have separated heterotrophic nanoplankton (Hnano) from autotrophic nanoplankton (Pnano), Hnano remineralization could have been calculated by comparing their uptake with its particulate C:P ratios, similar to the method by which Goldman calculated bacterial remineralization (Equation 2-1). Therefore, if the C:P ratio of the Hnano food source, after respiration is accounted for, is similar to the C:P ratio of Hnano, remineralization will not occur.

Although we do not know the C:P ratio of the Hnano in our experiment, Fenchel (1987) suggests that they are similar to those in bacterial biomass. This ratio is somewhat lower than the Redfield ratio that is usually attributed to Pnano (Lancelote and Billen 1986; Wheeler and Kirchman 1986). It is reasonable to assume that the range of values which the Hnano C:P can take on is narrower than that of its food source, because organisms tend to conserve a limiting resource and to excrete or respire an overabundant resource (Goldman 1987; Fenchel 1987). Homeostasis would tend to dictate that the composition (C:N; C:P) of the predator has a central tendency. This principle was apparent in our experimentation in the case of

bacteria. The coefficient of variation for the C:P ratio was greater in the whole water sample (composed mostly of phytoplankton) than in the bacteria.

Fenchel (1987) suggested that the amount of phosphorus remineralization was dependent both on the predator's gross growth efficiency (GGE) and on the difference between the predator and prey C:P ratios. If the GGE is constant, remineralization is less when Hnanos are feeding on phytoplankton (high C:P ratios) then when they are feeding on bacteria (low C:P ratio). The GGE does not, however, remain constant, but increases with food quality (Goldman et al. 1987). Food quality is defined by a balanced C:N:P ratio. By reducing the GGE when feeding on a low quality food such as phytoplankton (High C:N and C:P ratio), an Hnano can maintain the same cell specific remineralization rates that occur when feeding on a richer N and P food source at a higher GGE.

By regression analysis, we have established that most inorganic phosphorus flow went through nanoplankton POP to be grazed and remineralized directly by Hnanos, without bacteria acting as a significant intermediary in the remineralization process. Since POP in the nanoplankton was proportional to remineralization and since the Hnano were responsible for 90 percent or more of the total remineralization, the Hnano must be grazing on phytoplankton in the microcosms. They may also be grazing on the bacteria. This would not result in much remineralization because bacterial biomass was low compared to total biomass.

It is generally accepted that 10 percent of net primary production goes into production of PDOC used by bacteria (Ward 1984). Our results show

that by utilizing this PDOC, bacteria may be responsible for up to 10 percent of total remineralization. By grazing the rest of this primary production, the Hnano remineralize 90 percent or more of the total phosphorus.

6.2 Hypothesis 2, the effect of N/P supply ratios

As discussed above, the GGE changes with food quality in such a way that remineralization of a nutrient tends to remain constant. Therefore, since the high C:N and C:P ratios can be adjusted for with the GGE, the prey's N:P ratio may be more important to remineralization rates than its carbon to nutrient ratio.

Low supply N:P ratios resulted in increased remineralization of phosphorus by both bacteria and Hnano. Although there were no treatments with greater than the 16:1, N:P Redfield ratio, and thus no high N:P treatments, we can expect that high supply ratios would result in more remineralization of nitrogen by bacteria and Hnano, and in reduced remineralization of phosphorus. The microbial community uses this strategy to conserve the mineral nutrient in least supply. The dependence of bacteria on PDOC assures that bacterial remineralization will be small in comparison to total remineralization.

6.2.1 The effect of high N:P supply ratios

The 16:1 N:P supply ratios (low P) resulted in reduced total P-remineralization and in more bacterial uptake of phosphorus than did the 5:1 N:P treatment (high P). Bacteria have a low half-saturation constant for nutrient uptake because of their high surface-to-volume ratio

(Thingstad and Pengerud 1985) and, therefore, effectively compete with phytoplankton for nutrients (Bartbak and Thingstad 1985). Bartbak and Thingstad (1985) and Currie and Kalff (1984) suggests that this form of interspecific competition may be a major interaction that bacteria have in an estuarine and freshwater ecosystem. Our results show that bacteria take up inorganic phosphorus more often, and in greater quantities, than they remineralize phosphorus. Vadstein et al. suggest that bacteria have a substantially higher phosphorus requirement than do phytoplankton (1988). If this is true, it could account for a greater incidence of phosphorus uptake than remineralization even when supply ratio of N and P were at or less than Redfield ratios.

If the available organic substrates are phosphorus-poor relative to their biomass, bacteria will supplement their phosphorus requirements by the uptake of inorganic phosphorus (Caron et al. 1987). To a large extent, bacteria in estuaries and coastal waters depend on substrates that are poor in mineral nutrients, such as dead tissue or release products from vascular plants, macro-algae, or phytoplankton. In such situations, their inorganic phosphorus requirement will increase (Fenchel 1987).

6.2.2 The effect of low N:P supply ratios

Low supply ratio of N:P causes an increase in the community's growth dependence on N. The nitrogen limitation in the microcosms results in dissolved DIN:DIP ratios of inorganic nutrients that are considerably lower than the nutrient input ratios. During nitrogen limitation, the community of organisms strive to utilize all available N for growth. Phosphorus, above what is needed for assimilative and dissimilative metabolism, is released

maintaining a homeostasis between cellular N and P. Consequently, in these experiments, the effect of low N:P supply ratios was an increase in total P-remineralization and large DIP pools during nitrogen limited growth.

N:P supply ratios in the range encountered in these experiments rarely caused bacteria to remineralize phosphorus. Because of a higher P requirement compared to Pnano (Howarth 1988), the bacteria often took up DIP even when exposed to low N:P supply ratios. Bacterial P-uptake was, however, less under these conditions than when the N:P supply ratios were higher.

6.3. Hypothesis 3, the effect of dilution rate on the remineralization rate

The dilution rate did not have a direct effect on remineralization rate, but rather, by washing out slow growing species, it affected how much biomass was present. In the summer, the major heterotrophic nanoplankton grazers were washed out of the higher dilution rate treatments. This resulted in a release from grazing pressure and, therefore, greater biomass in the high dilution rate in the summer. The October 28 through January 13 experiment had greater biomass in the lower dilution rate, presumably because of a reduction in the growth potential with lower temperature (Eppley 1972). The remineralization rate in these experiments was proportional to the amount of biomass present and was, therefore, greater in the high dilution rate treatment in the summer and early October experiments, and greater in the low dilution rate treatment in the late October through January experiments.

6.4. The effect of dilution rate on growth rate

A closer relationship was expected between dilution rate and community growth rate in these experiments. It was assumed that the Pnano stock would have dominated the community, and that the microcosm would act essentially like a chemostat, with dilution rate being equivalent to growth rate. By fixing growth rate with the dilution rate, the effect of community growth rate on remineralization could have been determined. There was, however, no relationship between dilution rate and growth rate, as measured as ^{14}C production and POC. As it turned out, the growth dynamics of the microcosm community were not dominated by the Pnano, but were affected by several distinct populations or stocks which loosely can be aggregated into either Pnano, cyanobacteria (Ppico), Hnano, or heterotrophic bacteria (Hpico). All of these stocks interacted to affect the Pnano growth rate, either by grazing (Hnano) (Sherr et al. 1988) or by competing for inorganic nutrients (Hpico) (Bratbak et al. 1985). In this experiment, it was not possible to separate the measurements of production for each of these stocks. A relative measure of grazing was made by comparing growth rate and dilution rate in each treatments. This showed that grazing affected both biomass concentration and production rates. Competition for phosphorus caused phytoplankton growth and biomass to be reduced. Approximately 10 percent of the inorganic P in these experiments was utilized by the bacteria instead of going into primary production. When bacteria are utilizing PDOM, the uptake of inorganic nutrients makes up for any additional P and N bacterial requirement.

6.4.1 Conformity of the microcosm community to a 3 compartment model

There are several populations in the microcosm community which are growing at different rates, competing for nutrients, and interacting through grazing relationships. To put these processes in perspective, a three compartment model of bacteria (Hpico), phytoplankton (Pnano), and heterotrophic protozoa (Hnano) has been constructed. Gold's (1977) treatment of growth in a chemostat has been modified to fit the community of organisms found in the microcosm system.

Microbial growth or death follows a linear rate law; that is, the rate of change of a variable is proportional to its own value. This assumes that the population is homogeneous and randomly dividing. The equation then has the form :

$$\frac{dN}{dt} = k\ N \qquad \text{Equation 6-1}$$

The integration or analytical form of this equation is :

$$N_t = N_0\, e^{k\,(t-to)} \qquad \text{Equation 6-2}$$

where N_t is the population size at time t, and N_0 is the population size at t_0, and k is a (growth - death) rate in reciprocal time (t^{-1}).

In a microcosm, the community of organisms is composed of many populations, each with its own intrinsic growth and death rate parameters. If n_i is the number in the ith group, and k_i is the corresponding rate parameter, then for each group we have :

$$\frac{dn_i}{dt} = k_i \; n_i \qquad \text{Equation 6-3}$$

If we assume that populations do not interact, and that r groups exist, then the total number in the community is :

$$N = n1 + n2 + \cdots + n_r \qquad \text{Equation 6-4}$$

In this treatment we assume that competition for the limiting resource combined with the effect of dilution rate result in three relatively homogeneous groups within the microcosms: the Hpico, the Pnano, and Hnano. Thus, the rate of change of N is the sum of the rates for the n_i populations :

$$\frac{dN}{dt} = \frac{dn_{Hpico}}{dt} + \frac{dn_{Pnano}}{dt} + \frac{dn_{Hnano}}{dt} \qquad \text{Equation 6-5}$$

The essential difference between a multiple species and a single species culture is that in a microcosm with several populations, the make-up of the species is continually changing and the relative rate of change for the community as a whole is not constant.

The effects of nutrient concentration, competition, dilution, or predation are not considered in the exponential relationship shown in Equation 6-5. To make this equation more realistic, these components must be added. If it is assumed that a given amount of population growth requires a fixed amount of limiting nutrient, then growth is proportional to the rate at which the limiting nutrient is consumed. This is the familiar Michaelis-Menten relationship:

$$\mu = \mu_{max} \frac{S_g}{K_s + S_g} \qquad \text{Equation 6-6}$$

where μ is growth rate, μ_{max} is the maximum growth rate (reciprocal time), S_g is the nutrient concentration, and K_s is the value of S_g needed for half-saturated growth.

The effect of dilution can be ascertained if we mass balance inputs to the microcosm with removal. The change in the amount of microorganisms in a flow-through microcosm equals cell production resulting from the inflow of nutrients and water, minus the removal of organisms and water by overflow :

$$(dN/dt) = \text{production - rate of removal} \qquad \text{Equation 6-7}$$

Production is the differential rate of increase (equation 6-1) combined with the Michaelis-Menten relationship :

$$\frac{dN}{dt} = k \mu N \qquad \text{Equation 6-8}$$

The rate of removal is the volume removed per unit of time multiplied by the concentrations :

$$\text{rate of removal} = F \frac{N}{V_g} \qquad \text{Equation 6-9}$$

where F is flow rate and V_g is volume. Combining the Michaelis-Menten relationship 6-5, equation 6-8, and 6-9 for each n_i population and substituting into equation 6-7, results in equation 6-10 (Gold 1977):

$$\frac{dn_i}{dt} = \left(\frac{k\,\mu_{max}\,S_g}{K_S+S_g} - \frac{F}{V_g}\right) n_i \qquad \text{Equation 6-10}$$

As explained by equations 6-9 and 6-10, individual sub-populations n_i attain concentration levels consistent with their growth rate, substrate concentration, and K_S value. It can be shown that if a nutrient is limiting, the growth rate is equal to dilution rate for each n_i population as long as dilution rate does not exceed the maximum growth rate (Gold 1977).

