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Contrasts between Tidal Freshwater and Estuarine Phytoplankton Growth on Intracellular and Recycled Nutrient Pools Over a Summer-Winter Seasonal Transition

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CONTRASTS BETWEEN TIDAL FRESHWATER AND ESTUARINE PHYTOPLANKTON GROWTH ON INTRACELLULAR AND RECYCLED NUTRIENT POOLS OVER A SUMMER-WINTER SEASONAL TRANSITION

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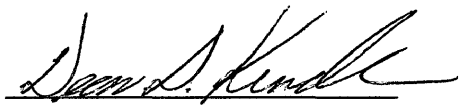

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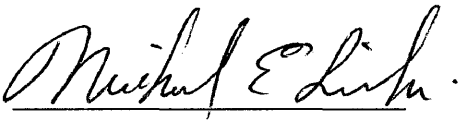

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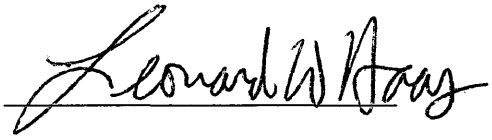

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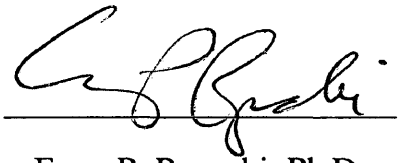

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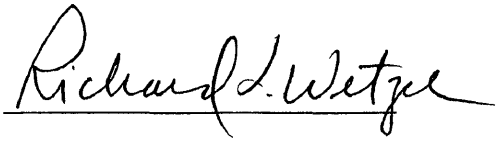

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Abstract

Trends in nutrient limitation and use of intracellular, new and recycled extracellular pools for phytoplankton growth were examined at tidal freshwater and estuarine sites on the York River , a temperate, tidally mixed river of the Chesapeake Bay, during the 1990 summer to winter seasonal transition. Eight twenty-four hour batch culture dilution incubations were selectively nutrient enriched to assess short term phytoplankton growth dependencies on three qualitatively different pools of N and P: 1) intracellular, 2) new extracellular and 3) recycled extracellular. These experiments showed a relaxation of N-limitation at both sites and a shift to P-limitation in the tidal freshwater region over the seasonal transition. The tidal freshwater phytoplankton community shifted from dependency on intracellular pools in the summer to recycled and new extracellular pools in the winter, while the estuarine phytoplankton reduced their reliance on recycled and new extracellular pools and shifted to intracellular pools. Differences between use of N and P pools were small. During late summer and fall, phytoplankton in the tidal freshwater region are N-limited possibly as a result of low ambient ammonium levels and low cellular nitrate reduction. The unexpected dominance of intracellular pools in the tidal freshwater region is likely the consequence of uncoupled nitrate uptake and assimilation plus low grazing pressure on the phytoplankton. By early winter, N-limitation relaxes as river discharge and grazing pressure increase leading to rising ambient levels of ammonium and nitrate. Increased grazing pressure and ammonium levels may have also influenced the switch from intracellular to recycled extracellular pools. By contrast, the estuarine phytoplankton community enjoys a more stable nutrient environment which is highly dependent on the recycled nutrients during the late summer. In the estuary low winter temperatures cause low growth and grazing rates. The reduction in these rates likely cause the switch in growth dependency from recycled to intracellular pools and the relaxation of N-limitation.

Contrasts between tidal freshwater and estuarine phytoplankton growth on intracellular and recycled nutrient pools over a summer-winter seasonal transition.

Introduction

Phytoplankton growth is traditionally considered to be under nitrogen control (N-limited) in marine systems and phosphorus control (P-limited) in freshwater systems (e.g. Hecky and Kilham 1988). This dogma offers little insight into the dynamic regime of an estuary, which is neither completely marine nor fresh (Boynton et al. 1980, Schindler 1981). It remains a subject of debate as to whether, when, and where estuarine phytoplankton become either N or P-limited (Caraco 1988, Webb 1988). Recent studies have demonstrated both N and P-limitation in some estuaries and brackish lakes (D'Elia et al. 1986, Caraco et al. 1987, Webb and Eldridge 1988, Eldridge 1989). Two of these studies (D'Elia et al. 1986; and Webb and Eldridge 1988, Eldridge 1989) found a seasonal pattern of nutrient limitation where P-limitation dominated during the winter, and N-limitation dominated during the summer.