Therefore, at steady state each population will have the same growth rate; populations in which growth rate does not equal dilution will wash out of the culture. Since substrate concentration is reduced by each n_1 population, and since each n_i population has a distinct K_S value for taking up the limiting nutrient, each sub-population will compete for the limiting nutrient.

In many of the experiments, the microcosm community did come to a condition of relatively constant composition (Figure 5-1; 5-2), indicating that each sub-population attained a concentration level consistent with its growth rate, substrate concentration, and K_S value. The growth rate of Hpico in some experiments correlated with dilution rate; the growth rates of the other stocks, however, did not correlate with their dilution rates. Additionally, the growth rates of one stock often differed from those of the other stocks.

In many ways the loss of biomass by predation is analogous to the effect of dilution rate, so it might be expected that some of the differences in the growth rates of the three stocks may be due to grazing. The effect of a predator in the microcosm can be modelled heuristically with the

Lotka-Volterra equation. These equations lack structural stability, but they are useful, however, to the understanding of the microcosm system's behavior (Gold 1977).

$$\frac{dni}{dt} = Ai\ ni - Bi\ \ ni\ \pi \qquad \text{equation 6-11}$$

$$\frac{dnj}{dt} = -a\ nj + Bj\ ni\ nj \qquad \text{equation 6-12}$$

In these equations, ni is the size of the prey population; nj is the size of the predator population; and Ai and Aj are intrinsic rates of increase of each population in the absence of the other populations. Ai is equivalent to equation 6-10 for the two prey stocks (Pnano and Hpico), and Bj is equivalent to equation 6-10 for the predator stock. In these equations, the effect of dilution rate on the prey equation 6-11 must be included in the production term (Ai ni), but since the predator reacts to prey density, dilution rate does not affect the loss term (Bi ni π). In the predator equation 6-15, both, the intrinsic death rate (-a nj) and the production rate (Bj ni nj) terms are affected by dilution. Substituting equation 6-10 for Ai generates equations 6-13, 6-14, and and equation 6-10 for Bj generates equation 6-15 which includes the effect of dilution, substrate concentration, and grazing for each stock.

term 1 term 2

$$\frac{dn_{Hpico}}{dt} = \left(\frac{k\,\mu_{max}\,S_g}{k_S+S_g} - \frac{F}{V_g}\right) n_{Hpico} - (Bi\ n_{Hpico}\, n_{Hnano})$$

equation 6-13

$$\frac{dn_{Pnano}}{dt} = \left(\frac{k\,\mu_{max}\,S_g}{k_S+S_g} - \frac{F}{V_g}\right) n_{Pnano} - (Bi\ n_{Pnano}\, n_{Hnano})$$

equation 6-14

$$\frac{dn_{Hnano}}{dt} = -(a\, n_{Hnano}\frac{F}{V_g}) + \left(\frac{k\,\mu_{max}\,ni}{k_{ni}+ni} - \frac{F}{V_g}\right) ni\, n_{Hnano})$$

equation 6-15

The three stocks in equation 6-13 through 6-15 interact through both grazing pressures and competition for a limiting nutrient or substrate.

In a system, natural or experimental, where both competition for a limiting resource and grazing can occur, Sommer (1988) suggests that three outcomes are possible. First, predation may be too weak to change the outcome of competition. Second, predation may be so strong, that prey can't exploit their resources sufficiently to make competition detectable. Third, predation may change the balance between competing prey organisms. The first alternative is likely to occur when the predator populations are strongly exploited, in which case term 2 of equation 6-13 and 6-14 would be small when compared to term 1, since losses to

predation are minimal. This case is unlikely in a microcosm system that excludes metazoans. The second alternative requires that none of the prey populations are resistant to predation, and that a steady state develops in which prey populations reproduce at their resource-saturated rates. In this case, term 2 is equivalent to term 1 in equations 6-13 and 6-14. In the third alternative, high predation losses increase the resource requirements for production until they equal mortality. The smaller the resource requirement, the better the competitive ability of a given species for a given resource (Tilman 1982; Sommer 1988). Alternative 3 requires that both the production term (1) and the grazing term (2) in equation 6-13 and 6-14 are regulated by the predator. Grazing on a stock results in an increased growth rate for the prey. This process results in a homeostasis between growth and predation without a requirement that the prey populations be equally susceptible to predation. Within the limits of μmax, the prey organism's growth rate is adjusted to make up for losses caused by predation. The third alternative, seems to explain our data the best.

Grazing pressure was responsible for the differences in biomass in the two dilution rate treatments, at least in the July through the October experiments. This was most obvious in the July 29 experiment, in which a heterotrophic dinoflagellate was present only in the low dilution rate experiment. Because of the large size of this dinoflagellate, allometric considerations may explain its slow growth rate as compared to that of the smaller forms of Hnano found in both dilution rates in the July experiment (Kuosa and Kivi 1989). This heterotrophic dinoflagellate was washed out of the microcosm at the higher dilution rate, releasing Pnano species from predation pressure and consequencely allowing Pnano abundance to

increase. This resulted in greater biomass in the high dilution rate. Growth rate of Pnano as measured in the tracer experiments was greater in the low dilution rate, having presumably been stimulated by grazing. Term 1 in equation 6-14 must, thus be coupled to the grazing rate. This can be possible if S_g increased as a result of sloppy feeding of the predator (ultimately resulting in more nutrients) and/or the μmax of a younger population of Pnano, resulting from grazing, is higher.

These experiments show all the components of Sommer's third alternative. Increased growth rate with grazing and unequal resistance to predation by competing stocks. The grazing rate on the Pnano stock were different in the two dilution rates and the bacteria were grazed at a constant rate of about 2 percent of their biomass per hour.

6.5 Trophic structure effects on remineralization

Physiological tolerances, food supply, and predation determine the potential success of an individual organism. If heterotrophic growth is limited by the quantity or quality of food, then population size and, therefore, community composition are determined by factors regulating the dynamics of prey organisms in the next lower trophic level. This form of regulation is called resource or bottom-up control. However, if enough nutrition is available to make growth resource-independent, population size may be governed by predation from the next higher trophic level. This is called sink or top-down control. The magnitude and distribution of predation pressure can alter the structure of pelagic food webs. Changes in community composition within one trophic level reverberate through the

food web and significantly alter the structure of the entire food web (Verity 1988).

Predator-prey interactions of this type may effect phosphorus remineralization rates, and as has previously been discussed, substrate level interaction play an important role in phosphorus remineralization. Remineralization within the microcosms was caused predominantly by heterotrophic nanoplankton. The rate of remineralization was a function of phosphorus supply, that is, the amount of phosphorus in the POP resources used by the Hnano. The amount of remineralization was not proportional to the density of Hnano, since there was no significant correlation in the data between either the Pnano/Hnano ratio or the Hnano density and remineralization. Thus, the P-remineralization by Hnano was to a large extent resource-controlled (bottom-up); the remineralization rate was unaffected by Hnano density, but was controlled by supply ratios of nutrients. A reduction in Hnano does not mean that the remineralization rate of phosphorus is reduced.

Because metazoan predators of Hnano were excluded in these experiments by filtering York River water through a 60 μm cartridge filter, sink control could not be examined. If introducing a metazoan into a microcosm stimulates metabolism by either keeping the Hnano in a state of physiological youth or increasing DOP concentrations by sloppy feeding, then a top-down control might be possible. However, a metazoan would not control remineralization merely by controlling the number of Hnano present. It is possible to draw some conclusions concerning sink control using data from microcosm experiments in which metazoans were included.

Roman, et al. conducted microcosm experiments in which copepods were not excluded (1988). In those experiments, 12 to 87 percent of the daily copepods carbon needs were satisfied by grazing on heterotrophic flagellates. This corresponded with 13 to 43 percent of the daily Hnano production. Regeneration rates of NH_4^+ and Hnano production were higher in the experiments with copepods than in those without copepods. Based on weight-specific metabolism, Roman et al. concluded that the copepods did not contribute significantly to ammonium regeneration (1988). Rather, they suggested that copepods stimulated bacterial production and nutrient regeneration by producing DOM and dissolved free acid amino nitrogen (DFAN) by sloppy feeding during grazing. In addition, 10 to 15 percent of the nitrogen excreted by the copepods can be released as DFAN (Bidiare 1983). The release of DFAN by the grazers provided a nitrogen rich substrate for the bacteria that is translated into an increase in the size of bacterial nitrogen pool. Any excess nitrogen could then be either directly remineralized by the bacteria or be released from the Hnano which grazed on the bacteria. Our experiments show that bacteria are not usually responsible for phosphorus remineralization; since similar processes govern the dynamics of phosphorus and nitrogen remineralization, the remineralization noted by Roman et al. was probably the result of Hnano remineralization.

Although grazing did reduce Hnano stocks, Roman et al. noted that the highest loss rate of bacteria and highest growth rate of Hnano occurred in the microcosms with macrozooplankton. Biomass and rate processes are not necessarily correlated; a lower number of Hnano in the treatments with copepods could carry out the additional remineralization if increased

metabolic activity resulted in a higher Hnano cell specific remineralization and grazing rate. Even under top-down (sink) control we should not expect the observed number of Hnano cells to correlate with remineralization. Only when resource supply ratios and growth rate remain the same and grazing pressure changes, will concentrations of Hnano and remineralization be related.

Hamilton and Taylor (1987) working with epilimnetic water, tested the hypothesis that removal of zooplankton would decrease phosphorus turnover time and that addition of zooplankton would increase phosphorus turnover time. As might be expected from the results of my experimental work and Roman et al., their data did not support this hypothesis (1987). No relationship between phosphorus turnover time and zooplankton concentration was found. Resource supply ratios of N and P, and growth rates interact to complicate the relationship between numbers of zooplankton and phosphorus turnover rate.

6.6 C, N, and P conversions and growth rate

A linkage existed between supply ratios of nutrients, particulate ratios, and remineralization of phosphorus. This results from the transfer of phosphorus from one nutrient pool to the next, as if the nutrient pools built up and, to preserve cellular homeostasis, overflows into the next pool. This makes the relative proportions of the C, N, and P in one pool at least a partial determinate of what will be found in successive pools (nutrient > particulate phase > remineralized nutrient). This scheme is complicated by the effect that growth rate has on the size of the particulate pools of C, N, and P. Increased growth rate causes whole water particulate ratios to have

relative proportions of C, N, and P approximating Redfield ratios; increased bacterial growth rate causes bacterial particulate ratios to have relative proportions of C:P and C:N ratios lower than Redfield ratios. These lower ratios are the result of a greater N and P requirement relative to C in bacteria (Fenchel 1987). In both bacteria and total biomass, the particulate ratios varies with supply ratios at growth rates below 1 day^{-1}, and asymptote to a fixed ratio at growth rates above 1 day^{-1}.

There was no relationship between C:P uptake ratios and growth rate, or between P released and growth rate (Figure 5-13A and 5-14 B) in these experiments. There was, however, a relationship between the C:P particulate ratio and growth rate as discussed above. To mass balance phosphorus, any phosphorus that is taken up in excess of the amount found in the particulate phase must be released. This can be expressed in the following relationship revised from (Lancelot and Billen 1985).

$$\frac{\text{organic C uptake}}{\text{C/Pu}} = \text{P remineralization} + \frac{\text{organic C uptake}}{\text{C/Pp}}$$

where C/Pu is the uptake ratio and C/Pp is the particulate ratio. This suggests that even if there is no relationship between growth rate and uptake ratios of P/C, there should be a relationship between the P/C uptake ratio and remineralization scaled to carbon biomass. Figure 6-1 shows that this is indeed the case. These two parameters are significantly, although somewhat loosely, related.

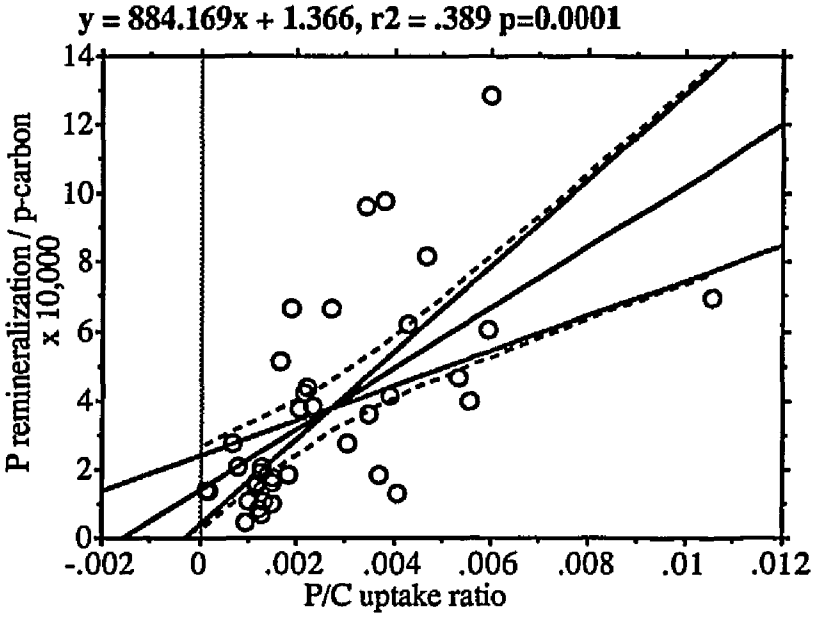


Figure 6-1 The relationship between the release of phosphorus per unit carbon and the P/C uptake ratio in the the microcosms for July through Dec 12, 1988 experiments.

Phosphorus remineralization can be viewed as a community response to the supply of nitrogen and phosphorus (Figure 6-1). No single group of organisms cause remineralization. It is instead the result of trophic level interactions among all populations. There are several types of interactions that effect remineralization. Competition between bacteria and phytoplankton for a nutrient can reduce the growth rate of both stocks. As carbon production rates are reduced by this competition, there is an excess of a second non-limiting nutrient relative to carbon, which is processed through the microbial food web and ultimately released in an inorganic form. Predation and supply ratios of nutrients near Redfield ratios are two factors which enhance growth rate. As carbon production rates increase, nutrients are conserved or released at rates commensurate with there relative supply.

On a community scale remineralization is the result of a mass balance between uptake of nutrients and nutrients that remain in intracellular synthetic pools. Remineralization being the difference of the two. There are of course dissolved organic releases of nutrient of which some is refractory and is lost to the biotic system. Most of the dissolved organic nutrients are rapidly utilized by the bacteria and constitute a relatively small pool.

Chapter 7

Implications of Microcosm results for the Lower York River

7.0. General

For these microcosm experiments, we focused on questions relevant to our understanding of the effect of nutrient loading in estuaries. Microcosms do not incorporate the same spatial and temporal scales found in estuaries; therefore, results of these experiments cannot be used as direct predictors of processes in estuaries. However, interpretation of microcosm results based upon our knowledge of nutrient supply, circulation, water residence time, and other physical forcing functions of an estuary, allow us to draw some parallels between microcosm studies conducted on the York River and processes in the York River Estuary. The York River is a sub-estuary of the Chesapeake Bay. It exhibits strong seasonal variations in freshwater flow and nutrient regimes.

7.1. Nutrient in the York River Estuary

Sewage inputs are thought to account for 13 percent of the total nitrogen and 33 percent of total phosphorus inputs into the York River. In the winter and spring, nutrients with a high N:P ratio (N:P = 28 in 1986) enter the estuary with the large input of freshwater (2300 CFS). During

the summer, freshwater with low N:P ratios entered the river at low flow rates (700 CFS). The ratio of DIN to DIP in the estuary varies seasonally, with a maximum of approximately 19:1 at the time of peak freshwater flow in the winter and a minimum of 12:1 at the time of minimum freshwater flow in the late summer (Virginia Water Control Board 1986). This suggests that primary production may switch from phosphorus limitation in the winter to nitrogen limitation in the summer, a finding supported by bioassay data (Webb 1988). Phytoplankton react to this alteration between nitrogen limitation and phosphorus limitation with decreased N:P particulate ratios in the summer and increased N:P particulate ratios in the winter (Virginia Water Control Board 1986).

7.2 Circulation

The York River is formed by the confluence of the Pamunkey and Mattapoi Rivers about 50 km upriver. The mean tide range at the mouth is 0.7 m and the surface salinity at this point range from 15-24 ppt (Haas 1977).

The York River estuary oscillates between conditions of considerable vertical stratification and of homogeneity on a cycle closely correlated with the spring-neap tidal cycle. This is caused by an influx of less saline water into the mouth of the York River from further up the Chesapeake Bay. Destratification events persist up to three days and result in near vertical homogeneity (Hayward et al. 1986).

7.3. Implications

The microcosm experiments provided evidence that supply ratios of nitrogen and phosphorus affect the rate of phosphorus remineralization. Thus, seasonal alteration of the N:P supply ratios to the York River, according to our experimental results, would affect the rate of phosphorus remineralization. In the late spring or early summer a reduction of the N:P supply occurs as nitrogen inputs caused by runoff and ground water are reduced. This results in an excess of phosphorus beyond Redfield ratios. This excess phosphorus is distributed among the various phosphorus pools and ultimately results in rapid remineralization of phosphorus back into the water column. This implies that the increased phosphorus in all these pools can be utilized when nitrogen becomes more plentiful. The October increase in biomass found in the microcosm may have resulted in part from utilization of phosphorus stored during periods of nitrogen limitation. This may be one mechanism by which spring and autumnal blooms may be intensified in estuaries.

Run-off, wind, and tidally induced mixing can cause dilution of microbial stocks. Our results indicated that this dilution could bring about increased remineralization and recycling of phosphorus. In the York River, this mechanism could make inorganic nutrients available during transitions between the spring-neap tidally induced destratification-stratification event. Ducklow noted increased production during these events in the York River (1982).

References

Admiraal, W. and M. J. W. Veldhuis. 1987. Determination of nucleosides and nucleotides in seawater by HPLC; application to phosphatase activity in cultures of the alga Phaeocystis pouchetii. Mar. Ecol. Prog. Ser. 36: 277-285.

Ammerman, J. W. and F. Azam. 1985. Bacterial 5'- Nucleotidase in aquatic ecosystems: A novel mechanism of phosphorus regeneration. Science 227: 1338-1340.

Andersen, O. K, J. C. Goldman, D. A. Caron and M. R. Dennett. 1986. Nutrient cycling in a microflagellate food chain: III. Phosphorus dynamics. Mar. Ecol. Prog. Ser. 31: 47-55.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257-263.

Bell, W. H. 1980. Bacterial ulitization of algal extracellular products. 1. The kinetic approach. Limnol. Oceangr. 25(6): 1007-1020.

Berman, T. and M. Stiller. 1977. Simultaneous measurement of phosphorus and carbon uptake in Lake Kinneret by multiple isotopic labeling and differential filtration. Microb. Ecol. 3: 279-288.

Bidiare, R. R. 1983. Nitrogen excretion by marine zooplankton. In: D. C. Carpenter, (ed.), Nitrogen in the Marine Environment. Academic Press, New York. pp. 385-409.

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

Bratbak, G. 1987. Carbon flow in an experimental microbial ecosystem. Mar. Ecol. Prog. Ser. 36: 267-276.

Caperon, J., D. Schell, J. Hirota and E. Laws. 1979. Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a ^{15}N isotope dilution technique. Mar. Biol. 54: 33-40.

Caron, D. A., J. C. Goldman, O. K. Andersen and M. R. Dennett. 1985. Nutrient cycling in a microflagellate food chain: II. Population dynamics and carbon cycling. Mar. Ecol. Prog. Ser. 24: 243-254.

Christian, R. R. and R. L. Wetzel. 1978. Interactions between substrate, microbes and consumers of Spartina detritus in estuaries. In: M. Wiley (ed.), Estuarine Interactions. Academic Press, New York. pp. 93-113.

Chrost, R. H. and M. A. Faust. 1983. Organic carbon release by phytoplankton: Its composition and utilization by bacterioplankton. J. Plankton Res. 5(4): 477-493.

Currie, D. J. and J. Kalff. 1984. A comparison of the ability of freshwater algae and bacteria to aquire and retain phosphorus. Limnol. Oceanogr. 29(2): 298-310.

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

D'Elia , C. F., J. Taft, J. T. Smullen and J. Macknis. 1983. Nutrient enrichment. In: Chesapeake Bay Program Technical Studies: A Synthesis. United States Environmental protection Agency, Washington D.C. U.S. Government Printing Office -606-490 pp. 36-265.

D'Elia, C. F. 1985. Nutrient enrichment effects in Chesapeake Bay: The nitrogen vs. phosphorus controversy. In: The Fate & Effects of Pollutants. A Symposium. University of Maryland Sea Grant, College Park UM-SG-TS-85-02 pp. 3-16.

D'Elia, C. F., J. G. Sanders and W. R. Boynton. 1986. Nutrient enrichment studies in a coastal plain estuary. Phytoplankton growth in large-scale, continuous cultures. Can. J. Fish. Aquat. Sci. 43: 397-406.

Ducklow, H. W. 1982. Chesapeake BAy nutrient and plankton dynamics. 1. Bacterial biomass and production during spring tidal destratification in the York River, Virginia, estuary. Limnol. Oceanogr. 27(4): 651-659.

Ducklow, H. W., D. A. Purdie, P. J. LeB. Williams and J. M. Davies. 1986. Bacterioplankton: A sink for carbon in a coastal marine plankton community. Science 232: 865-867.

Eppley, R. W. 1968. An incubation method for estimating the carbon content of phytoplankton in natural samples. Limnol. Oceanogr. 13: 574-582.

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. 70: 1062-1097.

Estrata, M., M. Alcaraz and C. Marrase. Effects of turbulence on the composition of phytoplankton assemblages in marine microcosms. Mar. Ecol. Prog. Ser. 38: 267-281.