Recent research has demonstrated that phytoplankton communities may utilize N and P for growth from three distinct pools, intracellular, new and recycled extracellular (Demanche et al. 1979, Dortch 1982, Dortch et al. 1984, Dortch et al. 1985, Andersen et al. 1991). Dortch et al. (1984) and Miyata et al. (1986) show that intracellular amino acids and orthophosphate represent readily available sources of N and P for phytoplankton growth and the intracellular concentrations of these compounds predict the nutrient status of the cells. Field studies using such indices for determining nutrient limitation require involved procedures and are difficult to interpret due to species specific variations in biological storage forms (Dortch et al. 1985, Miyata and Hattori 1986, Dortch and Postel 1989).

The intracellular pool represents organic and inorganic nutrients in the cell (Demanche et al. 1979, Dortch 1982, Andersen et al. 1991). Phytoplankton that experience short term fluctuations in extracellular nutrient concentrations may, to some degree, sequester nutrients intracellularly, in excess of their short term growth needs (Dortch et al. 1985,

Dortch et al. 1990, Andersen et al. 1991). The recycled pool represents those extracellular nutrients resulting from water column processes, such as grazer remineralization (Eppley and Peterson 1979). The new extracellular nutrients result from advective inputs into a water column system and are not of recent biomass origin within that system (Eppley and Peterson 1979). Nutrient limitation is linked to how biological and physical conditions; such as, uptake and assimilation physiology, grazing pressure, light, temperature, and advection, affect the nutrient concentrations in the three nutrient pools and the ability of the phytoplankton to use those nutrients (Elser et al. 1988, Dortch and Postel 1989, Vanni and Temte 1990, Moegenburg and Vanni 1991). Consequently, phytoplankton use of specific nutrient pools directly relates to the dominant biological and physical processes in their environment (Dortch 1982).

Andersen et al. (1991) develop a modification the dilution technique described by Landry and Hassett (1982) to quantify phytoplankton growth dependency on these three pools. Their modification builds on the different and distinguishable effects dilution has on the concentration of nutrients in the three pools (Andersen et al. 1991). In their study on the Oslofjord, Norway, they found that certain pools of N and P dominate the nutrient supply for phytoplankton growth and the magnitude of the supply, as well as the dominant supply pool, differs between the several sites, depths, and dates sampled (see Fig. 7, Andersen et al. 1991). The differences in pools use between the sites, depths, and dates appear to relate to differences in nutrient supply and help explain nutrient limiting conditions during their sampling period on the Oslofjord (Andersen et al. 1991).

This study uses Andersen et al.'s (1991) dilution technique to compare the importance of the three nutrient pools in the tidal freshwater and the estuarine region of the York River system, Virginia, in an attempt to explain the summer to winter seasonal patterns of nutrient limitation. The tidal freshwater is an important but understudied region of river-estuary systems (Schuchardt and Schimer 1991). High concentrations of nutrients and

phytoplankton biomass occur in this region (Filardo and Dunstan 1985, Anderson 1986, Schuchardt and Schimer 1991). The physiology of tidal freshwater phytoplankton is expected to exploit the large available store of dissolved N and P compounds and growth and nutrient limitation patterns should be tied to both short and long term fluctuations in the concentrations of the new extracellular pool. However, results from this study show a growth reliance by tidal freshwater phytoplankton on intracellular pools of N and P in late summer and a switch to recycled extracellular N and P pools in early winter. Interestingly, the expected switch from N to P-limitation was concurrent with the change in pool reliance. The seaward, more saline, region of the river-estuary system by contrast receives decreased new nutrient inputs from upstream advection (Filardo and Dunstan 1985, Anderson 1986, Fisher et al. 1988, Schuchardt and Schimer 1991). Consequently, within this region, phytoplankton are expected to have mostly recycled extracellular nutrient pools available, with new extracellular nutrient pools possibly available from periodic vertical mixing (Webb and Haas 1976, Webb and D'Elia 1980, Webb and Eldridge 1988, Eldridge 1989). Results from this study show a switch from recycled extracellular pools to intracellular pools of N and P. Although the magnitude of N-limitation was reduced, no seasonal switch to P-limitation was observed in the estuarine region of the river-estuary system.