Falkowski, P. G. and T. G. Owens. 1980. Light-shade adaption, two strategies in marine phytoplankton. Plant Physiol. 66: 592-595.

Fenchel, T. and T. K. Backburn 1979. Bacteria and mineral cycling. Academic Press, New York. pp. 101-126.

Fenchel, T. 1982. Ecology of heterotrophic microflagellate. IV. Quantitative occurrence and importance as bacterial comsumers. Mar. Ecol. Prog. Ser. 9: 35-42.

Fenchel, T. 1987. Ecology of protozoa: The biology of free living phagotrophic protists. Science Tech Publishers. Madison, Wisconsin. pp. 1-197.

Furman, J. A., A. G. Horrigan, and D. G. Capone. 1988. Use of 13 N tracer for bacterial and algal uptake of ammonium from seawater. Mar. Ecol. Prog. Ser. 45: 271-278.

Geider, R. 1988. Estimating the growth and loss rates of phytoplankton from time-series observations of 14 C bicarbonate. Mar. Ecol. Prog. Ser. 43: 125-138.

Glibert, P. M. 1982. Isotope dilution models of uptake and remineralization of ammonium by marine plankton. Limnol. Oceanogr. 4: 639-650.

Glibert, P. M. 1982. Regional studies of daily, seasonal and size fractionation variability in ammonium remineralization. Mar. Biol. 70: 209-222.

Gold, H. J. 1977. Mathematical modeling of biological systems - an introductory guidebook. Wiley, New York. pp.1-357.

Goldman, J. C. 1984. Ocean nutrient cycles. In: M. J. R. Fasham (ed.), Flows of Energy and Material in Marine Ecosystems. Plenum press, New York. pp. 137-170.

Goldman, J. C., D. A. Caron and M. R. Dennett. 1987. Nutrient cycling in a microflagellate food chain: IV. Phytoplankton-microflagellate interactions. Mar. Ecol. Prog. Ser. 38: 75-87.

Goldman, J. C., J. J. McCarthy, and D. C. Peavey. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature 279: 210-215.

Goldman, J. C., K. R. Tenore and H. I. Stanley. 1973. Inorganic nitrogen removal from wastewater: Effect on phytoplankton growth in coastal marine waters. Science 180: 955-956.

Goldman, J.C. 1980. Physiological processes, nutrient availability, and the concept of relative growth rate in marine phytoplankton ecology. In: Primary Production in the Sea. P. G. Falkowski (ed.), Plenum Press, New York and London. pp. 179-194.

Goldman, J. C. and M. R. Dennett. 1985. Susceptibility of some marine phytoplankton species to cell breakage during filtration and post-filtration rinsing. J. Exp. Mar. Biol. Ecol. 86: 47-58.

Goldman, J. C., D. A. Caron and M. R. Dennett. 1987. Regulation of gross growth efficiency and ammonium regulation in bacteria by substrate C:N ratio. Limnol. Oceanogr. 32(6): 1239-1252.

Gude, H. 1985. Influence of phagotrophic processes on the regeneration of nutrients in two-stage continuous culture systems. Microb. Ecol. 11: 193-204.

Haas, L. W. 1977. The effect of the spring-neap tidal cycle on the vertical salinity structure of the James, York and Rappahannock Rivers in Virginia, U.S.A. Estuar. Coast. Mar. Sci. 5: 485-496.

Haas, L. W. 1983. Improved epifluorescence microscopy for observing plankton micro-organisms. Ann. Inst. Oceanogr. Paris. 58: 261-266.

Haas, L. W. and K. L. Webb. 1979. Nutritional mode of several non-pigmented microflagellates from York River estuary, Virginia. J. Exp. Mar. Biol. Ecol. 39: 125-134.

Hagstrom, A., F. Azam, A. Andersson , J. Wikner and F. Rassoulzadegan. 1988. Microbial loop in an oligotrophic pelagic marine ecosystem: Possible roles of cyanobacgeruia and nanoflagellates in the organic fluxes. Mar. Ecol. Prog. Ser. 49: 171-187.

Hamilton, D. T., and W. D. Taylor. 1987. Short-Term effects of zooplankton manipulation on phosphorus uptake. Can. J. Fish. Aquat. Sci. 4: 1038-1044.

Harris, G. P. 1986. The measurement of productivity and growth rates. In: Phytoplankton Ecology: structure, function and fluctuation. Chapman and Hall N. Y. pp. 112-136.

Harrison, W. G. 1983. Uptake and recycling of soluble reactive phosphorus by marine microplankton. Mar. Ecol. Prog. Ser.10: 127-135.

Hayward, D., L. W. Haas, J. D. Boon, K. L. Webb and K. D. Friedland. 1986. Empirical models of stratification variations in the York River Estuary, Virginia, USA. In: J. Bowman, M. Yentsch, and W. T.Peterson (eds.), Tidal mixing and plankton dynamics. Lecture notes on Coastal and Estuarine Studies, Vol. 17. Springer-Verlag. Berlin. pp. 346-367.

Hilmer, T. and G. C. Bate. 1989. Filter types, filtration and post-filtration treatment in phytoplankton production studies. J. Plankton Res. 11(1): 49-63.

Horrigan S. G., A. Hagstrom, I. Koiko and F. Azam. 1988. Inorganic nitrogen utilization by assembleages of marine bacteria in seawater culture. vol. 50 pp. 147-150.

Howarth, H. W. 1988. Nutrient limitation of net primary production in marine ecosystems. Ann. Rev. Ecol. 19: 89-110.

Jeffrey, S. W. and G. F. Humphrey. 1975. New Spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. 167: 191-194.

Johannes, R. E. 1965. Influience of marine protozoa on nutrient regeneration. Limnol. Oceanogr. 10: 201-214.

Jones, J. G., B. M. Simon and C. R. Cunningham. 1983. Bacterial uptake of algal extracellular products: An experimental approach. J. of Appl. Bacteriol. 54: 355-365.

Jonge, V. N. 1980. Fluctuations in the organic carbon to chlorophyll a ratios for estuarine benthic diatom populations. Mar. Ecol. Prog. Ser. 2: 345-353.

Kemp, W. M. and W. R. Boynton. 1984. Spatial and temporal coupling of nutrient inputs to estuarine primary production: The role of particulate transport and decomposition. Bull. Mar. Sci. 35: 522-535.

King, F. D. 1987. The nitrogen recycling efficiency in steady-state oceanic environments. Deep-Sea Res. 34(5/6): 843-856.

Koepfler, E. T. 1989. Heterotrophic bacterial production as measured by the tritiated thymidine incorporation method: Relationships to biological and abiological factors in estuarine environments. Dissertation presented to the School of Marine Science, The College of William and Mary in Virginia. pp. 1-136.

Kuosa, K. and K. Kivi. 1989. Bacteria and heterotrophic flagellates in the pelagic carbon cycle in the north Baltic Sea. Mar. Ecol. Prog. Ser. 53: 93-100.

Laake, M., A. B. Dahle, K. Eberlein and K. Rein. 1983. A modelling approach to the interplay of carbohydrates, bacteria and non-pigmented flagellates in a controlled ecosystem experiment with Skeletonema costatum. Mar. Ecol. Prog. Ser. 14: 71-79.

Lancelot, C. and G. Billen. 1985. Carbon-nitrogen relationships in nutrient metabolism of coastal marine ecosystems. In: H. W. Jannasch and P. T. LeB. Williams (eds.), Adv. Aquat. Microbiol. 3: 263-321.

Larsson, U. and A. Hagstrom. 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. Mar. Biol. 52: 199-206.

Laws, E. A. and J. W. Archie. 1981. Appropriate use of regression ananlysis in marine biology. Mar. Biol. 65: 13-16.

Laws, E. A. and D. G. Redalje. 1979. Effect of sewage enrichment on the phytoplankton population of a subtropical estuary. Pac. Sci. 33: 129-144.

Lean, D. R. S. and C. Nalewajko. 1979. Phosphorus turnover time and phosphorus demand in large and small lakes. Arch. Hydrobiol. Beih Ergebn Limnol. 13: 120-152.

Leffler, J. W. 1980. Microcosmology: Theoretical applications of biological models. In: J. P. Giesy (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, CONF-781101, National Technical Information Service, Springfield, Va. pp. 14-29.

Li, W. K. W., D. V. Subba Rao, W. G. Harrision, J. C. Smith, J. J. Cullen, B. Irwin and T. Platt. 1983. Autotrophic piciplankton in the tropical ocean. Science 219: 292-295.

Li, W. K. W. 1986. Experimental Approaches to field measurements: Methods and interpretation. In: Photosynthetic Picoplankton. T. Platt and W. K. W. Li. Can. Bull. Fish. Aquat. Sci. 214: 251-286.

Lung, W. S. 1986a. Assessing phosphorus control in the James River basin. J. Environ. Engr. 112: 44-60.

Lung, W. S. 1986b. Phosphorus loads to the Chesapeake Bay: A perspective. J. Water Pollut. Control Fed. 58: 749-756.

Maestrini, S. Y., D. J. Bonin, and M. R. Droop. 1985a. Phytoplankton as indicators of sea water quality: Bioassay approach and protocols. In: L. E. Shubert (ed.), Algae as Ecological Indicators. Academic Press, New York. pp. 71-132.

Maestrini, S. Y., D. J. Bonin, and M. R. Droop. 1985b. Test algae as indicators of sea water quality: Prospects. In: L. E. Shubert (ed.), Algae as Ecological Indicators. Academic Press, New York. pp. 133-188.

Malone, T. C., W. M. Kemp, H. W. Ducklow, W. R. Boynton, J. H. Tuttle and R. B. Jonas. 1986. Lateral variation in the production and fate of phytoplankton in a partially stratified estuary. Mar. Ecol. Prog. Ser. 32: 149-160.

Marita, R. Y. 1986. Autecological studies and marine ecosystems. In: R. L. Tate,III (ed.), Microbial Autecology: A Method for Envioronmental Studies. John Wiley and Sons, Inc. pp. 147-181.

Martin, J. H., G. A. Knauer, D. M. Karl and W. W. Broenkow. 1987. VERTEX: Carbon cycling in the northeast Pacific. Deep-Sea Res. 34(2): 267-285.

Officer, C. B., R. B. Biggs, J. L. Taft, L. E. Craonin, M. A. Tyler and W. R. Boynton. 1984. Chesapeake Bay anoxia: Origin, development, and significance. Science 223: 22-27.

Parslow J. S., G. J. Doucette, F. J. R. Taylor and P. J. Harrison. 1986. Feeding by the zooflagellate Pseudobodo sp. on the picoplankton prasinomonad Micromonas pusilla. Mar. Ecol. Prog. Ser. 29: 237-246.

Parsons, T. R., L. J. Albright, F. Whitney, C. S. Wong, and P. J. Williams. 1981. The effect of glucose on the productivity of seawater: An experimental approach using controlled aquatic ecosystems. Environ. Res. 4: 229-242.

Parsons, T. R., M. Takahashi, B. Hargrave. 1984. Chemical composition. In; Biological Oceanography Processes. Pergamon Press NY. pp.48-60.

Parsons, T. R., Y. Maiita, and C. M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press. pp. 1-173.

Patten, B. C. 1971. Primer for ecological modeling and simulation with analog and digital computers. In: B. C. Patten (ed.), Systems Analysis and Simulation in Ecology, v.I., Academic Press, New York. pp. 3-121.

Pengerud, B. E. F. Skjoldal and T. F. Thingstad. 1987. The reciprocal interaction between degradation of glucose and ecosystem structure. Studies in mixed chemostat cultures of marine bacteria, algae, and bacterivorous nanoflagellates. Mar. Ecol. Prog. Ser. 35: 111-117.

Perkin-Elmer. 1978. Instruction manual. 240B Elemental Analyzer 1 993-9379. Norwalk Conn.

Perry, M. G. 1972. Alkaline phosphatase activity in subtropical Central North Pacific waters using a sensitive fluorometric method. Mar. Biol. 15: 113.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. Bioscience. 24: 499-504.

Pomeroy, L. R. 1988. Energetics of microbial food webs. Hydrobiologia. 159: 7-18.

Ray, R. T. 1986. The role of picoplankton in phytoplankton dynamics of a temperate coastal plain estuary. Thesis. Presented to the School of Marine Science. The College of William and Mary in Williamsburg, Virginia. pp. 1-84.

Raven, J. A. and J. Beardall 1981. Respiration and photorespiration. Platt [ed] Physiological bases of phytoplankton ecology. Can. Bull. Fish. Aquatic. Sci. 210 pp. 55-82.

Redfield, R. C. 1958. The biological control of chemical factors in the environment. Am. Sci. 46: 205-221.

Roman, M. R., H. W. Ducklow, J. A. Furman, C. Garside, P. M. Glibert, T. C. Malone and G. B. McManus. 1988. Production , consumption and nutient cycling in a laboratory mesocosm. Mar. Ecol. Prog. Ser. 42: 39-52.

Romanenko, V. I., J. Overbeck and Y. I. Sorokin. 1972. Estimation of production of heterotrophic bacteria using ^{14}C. In: Y.I. Sorokin and H. Kadota, (eds.), Techniques for the Assesment of Microbial Production and Deposition in Fresh Waters, Handbook No. 23 pp. 82-85.

Sanders, J. G., S. J. Cibik, C. F. D'Elia and W. R. Boynton. 1987. Nutrient enrichment studies in a coastal plain estuary.: Changes in phytoplankton species composition. Can. J. Fish. Aquat. Sci. 44: 83-90

Schelske, C. L. 1984. In situ and natural phytoplankton assemblages bioassays. In: L. E. Shubert (ed.), Algae as Ecological Indicators. Academic Press, New York. pp. 15-47.

Sherr, B. F., E. B. Sherr and C. S. Hopkinson. 1988. Trophic interactions within pelagic microbial communities: Indications of feedback regulation of carbon flow. Hydrobiologia 159: 19-26.

Sherr, B. F., E. B. Sherr, T. L. Andrew, R. D. Fallom and S. Y. Newell. 1986. Tropic interactions between heterotrophic protozoa and bacterioplankton in estuarine water analyzed with selective metabolic inhibitors. Mar. Ecol. Prog. Ser. 32: 169-179.

Sherr, E. B., B. F. Sherr and G. A. Paffenhofer. 1986. Phagotrophic protozoa as food for metazoan: A "missing" trophic link in marine pelagic food webs? Marine Microbial Food Webs 1(2): 61-81.

Sieburth, J. and K. W. Estep. 1985. Precise and meaningful terminology in marine microbial ecology. Marine Microbial Food Webs 1: 1-16.

Sieracki, M. E., L. W. Haas, D. A. Caron and E. J. Lessard. 1987. Effect of fixation on particle retention by microflagellates: Underestimation of grazing rates. Mar. Ecol. Prog. Ser. 38: 251-258.

Smith, R. E. and T. Platt. 1984. Carbon exchange and ^{14}C tracer methods in a nitrogen - limited diatom, Thalassiosira pseudonana. Mar. Ecol. Prog. Ser. 16: 75-87.

Smith, S. V. and F. T. Mackenzie. 1987. The ocean as a net heterotrophic system: Implications from the carbon biochemical cycle. Global Biochem. Cycles 1(3): 187-198.

Sommer, U. 1986. Phytoplankton competition along a gradient of dilution rates. Oecologia (Berlin) 68: 503-506.

Sommer, U. 1988. Phytoplankton succession in microcosm experiments under simultaneous grazing pressure and resource limitation. Limnol. Oceanogr. 33(5): 1037-1054.

Sommer, U. 1989. Nutrient status and nutrient competition if phytoplankton in a shallow, hypertrophic lake. limnol. Oceanogr. 34(7) : 1162-1173.

Spies, A. and T. R. Parsons. 1985. Estuarine microplankton: An experimental approach in combination with field studies. J. Exp. Mar. Biol. Ecol. 92: 63-81.

Steele, J. H. and I. E. Baird. 1961. Relations between primary production, chlorophyll and particulate carbon. Limnol. Oceanogr. 6: 68-78.

Steele, J. H. and I. E. Baird. 1962. Carbon-chlorophyll relations in cultures. Limnol. Oceanogr. 7: 101-102.

Stewart, A. J. and R. G. Wetzel. 1986. Cryptophytes and other microflagellates as couplers in planktonic community dynamics. Arch. Hydrobiol. 106: 1-19.

Suttle, C. A., A. M. Chan, W. D. Taylor and P. J. Harrison. 1986. Grazing of planktonic diatoms by microflagellates. J. Plankton Res. 8: 393-398.

Suttle, C. A. and P. J. Harrison. 1988. Ammonium and phosphate yptake rates, N:P supply ratuis, and evidence for N and P limitation in some oligotrophic lakes. Limnol. Oceangr. 33(2): 186-202.

Syrett, P. J. 1981. Nitrogen metabolism of microalgae. In: Physiological bases of phytoplankton ecology. Can. Bull. Fish and Aquat. 210, pp.182-210

Taft, J. L., E. O. Hartwig and R. Loftus. 1980. Seasonal oxygen depletion in Chesapeake Bay. Estuaries 3: 242-247.

Technicon Industrieal Systems. 1976. Technicon AutoAnalzer 11 Industrial Method No. 102-70W/B, Nitrite in Water and Wastewater.

Technicon Industrial Systems. 1972. Technicon AutoAnalzer 11 Industrial Method No. 158-71W, Nitrate and Nitrite in Water and Wastewater.

Thingstad, T. F. and B. Pengerud. 1985. Fate and effect of allochthonous organic material in aquatic microbial ecosystems, an analysis based on chemical theory. Mar. Ecol. Prog. Ser. 21: 47-62.

Tilman, D. 1982. Resource competition and community structure. Princeton.

U.S. EPA. 1974. Methods for chemical analysis of water and wastes. pp. 168-174.

Vadstein, O., and A. Jensen. 1988. Growth and phosphorus status of limnetic phytoplankton and bacteria. Limnol. Oceanogr. 33(4): 489-503.

Vargo, G. A. and E. Shanley. 1985. Alkaline phosphatase activity in the red-tide dinoflagellate Ptychodiscus brevis. Mar. Ecol. 6(3): 251-264.

Verity, P. G. 1987. Factors driving changes in the pelagic trophic structure of estuaries with implications for the Chesapeake Bay. In: Perpectives on the Chesapeake Bay: Recent Advances in Estuarine Science EPA/CBP/TRS. pp.35-56.

Virginia Water Control Board. 1987. Tributary water quality 1986 characterization report. Chesapeake Bay Office, Richmond, VA. pp 1-101.

Ward, B. B. 1984. Photosynthesis and bacterial utilization of phytoplankton exudates: Results from pre- and post-incubation size fractionation. Oceanol. Acta.7(3): 337-343.

Waterbury, J. B., S. W. Watson, F. W. Valois and D. G. Franks. 1986. Biological and ecological characterization of the marine unicellular cyanobacteria Synechococcus. In: Photosynthetic picoplankton. Can. Bull. Fish. Aquat. Sci. 214: 71-120.

Webb, K. L. and P. M. Eldridge. 1988. Nutrient limitation in a coastal plain estuary: Seasonal and salinity effects. Presented at AGU Meeting New Orleans, LA. January. Eos. 68(50): 12A-05.

Webb, K. L. 1988. Comment on "Nutrient limitation of phytoplankton growth in brackish coastal ponds" by Caraco, Tamse, Boutros and Valiela (1987). Can. J. Fish. Aquat. Sci. 45(2): 380-381.

Weger, H G. 1989. Respiratory losses in the lioght in a marine diatom: Measurements by short-term mass spectrometer. Limnol. Oceanogr. 34(7): 1153-1161.

Wheeler, P. A. and D. L. Kirchman. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol. Oceanogr. 31(5): 998-1009.

Williams, P. J. LeB. 1984. Bacterial production in the marine food chain: The emperor's new suit of clothes? In: M. J. R. Fasham (ed.), Flows of Energy and Marine Ecosystems. Plenum Press, New York. pp. 271-300.

Winberg, G. G. 1968. Methods for the estimates of production of aquatic animals. (Trans. by Duncan, 1971). Adv. Ecol. Res. Academic Press, London-New York pp. 1-175.

Zar, J. H. 1984. Biostatistical analysis. Prentice-Hall international, Inc, London. pp.1-717.

Zevenboom, W. 1986. Ecophysiology of Nutrient uptake, Photosynthesis and Growth. In: T. Platt and W. K. Li (eds.), Photosynthetic Picoplankton. Can. Bull. Fish. Aquat. Sci. pp. 214-583.

Appendix A

Experimental design and procedures for chlorophyll and nutrient analysis

Appendix A

1. Analysis of particulate carbon and nitrogen and phosphorus.

Duplicate samples for particulate nitrogen, phosphorus and carbon analysis were filtered in 100 to 200 ml aliquots onto 25 mm Whatman GF/F filters and stored frozen .

1.1 A Model 240B Elemental Analyzer (Perkin-Elmer 1978) was used to determine carbon and nitrogen on the filters.

1.2 Samples for particulate phosphorus were analyzed using an Orion Autoanalyzer. Filters and sample were combusted at 550 °C and placed in 1.0 N hydrochloric acid. Ammonium molybdate and potassium tartrate react in acid to form an antimony-phospho-molybdate complex. This in reduced to a blue-colored complex by ascorbic acid. Detection limit of this procedure is 0.001 $mg\text{-}l^{-1}$.

2. Analysis of dissolved carbon and nitrogen and phosphorus.

2.1 Carbonate alkalinity determination: A 100 ml. aliquot of seawater was mixed with an aliquot of standard acid. The pH before and after the treatment was used to calculate the total alkalinity and the carbonate alkalinity (Parsons et al.1984).

2.2 Samples for dissolved inorganic nitrogen and phosphorus nutrients were filtered through 47 mm GF/F filters and frozen for later analysis with a Technicon Autoanalyzer (D'Elia et al. 1983). Every fifth sample was replicated to determine the precision of the technique.

2.2.1 Orthophosphorus (Ascorbic acid method)

Ammonium molybdate and antimony potassium tartrate react with orthophosphate in an acid H_2SO_4 environment to form an antimony-phosphmolybdate complex which is reduced to a blue-colored complex by

ascorbic acid (Technicon Industrial Systems 1976). Detection limit of this procedure is 0.001 mg-l^{-1}.