Background

The conventional concept of estuaries is that they form in semi-enclosed coastal bodies of water which have free connection to the open sea and entering sea water is measurably diluted by freshwater (Cameron and Pritchard 1963). In estuaries, a range of salinity and nutrient concentrations occur based on the relative tidal and river flows. A variety of factors influence phytoplankton growth and biomass in the estuary. The availability of light and nutrients, the rate of grazing, and the extent of advection due to river flow all effect how successful phytoplankton populations are in the estuary (Ketchum 1954, Filardo and Dunstan 1985, Harding et al. 1986, Fisher et al. 1988).

The abundances of the macro-nutrients N and P play a dominant role in determining the growth rates for both marine and freshwater phytoplankton communities (e.g. Hecky and Kilham 1988). The N to P molar ratio of 16:1 represents the optimal nutrient supply rate for phytoplankton (Redfield 1958). Under Redfield's condition phytoplankton which have the proper molar ratio of N to P in their cellular composition are healthy (Parsons et al. 1984). Conditions of N and P limitation occur when either N or P are in large excess of the other, relative to Redfield's ratio. Under these conditions growth is limited by the nutrient present in lower than optimal concentration (Ryther and Dunstan 1971).

The concept of nutrient limitation is somewhat confusing and complicated. Liebiz (1840) originated the concept of nutrient limitation of primary production by stating, "growth of a plant is dependent on the minimum amount of food stuff." Unclear for marine systems, in which phytoplankton share a dynamic and common medium, is whether nutrients control phytoplankton growth on strictly the cellular scale or, rather, on a community scale by limiting total biomass production. For example, nutrient limitation may represent a physiological condition where the concentration of the one or more nutrients is below a threshold concentration, on a per cell basis, necessary for cellular growth. Thus, individual phytoplankters are physiologically impaired, lacking sufficient nutrients to grow. The existence of "physiological nutrient limitation" in commonly considered nutrient enriched systems, such as rivers and estuaries, is paradoxical since such systems have, by definition, an excess of nutrients necessary for phytoplankton cellular growth. There is, in fact, little evidence that natural phytoplankton populations experience "physiological nutrient limitation" in either nutrient rich or poor environments (e.g. Hecky and Kilham 1988).

The concept of nutrient limitation of biomass offers an alternative understanding of the nutrient-to-growth relationship. "Biomass nutrient limitation" results from a phytoplankton

community's inability to increase its biomass due to an insufficient supply ratio of one nutrient as compared to another. The limiting nutrient's concentration determines the community's realized size (Ryther and Dunstan 1971). Therefore, it makes more sense to consider nutrient limitation of biomass in aquatic systems. Using Redfield's ratio to describe the optimal supply ratio for phytoplankton growth, a condition where the ratio is greater than 16:1 represents P-limitation and less than 16:1, N-limitation. Although supply ratios of N to P affect algal growth, Hecky and Kilham (1988) suggest that the traditionally considered dissolved inorganic nutrient supply ratios do not accurately reflect the nutritional state of the phytoplankton community. Rather, they argue that physiological indices, such as elemental ratios of N and P in biomass, provide more realistic predictions of phytoplankton nutrient limitation (Hecky and Kilham 1988).

From a community perspective, the physiological aspect of nutrient limitation relates to how phytoplankton respond, through uptake and assimilation of nutrients, to their extracellular nutrient environment. Phytoplankton which experience unbalanced nutrient concentrations often reflect the imbalance in cellular nutrient to carbon ratios (Goldman et al. 1979, Dortch et al. 1985, Miyata et al. 1986). For example, intracellular pools have been shown to develop in cultured populations of diatoms and dinoflagellates that are nutrient starved and then placed in nutrient rich environments (Demanche et al. 1979, Collos 1982, Dortch et al. 1984, Miyata and Hattori 1986). Nutrients are generally used by phytoplankton cells in an energy dependent two step process starting with uptake, often represented by Michaelis-Menten kinetics, and ending with assimilation, the process of incorporating those nutrients into biomass (Droop 1983). Highly oxidized nutrients such as nitrate require an additional energy dependent intermediate step of reduction. Sequestering of N or P likely results when phytoplankton have discontinuous uptake, reduction or assimilation rates or when reduction or assimilation are the rate limiting steps (Demanche et al. 1979, Collos 1982, Dortch 1982, Droop 1983, Dortch et al. 1984,