2.2.2 Nitrate and nitrite (NO_3 + NO_2) diazotization method

Nitrate is reduced to nitrite in a copper-cadium reduction column. The nitrite ion the reacts with sulfanilamide under acid conditions to form a diazo compound. This compound is complexed with N-napthylethylenediamine dihydrochoride to form a reddish-purple azo dye (Parsons et al. 1984). The detection limit of this procedure is 0.001 mg-l^{-1}

2.2.3 Nitrite (NO_2)

This procedure is the same as the diazotization method 2.2.2 except the sample is not reduced in a copper-cadium column (Technicon Industrial Systems 1972). The detection limit of this procedure is 0.001 mg-l^{-1}

2.2.4 Filtered Ammonium (NH_4^+)

Alkaline phenol and hypochlorite react with ammonium to form indophenol blue that is proportional to the ammonium concentration. The blue color formed is intensified with sodium nitroprusside (U.S. EPA). Detection limit of technique is 0.013 mg-l^{-1}.

3 Chlorophyll A determinations.

Chlorophyll a concentration was measured by filtering 5 ml of sample through a Whatman GF/F filter (25mm) which was immediately placed in a light -proof, capped vial containing 8 ml of 45 percent dimethyl sulfoxide, 45 percent acetone, 10 percent distilled water and 0.1 percent diethylamine (Webb and Hayward, unpublished). Samples were extracted in the dark overnight at room temperature. Chlorophyll was determined on a Turner Designs fluormeter, calibrated with a Cary 15 scanning

spectrometer using a chorophyll a standard (Spinach, Sigma Scientific Co.) and the trichromatic equations of Jeffrey and Humphry (1975).

4 Epifluorescence microscope counting technique.

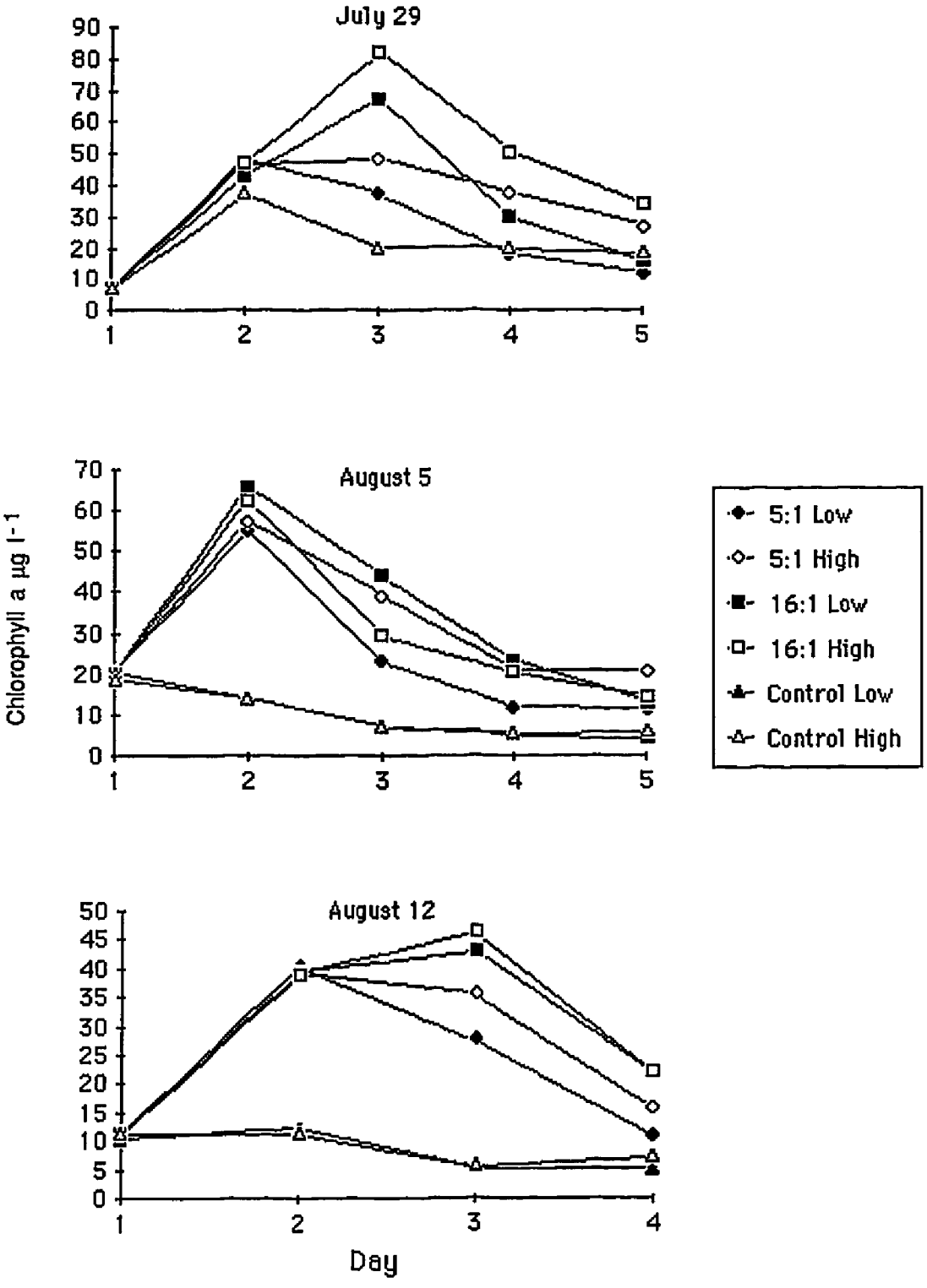
Two ml of sample was collected by micropipet from either 5 cm below the surface of each microcosm or from the center of an appropriately size fractionated sample. They were dispensed into 5 ml test tubes containing 100 µl of 6 percent gluteraldehyde. Proflavin (0.00132 percent w/v, final concentration) and DAPI was added 5 minutes before the sample is filtered onto black stained 0.2 µm Nuclepore™ filters (Haas 1983). Slides were examined under a Zeiss Standard epifluorescence microscope equipped with a Neofluar 63/1.4 oil immersion objective and a Mercury AC 50W light source. A blue and a green light filter system is used for activation light (band pass 450-490 nm and <566 nm filters respectively). The light subsequently passes through an FT 510 chromatic beam splitter and then either through a long pass 520 barrier filter, or through a long pass 590 barrier filter. Bacteria was counted under ultraviolet excitation. Heterotrophic eucaryotes, diatoms and phytoflagellates were counted under blue excitation. Identifications were made to genus level where possible within these major categories. Counts of phycoerythrin-containing cyanobacteria (genus Synechococcus) were made under the green and blue light filter systems. Cell dimensions of randomly selected cells, usually 30 to 40 cells of each species, were measured by calibrated ocular micrometer for each experiment. Photosynthetic and non photosynthetic area was determined by image analysis or by standard microscopic techniques. An average dimension in each measurement plane was determined and the volume of an equivalent

solid for photosynthetic and non photosynthetic components were calculated using standard geometric formulas.

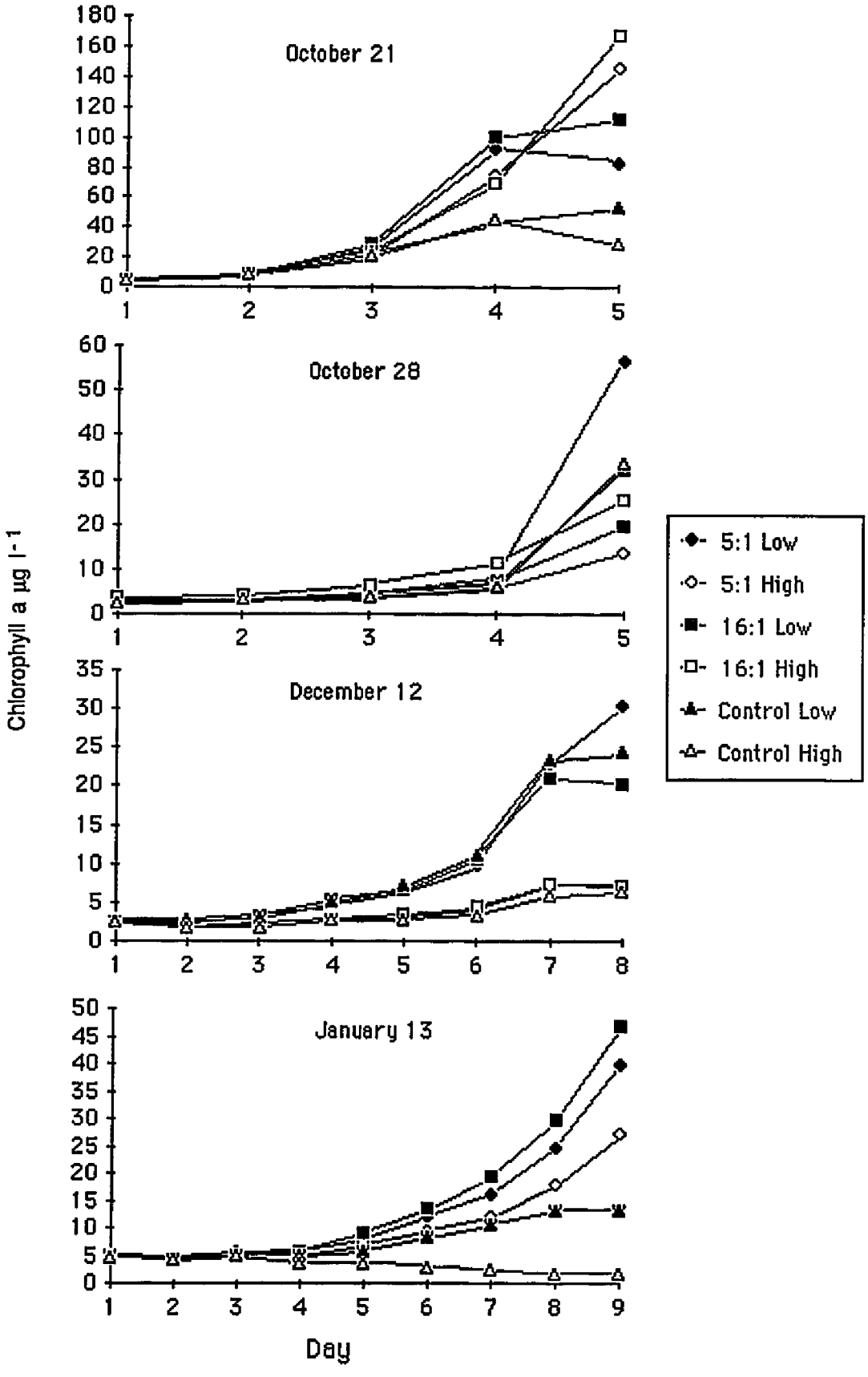
Appendix B

Time series Figures of chlorophyll (a), for all experiments, from inoculation of the microcosms until tracer incubations were begun.

Appendix B-1


July 29
August 5
August 12
Chlorophyll a µg l-1
Day
5:1 Low
5:1 High
16:1 Low
16:1 High
Control Low
Control High

Appendix B-2


October 21
October 28
December 12
January 13
Chlorophyll a µg l-1
Day
5:1 Low
5:1 High
16:1 Low
16:1 High
Control Low
Control High

Appendix C

Physical, chemical, and biological data from seven microcosm experiments during 1988 and 1989

1 Jul 29
2 Aug 5
3 Aug 11
4 Oct 21
5 Oct 28
6 Dec 12
7 Jan 13

Treatment	dil	set	temp	Oxygen	sal.
'5:1	low	1	27.0	7.06	21
'5:1	high	1	27.0	7.32	21
'16:1	low	1	27.0	7.27	21
'16:1	high	1	27.0	7.65	21
Control	low	1	27.0	7.34	21
Control	high	1	27.0	6.63	21
'5:1	low	2	29.5	7.25	24.5
'5:1	high	2	29.5	749	24.5
'16:1	low	2	29.5	6.26	24.5
'16:1	high	2	29.5	6.73	24.5
Control	low	2	29.5	6.69	24.5
Control	high	2	29.5	6.71	24.5
'5:1	low	3	30.0	7.06	22
'5:1	high	3	30.0	6.89	22
'16:1	low	3	30.0	7.14	22
'16:1	high	3	30.0	6.46	22
Control	low	3	30.0	7.22	22
Control	high	3	30.0	6.89	22
'5:1	low	4	15.5	8.9	23
'5:1	high	4	15.0	8.18	23.5
'16:1	low	4	15.5	* *	23
'16:1	high	4	15.0	9.52	23.5
Control	low	4	15.0	8.51	23.5
Control	high	4	15.0	9.56	23.5
'5:1	low	5	15.0	9.28	23.5
'5:1	high	5	15.0	9.32	24
'16:1	low	5	15.0	9.1	24
'16:1	high	5	15.0	9.06	24
Control	low	5	15.0	9.08	23.5
Control	high	5	15.0	9.28	23.5
'5:1	low	6	10.0	10.68	20
'5:1	high	6	10.0	5.47	23
'16:1	low	6	10.0	5.75	23
'16:1	high	6	10.0	11.19	23
Control	low	6	10.0	11.02	20
Control	high	6	10.0	10.6	20
'5:1	low	7	7.0	10.25	20
'5:1	high	7	6.5	8.84	20
'16:1	low	7	7.0	10.15	20
'16:1	high	7	7.0	10.19	20
Control *	low	7	6.5	10.74	20
Control *	high	7	6.5	10.09	20
			Deg Centigrade	Mg/l	ppt
				** no sample	

Treatment	pH	Alk	NO2+NO3	NH3	DIP µg/l
'5:1	8.55	1.16	3	1	49
'5:1	8.58	1.34	3	4	49
'16:1	8.59	1.15	3	2	2
'16:1	8.6	1.21	3	1	2
Control	8.54	1.33	3	1	1
Control	8.51	1.37	3	4	1
'5:1	8.48	1.83	3	6	16
'5:1	8.48	1.85	3	4	104
'16:1	8.86	1.34	3	5	4
'16:1	8.86	1.34	3	6	3
Control	8.41	1.72	3	4	2
Control	8.41	1.69	3	4	2
'5:1	8.58	1.57	6	1	57
'5:1	8.93	1.58	4	2	90
'16:1	8.88	1.47	6	2	3
'16:1	8.57	1.5	4	27	2
Control	8.23	1.77	3	3	2
Control	8.4	1.8	14	4	2
'5:1	8.38	1.8	5	1	31
'5:1	8.33	1.72	6	0	3
'16:1	8.26	1.82	8	9	2
'16:1	8.53	1.69	43	0	3
Control	8.31	1.8	1	0	2
Control	8.64	1.7	2	4	2
'5:1	8.17	1.94	119	33	115
'5:1	8.17	1.91	152	365	156
'16:1	8.17	1.96	143	583	83
'16:1	8.11	1.93	157	519	61
Control	8.01	2.01	5	35	2
Control	8.12	1.95	4	0	2
'5:1	8.1	2.08	111	123	103
'5:1	8.05	2	105	523	161
'16:1	8.08	1.92	108	423	67
'16:1	8.07	1.97	124	567	85
Control	9.08	2.09	46	14	1
Control	8.09	2.08	139	44	8
'5:1	7.88	2.05	75	190	113
'5:1	7.83	2.01	58	269	139
'16:1	7.88	2.02	67	403	46
'16:1	7.89	2.01	57	476	59
Control	7.89	1.94	63	14	1
Control	7.9	1.97	43	22	1
		Meq/l	ug/l	ug/l	ug/l

Treatment	P<1 Chl	P<1 C	P<1 N	P<1 P	WP Chl
'5:1	0.33	48.48	8.51	0.68	11.73
'5:1	0.29	48.03	7.22	0.23	27.04
'16:1	0.34	40.77	7.54	0.52	15.76
'16:1	0.29	74.02	11.10	0.42	33.92
Control	0.28	42.88	3.81	0.16	9.70
Control	0.98	59.98	10.91	0.23	18.48
'5:1	0.45	22.60	1.58	0.19	11.20
'5:1	0.51	59.93	6.97	0.26	20.48
'16:1	0.79	38.18	4.62	0.29	12.99
'16:1	0.94	51.26	6.19	0.26	14.56
Control	0.40	16.15	1.03	0.16	4.24
Control	0.26	23.22	4.16	0.13	6.07
'5:1	0.34	17.56	2.18	0.16	11.22
'5:1	0.51	21.28	2.37	0.19	15.84
'16:1	0.77	28.10	5.08	0.19	22.16
'16:1	0.44	21.41	3.52	0.16	22.24
Control	0.26	11.68	1.86	0.13	5.19
Control	0.49	12.51	2.96	0.16	7.36
'5:1	0.98	35.43	4.78	0.29	88.16
'5:1	0.71	24.95	2.52	0.19	145.60
'16:1	0.72	46.40	6.35	0.35	112.64
'16:1	0.86	19.49	1.88	0.19	167.20
Control	1.26	25.44	2.08	0.19	52.80
Control	0.68	21.74	2.54	0.19	28.56
'5:1	0.53	16.26	1.50	0.16	56.64
'5:1	0.49	41.23	1.99	0.13	13.59
'16:1	0.66	18.78	1.68	0.13	19.76
'16:1	0.52	15.83	1.01	0.13	25.44
Control	0.51	14.82	4.27	0.35	32.32
Control	0.46	20.75	1.18	0.13	33.44
'5:1	0.29	13.84	2.11	0.13	30.48
'5:1	0.43	12.41	2.33	0.10	7.28
'16:1	0.67	14.07	2.21	0.13	20.97
'16:1	0.30	13.84	2.55	0.13	7.84
Control	0.40	12.35	1.47	0.10	24.32
Control	0.24	6.94	1.40	0.06	6.53
'5:1	0.31	11.74	3.16	0.13	24.80
'5:1	0.36	8.49	2.17	0.19	27.12
'16:1	0.44	11.80	2.20	0.13	29.84
'16:1	0.29	7.43	2.08	0.13	13.19
Control	0.26	7.30	1.19	0.10	3.04
Control	0.19	5.48	1.59	0.10	1.59
	ug/l	µmol/l	µmol/l	µmol/l	µg/l

Treatment	WP-C	WP-N	WP-P	P<1C/P ratio	P WC/P ratio
'5:1	203.08	26.00	0.97	71.56	209.85
'5:1	341.06	32.46	1.65	212.71	207.31
'16:1	216.68	29.46	0.77	78.99	279.88
'16:1	378.47	41.27	0.65	176.52	586.63
Control	131.19	12.76	1.16	265.88	112.97
Control	247.45	25.86	0.35	265.62	697.36
'5:1	102.39	12.95	0.94	116.75	109.46
'5:1	155.43	27.08	1.65	232.23	94.48
'16:1	179.76	26.11	1.77	131.52	101.32
'16:1	299.59	30.72	1.65	198.64	182.10
Control	81.39	7.20	0.65	100.10	126.15
Control	117.40	11.80	0.55	179.95	214.08
'5:1	133.72	17.55	2.48	108.90	53.84
'5:1	116.09	19.07	0.84	109.92	138.42
'16:1	217.73	30.95	0.81	145.17	269.99
'16:1	190.80	29.15	1.84	132.75	103.77
Control	68.11	9.68	0.55	90.48	124.20
Control	92.72	10.65	0.52	77.57	179.64
'5:1	537.41	35.87	2.87	122.05	187.19
'5:1	442.10	47.82	2.35	128.93	187.74
'16:1	704.39	41.47	1.55	130.77	454.92
'16:1	402.38	54.09	2.00	100.69	201.19
Control	261.13	20.34	0.74	131.46	351.95
Control	149.96	12.19	0.42	112.31	357.61
'5:1	138.92	29.07	2.87	100.79	48.39
'5:1	82.66	12.08	2.35	319.55	35.10
'16:1	73.61	11.03	1.55	145.56	47.54
'16:1	59.88	7.28	2.00	122.68	29.94
Control	105.74	15.11	0.42	41.78	252.16
Control	89.66	13.94	0.65	160.85	138.97
'5:1	103.78	19.64	0.65	107.24	160.86
'5:1	47.71	6.69	0.42	128.28	113.78
'16:1	80.34	12.52	0.81	109.05	99.62
'16:1	47.03	6.41	0.39	107.24	121.50
Control	107.43	14.74	0.39	127.61	277.52
Control	35.39	4.14	0.19	107.59	182.84
'5:1	84.76	12.36	0.97	90.95	87.58
'5:1	69.74	12.08	0.81	43.86	86.47
'16:1	84.64	13.42	0.97	91.46	87.46
'16:1	50.02	7.97	0.52	57.57	96.92
Control	43.15	5.75	0.23	75.41	191.09
Control	29.01	7.55	0.10	56.61	299.79
	µmol/l	µmol/l	µmol/l		

Treatment	P<1C/N	PWC/N	P<1 N/P	PW N/P	C/Chlweight
'5:1	5.70	7.81	12.60	26.90	208
'5:1	6.65	10.51	32.00	19.70	151
'16:1	5.40	7.35	14.60	38.10	165
'16:1	6.67	9.17	26.50	64.00	134
Control	11.27	10.28	23.60	11.00	162
Control	5.50	9.57	48.30	72.90	161
'5:1	14.27	7.91	8.20	13.80	110
'5:1	8.60	5.74	27.00	16.50	91
'16:1	8.26	6.88	15.90	14.70	166
'16:1	8.29	9.75	24.00	18.70	247
Control	15.65	11.31	6.40	11.20	230
Control	5.58	9.95	32.20	21.50	232
'5:1	8.05	7.62	13.50	7.10	143
'5:1	8.99	6.09	12.20	22.70	88
'16:1	5.53	7.04	26.30	38.40	118
'16:1	6.09	6.55	21.80	15.90	103
Control	6.28	7.03	14.40	17.70	158
Control	4.22	8.71	18.40	20.60	151
'5:1	7.41	14.98	16.50	12.50	73
'5:1	9.90	9.24	13.00	20.30	36
'16:1	7.31	16.99	17.90	26.80	75
'16:1	10.38	7.44	9.70	27.00	29
Control	12.21	12.84	10.80	27.40	59
Control	8.55	12.30	13.10	29.10	63
'5:1	10.84	4.78	9.30	10.10	29
'5:1	20.73	6.84	15.40	5.10	73
'16:1	11.15	6.67	13.10	7.10	45
'16:1	15.61	8.22	7.90	3.60	28
Control	3.47	7.00	12.00	36.00	39
Control	17.59	6.43	9.10	21.60	32
'5:1	6.55	5.28	16.40	30.40	41
'5:1	5.33	7.14	24.10	15.90	79
'16:1	6.38	6.42	17.10	15.50	46
'16:1	5.44	7.34	19.70	16.60	72
Control	8.42	7.29	15.10	38.10	53
Control	4.94	8.54	21.80	21.40	65
'5:1	3.72	6.86	24.50	12.80	41
'5:1	3.91	5.77	11.20	15.00	31
'16:1	5.36	6.31	17.10	13.90	34
'16:1	3.57	6.28	16.10	15.40	46
Control	6.15	7.50	12.30	25.50	171
Control	3.45	3.84	16.40	78.10	219