Martinez 1991). The phytoplankton community's intracellular nutrient pool represents nutrients contained cumulatively in the phytoplankton cells. Dortch et al. (1985) and Miyata and Hattori (1986) hypothesized that intracellular nutrient pools allow communities to maintain stable growth rates during periods of nutrient deprivation through the mobilization of nutrients incorporated in ancillary compounds. Chemical analysis of cultured marine diatoms and dinoflagellates under nutrient deprivation show that intracellular N to P ratios may change drastically, either increasing or decreasing depending on whether N or P is being used, indicating depletion of intracellular pools (Harrison et al. 1977, Sakshaug et al. 1984, Miyata et al. 1986). In nature, phytoplankton which experience short term fluctuations in extracellular nutrient levels may develop intracellular pools to maintain stable growth rates (Dortch 1982, Dortch et al. 1985, Andersen et al. 1991).

Extracellular dissolved inorganic nutrients come from two sources (pools), new and recycled (Eppley and Peterson 1979). Dissolved extracellular N and P are normally considered to be in the ion forms of NH_4^+, NO_3^-, PO_4^{3-} (Valiela 1984). Traditionally, ammonium represented recycled N and nitrate, new N (Eppley and Peterson 1979). More generally, N and P allochthonous to the system being considered can be termed new nutrients. New nutrients may constitute the majority of extracellular nutrients available to the phytoplankton community. In such communities, grazing pressure is less significant to phytoplankton growth. Nutrient enriched and upwelling communities are examples of phytoplankton dependent on new nutrients. When these communities lack sufficient biomass exports or sinks, the biomass accumulates in the water column leading to blooms and possibly, over longer periods of time, to eutrophication (Paerl 1988). Phytoplankton communities that utilize new extracellular pools exclusive of recycled ones may be ultimately limited by light, temperature, or trace minerals.

The recycled pool develops out of the collective activities of phytoplankton, bacteria, and grazers in what has been termed the "microbial loop" (Azam et al. 1983). Bacteria scavenge dissolved organic material (DOM) released by phytoplankton, sloppy feeding of grazers, and lysis of organisms. In turn, heterotrophic flagellates graze the bacteria as well as phytoplankton, and remineralize N and P (Caron et al. 1985, Gude 1985, Goldman et al. 1987, Bloem et al. 1989). For the microbial loop to efficiently remineralize nutrients, heterotrophic protozoa must first graze the majority of the bacterial and phytoplankton biomass (Lehman 1980, Lehman 1984). The release of nutrients occurs when grazers possess different N:P ratios than their prey or demonstrate a low trophic transfer efficiency during digestion and assimilation (Lehman 1980, Lehman 1984, Goldman et al. 1987, Bloem et al. 1989, Eldridge 1989, Tranvik 1989). Phytoplankton communities that are highly dependent on remineralization for N and P must show a tight coupling between growth and grazing (Lehman 1980, Lehman 1984). Furthermore, such communities are conservative, tending to have neither significant biomass exports nor allochthonous nutrient inputs.

In estuaries, phytoplankton communities in regions of high river flow primarily use intracellular and new extracellular pools for growth. Such communities experience net down river advection which results in short residence times in the freshwater region and a rapid and consistent loss of biomass out of the freshwater (Ketchum 1951, Ketchum 1954). These freshwater communities cannot remineralize those nutrients which were tied up in the lost biomass. Consequently, if the freshwater population maintains a stable standing stock, the nutrients lost due to advected biomass must be replaced by some new source. Also, river flow provides a constant source of nutrients with the transport of dissolved nutrients from upstream and non-point source land runoff (Filardo and Dunstan 1985, Anderson 1986, Fisher et al. 1988). Light may also limit phytoplankton growth in turbid rivers and in the oligohaline (very low salt) regions of some coastal plain estuaries. This especially likely near the freshwater-saltwater interface where the landward bottom