Treatment	Cpre	CW	Cpost	Ppre	PW
'5:1	0.285	4.740	0.455	0.019	*0.072
'5:1	0.363	5.600	0.948	0.018	*0.067
'16:1	2.629	9.829	0.717	0.007	0.015
'16:1	2.985	12.882	1.588	0.014	0.017
Control	0.606	14.591	1.312	0.005	0.034
Control	3.423	13.662	2.725	0.010	0.016
'5:1	2.476	7.782	0.706	0.010	0.016
'5:1	0.790	11.395	0.770	0.015	0.039
'16:1	6.280	12.689	1.584	0.012	0.016
'16:1	6.660	12.447	1.952	0.015	0.019
Control	6.983	7.113	1.314	0.006	0.007
Control	9.428	19.641	2.096	0.009	0.013
'5:1	0.360	5.654	0.250	0.012	0.022
'5:1	0.455	6.705	1.817	0.012	0.037
'16:1	4.572	9.834	1.380	0.021	0.036
'16:1	3.248	15.440	1.982	0.010	0.018
Control	2.353	7.856	0.616	0.006	0.010
Control	1.251	6.197	0.997	0.002	0.008
'5:1	0.390	11.254	0.553	0.006	0.011
'5:1	0.078	29.111	0.572	0.008	0.044
'16:1	1.643	24.297	1.611	0.041	0.030
'16:1	3.110	40.050	1.208	0.045	0.032
Control	2.019	13.678	1.133	0.028	0.025
Control	1.298	10.592	0.695	0.015	0.023
'5:1	0.327	24.664	1.076	0.007	0.115
'5:1	0.276	4.511	0.374	0.007	0.027
'16:1	0.261	6.666	0.415	0.004	0.027
'16:1	0.442	11.384	0.455	0.004	0.043
Control	5.955	19.399	3.558	0.013	0.032
Control	3.348	18.862	2.197	0.013	0.036
'5:1	0.169	6.438	0.341	0.009	0.038
'5:1	0.253	1.522	0.282	0.010	0.016
'16:1	0.267	4.331	0.427	0.006	0.024
'16:1	0.156	2.826	0.952	0.012	0.015
Control	0.374	6.929	0.504	0.002	0.021
Control	0.195	1.153	0.281	0.001	0.004
'5:1	0.487	7.211	0.578	0.005	0.031
'5:1	0.889	11.248	0.988	0.005	0.027
'16:1	0.601	16.659	0.890	0.003	0.037
'16:1	0.590	5.560	0.645	0.004	0.015
Control	*15.014	*21.3	*11.84	0.002	0.004
Control	*1.585	*14.273	*4.461	0.001	0.002
	µmol/l-hr	µmol/l-hr	µmol/l-hr	µmol/l-hr	µmol/l-hr
		* high carbon 14 DPM,			*high dark
		was not used in some statistical analyses			uptake

Treatment	Ppost	Lpre-C/P	WL-C/P	Lpost-C/P	μ Post C
'5:1	0.012	15	66	38	0.009
'5:1	0.019	21	83	51	0.020
'16:1	0.001	395	668	623	0.018
'16:1	0.002	210	777	715	0.021
Control	0.005	120	432	241	0.031
Control	0.003	356	853	867	0.045
'5:1	0.003	256	473	230	0.031
'5:1	0.007	52	295	103	0.013
'16:1	0.002	512	788	845	0.041
'16:1	0.002	451	639	851	0.038
Control	0.001	1159	985	939	0.081
Control	0.001	1074	1526	1523	0.090
'5:1	0.005	31	262	47	0.014
'5:1	0.014	38	184	128	0.085
'16:1	0.003	214	274	426	0.049
'16:1	0.002	313	864	944	0.093
Control	0.002	399	778	369	0.053
Control	0.001	777	769	931	0.080
'5:1	0.002	63	1038	224	0.016
'5:1	0.004	9	656	134	0.023
'16:1	0.009	40	817	171	0.035
'16:1	0.003	69	1253	379	0.062
Control	0.005	71	544	240	0.045
Control	0.004	87	453	193	0.032
'5:1	0.007	48	215	144	0.066
'5:1	0.007	39	168	57	0.009
'16:1	0.003	65	251	122	0.022
'16:1	0.003	102	264	134	0.029
Control	0.005	471	615	664	0.240
Control	0.004	265	528	561	0.106
'5:1	0.007	20	168	47	0.025
'5:1	0.009	25	94	30	0.023
'16:1	0.004	47	178	101	0.030
'16:1	0.009	13	187	100	0.069
Control	0.002	181	326	293	0.041
Control	0.001	234	303	426	0.040
'5:1	0.003	108	236	208	0.049
'5:1	0.003	173	418	298	0.116
'16:1	0.003	239	456	308	0.075
'16:1	0.003	168	362	222	0.087
Control	0.002	8269	5560	5083	1.622
Control	0.001	1636	5838	4121	0.814
	μmol/l-hr				1/hr

Treatment	μ Whole C	μ Post P	μ Whole P		
'5:1	0.023	0.018	0.074		
'5:1	0.016	0.082	0.041		
'16:1	0.045	0.002	0.019		
'16:1	0.034	0.005	0.026		
Control	0.111	0.034	0.029		
Control	0.055	0.014	0.045		
'5:1	0.076	0.016	0.018		
'5:1	0.073	0.029	0.023		
'16:1	0.071	0.006	0.009		
'16:1	0.042	0.009	0.012		
Control	0.087	0.009	0.011		
Control	0.167	0.011	0.023		
'5:1	0.042	0.033	0.009		
'5:1	0.058	0.073	0.044		
'16:1	0.045	0.017	0.044		
'16:1	0.081	0.013	0.010		
Control	0.115	0.013	0.018		
Control	0.067	0.007	0.016		
'5:1	0.021	0.009	0.004		
'5:1	0.066	0.022	0.019		
'16:1	0.034	0.027	0.019		
'16:1	0.100	0.016	0.016		
Control	0.052	0.024	0.034		
Control	0.071	0.019	0.056		
'5:1	0.178	0.046	0.040		
'5:1	0.055	0.051	0.011		
'16:1	0.091	0.026	0.017		
'16:1	0.190	0.026	0.022		
Control	0.183	0.015	0.075		
Control	0.210	0.030	0.055		
'5:1	0.062	0.056	0.059		
'5:1	0.032	0.098	0.039		
'16:1	0.054	0.033	0.030		
'16:1	0.060	0.074	0.039		
Control	0.064	0.018	0.055		
Control	0.033	0.010	0.020		
'5:1	0.085	0.022	0.032		
'5:1	0.161	0.017	0.033		
'16:1	0.197	0.022	0.038		
'16:1	0.111	0.023	0.030		
Control	0.494	0.024	0.017		
Control	0.492	0.011	0.025		
	1/hr	1/hr	1/hr		

Treatment	Hnano P remin	<1µ-P remin	W P Remin		
'5:1	0.0702	0.0055	0.0757		
'5:1	0.0965	0.0142	0.1106		
'16:1	0.0387	-0.0079	0.0387		
'16:1	0.0397	-0.0068	0.0397		
Control	0.0492	0.0005	0.0497		
Control	0.0377	-0.0071	0.0377		
'5:1	0.0381	-0.0030	0.0381		
'5:1	0.1459	0.0042	0.1501		
'16:1	0.0350	-0.0102	0.0350		
'16:1	0.0485	-0.0075	0.0485		
Control	0.0085	-0.0117	0.0085		
Control	0.0320	-0.0103	0.0320		
'5:1	0.0527	0.0030	0.0557		
'5:1	* *	-0.0023	* *		
'16:1	0.0403	-0.0063	0.0403		
'16:1	0.0309	-0.0128	0.0309		
Control	0.0139	-0.0051	0.0139		
Control	0.0067	-0.0118	0.0067		
'5:1	0.0258	-0.0021	0.0258		
'5:1	0.0448	-0.0002	0.0448		
'16:1	0.0619	-0.0029	0.0619		
'16:1	0.0828	-0.0088	0.0828		
Control	0.0482	-0.0039	0.0482		
Control	0.0632	-0.0026	0.0632		
'5:1	0.1140	-0.0032	0.1140		
'5:1	0.1006	0.0055	0.1060		
'16:1	0.0088	0.0005	0.0093		
'16:1	0.0585	-0.0003	0.0585		
Control	0.0546	-0.0798	0.0546		
Control	0.0598	-0.0097	0.0598		
'5:1	0.0589	0.0040	0.0629		
'5:1	0.0260	0.0072	0.0332		
'16:1	0.0316	0.0003	0.0319		
'16:1	0.0213	0.0006	0.0219		
Control	0.0294	-0.0022	0.0294		
Control	0.0127	-0.0020	0.0127		
'5:1	0.0525	-0.0036	0.0525		
'5:1	0.1059	-0.0192	0.1059		
'16:1	0.0371	-0.0068	0.0371		
'16:1	0.0334	-0.0083	0.0334		
Control *	0.0058	-0.1547	0.0058		
Control *	0.0039	-0.0777	0.0039		
			**no value		
	µmol-P/l-hr	µm-P/l-hr	µm-P/l-hr		

Vita

PETER M. ELDRIDGE

EDUCATION

BA, Biology/Chemistry, Southampton College, Southampton, N.Y.
MS, Marine Science. Long Island University, Brookville, N. Y.
Thesis: The Diurnal Distribution of the dinoflagellate *Amphidinium sp.* in a water column.
Ph D, Microbial Ecology. College of William and Mary, Virginia Institute of Marine Science. Gloucester Pt., VA.
Dissertation: The effect of nitrogen limitation on phosphorus uptake and remineralization in continuous flow microcosms.

EXPERIENCE 1975-present

Virginia Institute of Marine Science, Gloucester Pt., VA. 1985-Present

Scripps Institute of Oceanography, San Diego,CA. 1982-1985

Lockheed Ocean Science Laboratory, Carlsbad, CA 1977-1982.

Scientist 3, Lawler, Matusky and Skelly Engineers Nyach, NY 1975 -1977

PROFESSIONAL AFFILIATIONS

The American Society of Limnology and Oceanography
Society of Environmental Toxicology and Chemistry.
Task Group on Early Life Stage Toxicity Tests, (ASTM).
American Phycological Society.

HONORS

Best Graduate Student Poster, ASLO-89, Fairbanks, Alaska
Deans list, Southampton College