current and the seaward river current meet. The convergence of the two currents results in a cycle of deposition and resuspension riverborne and sedimentary particles and may concentrate the suspended load to serveral orders of magnitude higher than regions either further up or down stream (Schubel 1968, Schubel and Biggs 1969). High turbidity zones, turbidity maxima, and resulting chlorophyll minima have been observed to be a general feature of coastal plain estuaries (Schubel 1968, Schubel and Biggs 1969, Morris et al. 1978, Morris et al. 1982, Anderson 1986, Harding et al. 1986, Owens 1986, Fisher et al. 1988). Light-limited phytoplankton may never effectively exhaust the extracellular pools in the freshwater regions of the estuary and accordingly their growth should not depend on the quick turnover of biomass to liberate nutrients. Lastly, the nutrient environment of the upper estuary is expectedly variable, changing temporally with the intensity of river discharge and land runoff and spatially with changes in river morphology and circulation.

In contrast to the high flow regions of the upper estuary, the lower estuary experiences lower turbidity and a higher intensity and quality of light which allows for higher growth rates (Harding et al. 1986). However, lower allochthonous nutrient input rates, and overall lower concentrations of dissolved nutrients (Filardo and Dunstan 1985, Anderson 1986, Fisher et al. 1988) makes efficient recycling of nutrients necessary during high growth rate periods. In such regions, recycled nutrient pools should dominate phytoplankton growth. The lower York River, Virginia, undergoes periods of stratification and destratification tied to the spring-neap tidal cycle (Haas 1977). The vertical mixing of the water column during destratification enriches the surface water by reintroducing nutrients previously trapped below the pycnocline (Webb and D'Elia 1980).

Dilution Theory

Landry & Hassett (1982) developed a technique to quantify grazing and growth rates of phytoplankton by diluting natural water (whole water-WW) with filtered, cell-free natural water (dilution water-DW). This "dilution technique" depends on three pivotal assumptions: (1) The growth of an individual phytoplankter is not affected by the concentration of phytoplankton (i.e. growth rate of the individual does not change with dilution); (2) the consumption of a phytoplankton cell is simply a function of the encounter probability of a grazer to its prey (grazing is a linear function of dilution); and (3) phytoplankton biomass is a product of its initial value and the exponential function of its growth rate , grazing rate , and time.

The standard equation for calculating phytoplankton growth rates from chlorophyll-a concentration (Chl-a) measurements is:

$$r = \frac{1}{t} \ln\left(\frac{P_t}{P_0}\right) = k\text{-}g, \qquad (1)$$

where the apparent growth rate (r) is calculated from the exponential change in Chl-a from its initial (P_0) to final value (P_t) over some time interval (t), and represents the difference between the optimal or gross growth rate of the phytoplankton (k) and the grazing rate (g). Therefore, based on this relationship of r to k and the assumptions given for dilution experiments, r should approach the value of k in a linear fashion with progressively more dilute incubated mixtures of WW and DW. Thus, r is negatively correlated to the fraction of WW (x) which represents the reduction of grazer abundance and overall grazing rate. Statistically then, a simple linear regression of r (dependent variable) against x (independent variable) predicts g, the negative slope of the regression line, and k, the y-intercept of the regression line where g is theoretically equal to 0. At an infinite dilution the predator is absent from the prey and, thus, the prey growth rate is maximal for its environmental

conditions. Non-optimal environmental conditions, such as nutrient limitation, and non-linear grazing may lead to violations of these assumptions and require special consideration (Burkill et al. 1987, Paranjape 1987, Gifford 1988, Gallegos 1989, Andersen et al. 1991).

Dilution of low-nutrient natural water may induce a condition of artificial nutrient limitation because the nutrients that support the algal cell are supplied through grazing and the grazer concentration is decreased as a linear function of dilution (Landry and Hassett 1982). To avoid this confounding complication, dilution experiments traditionally require the addition of nutrients sufficient to support phytoplankton growth independent of grazer remineralization over the time course of the experiment (Landry and Hassett 1982, Burkill et al. 1987, Paranjape 1987, Gifford 1988, Gallegos 1989). Andersen et al. (1991) take an opposite approach from Landry and Hassett (1982) and explore how dilution actually affects nutrient concentrations and phytoplankton growth rate independent of grazing. So, whereas Landry and Hassett (1982) originally intended to control for the impact of nutrients on phytoplankton growth in order to quantify the impact of grazing, Andersen et al (1991) attempt to control for grazing so that they can quantify the impact of intracellular, new and recycled extracellular pools of N and P on phytoplankton growth.

The first step for Andersen et al. (1991) is to quantify the grazing rate present in the natural water. If dilution assumptions about grazing hold true (Landry and Hassett 1982), then grazing remains constant in nutrient enriched and non-enriched dilutions (Andersen et al. 1991). Since g can only be accurately estimated when nutrient concentrations are controlled, Anderson et al.'s (1991) technique requires one fully nutrient enriched dilution series. The grazing and gross growth rates are calculated for the WW as described previously. Next, dilution series are selectively enriched with all necessary growth nutrients except for either N or P. This allows changes in phytoplankton growth rates over the incubation period to depend only on the natural pools of the nutrient *not* added. Since g calculated for the fully nutrient enriched dilution series is an estimate of the WW grazing

rate, the apparent growth rates for the selectively enriched dilution incubations are corrected for grazing by adding the fraction of g corresponding to the fraction of WW in each dilution:

$$\mu_x = r_x + gx, \tag{2}$$

where the grazing corrected growth rate (μ) equals the apparent growth rate (r) of some dilution x (fraction of WW) plus the proportional fraction of the WW grazing rate (g). Grazing is corrected for in this manner because the grazing pressure in each dilution is directly proportional to the number of grazers present in the dilution which is directly proportional to the fraction of whole water present. The manner by which μ changes as a function of dilution reflects the phytoplankton community's dependency on intracellular, new and recycled extracellular nutrient pools.

The interpretation of how changes in μ with dilution relate to N and P pool dependencies is based on how dilution affects the concentrations of the three pools. Recycled extracellular nutrient pool concentrations depend on the concentrations of both phytoplankton, the ultimate source of nutrients, and the grazers which actually recycle nutrients by grazing the phytoplankton. Since dilution lessens both phytoplankton and grazer concentrations by x, then the concentration of the recycled pool is reduced by x^2:

$$R_x = R_1 x^2. \tag{3}$$

In this equation the concentration of recycled extracellular nutrients (R), for some fraction of WW (x), is the initial concentration of the WW recycled pool (R_1) times the square of the dilution.

Intracellular pools are contained within individual phytoplankters so concentrations of both correspond directly to dilution. Consequently, the magnitude of intracellular pools in a dilution mixture decreases linearly as dilution increases:

$$I_x = I_1 x. \quad (4)$$

In this equation the concentration of intracellular nutrients (I) for a given fraction of WW (x) equals the initial WW intracellular nutrient concentration (I_1) multiplied by the fraction of WW present in the dilution.

New extracellular pool concentrations are assumed to be the same in WW and DW since effective filtering only removes cells and particulate material. Consequently, new extracellular nutrient pool concentrations remain constant with dilution:

$$E_x = E_1. \quad (5)$$

In this equation the concentration of new extracellular nutrients (E), for some fraction of WW (x), equals the initial WW new extracellular nutrient concentration (E_1).

Since apparent growth rates in the selective nutrient enriched dilutions are corrected for grazing, growth rate is proportional ($\propto$) to the sum of the nutrient concentrations in the three pools at a given irradiance and temperature:

$$\mu_x \propto [Rx^2 + Ix + E]. \quad (6)$$

Equation 6 simply demonstrates that μ, for some fraction of WW (x), is proportional to the combined nutrient concentrations in the intracellular, new and recycled extracellular pools. Therefore, if growth rate is measured as Chl-a production over some time interval, then the growth rate in a given fraction of WW is the sum of the Chl-a production resulting from the intracellular, new and recycled extracellular pools:

$$\Delta P_{x,t} = t\left(\Delta P_R x^2 + \Delta P_I x + \Delta P_E\right). \quad (7)$$

Equation 7 shows that Chl-a production, as a change in Chl-a concentration (ΔP) over the time period t for some fraction of WW (x), is the sum of the Chl-a production supported by

the recycled (R), intracellular (I), and new (E) pools of nutrients. Equation 7 also defines the proportional relationship of μ, in terms of ΔP for some t, to the three pools outlined in equation 6. It is important to note, that the contribution to ΔP of each pool changes differently with dilution (x). This differential relationship of each pool to ΔP with dilution allows for the inferences about growth dependency on each pool made by this technique.

Returning to equation 1, apparent growth rate equals the natural logarithm of the final Chl-a concentration (P_t) divided by the initial Chl-a concentration (P_0) over some time interval (t). The initial Chl-a concentration for each dilution is actually the initial Chl-a concentration of the WW multiplied by the fraction of WW present in that dilution (xP_0). It also follows that during the incubation the final Chl-a concentration for each dilution equals the initial Chl-a concentration plus the increase in the Chl-a concentration ($P_t = xP_0 + \Delta P$). Thus, for the selectively nutrient enriched dilutions, values of μ (equation 2) for each fraction of WW (x) can be estimated from a modification equation 1:

$$\mu_x = \frac{1}{t} \ln\left(\frac{P_t}{P_0}\right) = \frac{1}{t} \ln\left(\frac{xP_0 + \Delta P}{xP_0}\right) = \frac{1}{t} \ln\left(1 + \frac{\Delta P}{xP_0}\right). \quad (8)$$

Equation 8, by substituting ($xP_0 + \Delta P$) for (P_t) and (xP_0) for (P_0), demonstrates the relationship of μ for each dilution to the change of Chl-a per the initial Chl-a concentration in the dilution. Equation 8 can be expanded to describe the contribution of the three pools (R,I, and E) to μ for each dilution:

$$\mu_x = \frac{1}{t} \ln\left(1 + \frac{\Delta P_R x^2 + \Delta P_I x + \Delta P_E}{xP_0}\right). \quad (9)$$

Equation 9 is the substitution of Equation 7 into Equation 8 and further defines the proportional relationship of μ to the three pools as described in Equation 6.

$$\mu_x = \frac{1}{t} \ln\left(1 + \frac{\Delta P_R x + \Delta P_I + \Delta P_E x^{-1}}{P_0}\right). \quad (10)$$

Equation 10 shows the reduction of the numerator in Equation 9 by x. The reduction is a result of the fact that growth rates are cell specific and, consequently, a reduction by x in nutrient concentration within the three pools is offset by the concurrent x decrease in demand for the nutrients as phytoplankton concentrations are reduced by x. Growth coefficients (K_n, where n is I, E or R) can be substituted for the change in Chl-a divided by the initial Chl-a $\left(\frac{\Delta P_n}{P_0}\right)$ in each pool growth term in Equation 10. The substitution produces the following equation and is the nutrient growth model developed by Andersen et al. (1991):

$$\mu_x = \frac{1}{t} \ln \left(1 + K_R x + K_I + K_E x^{-1}\right). \tag{11}$$

Values for μ are calculated from the actual dilution incubations as described in Equation 2. Thus, to estimate the three pool growth coefficients, Equation 11 can be rearranged to yield a relationship of the growth coefficients to a transformed μ:

$$y = e^{\mu} - 1 = K_R x + K_I + K_E x^{-1}. \tag{12}$$

In Equation 12 the time (t) present in equation 11 is equal to 1 d (incubations took 24 h) and y equals the transformation of μ which allows for the removal of the natural logarithm and constant from the growth coefficients. Therefore values of y are calculated from values of μ and a multiple linear regression of y (dependent variable) against dilution (x) and the inverse of dilution (x^{-1}) (independent variables) estimates the growth coefficients for each pool.

To understand how the model and multiple linear regression of y against x work, consider Equation 11. For example, when intracellular nutrient pools support growth, calculated μ's for all the dilutions would cluster fairly closely to some common value. The multiple linear regression of these y's against x should only give a intercept value (K_I) and the regression line is a straight line with a slope equal to 0. Differently, when new

extracellular pools dominate nutrient supplies, the values of y increase by the inverse of dilution (x^{-1}) because the concentration of nutrients per cell increase exponentially with increasing dilution. The multiple linear regression of these y's against x will generate only a coefficient for x^{-1} variable (K_E) and the regression line will show an exponential and asymptotic increase as dilution increases. Finally, when recycled nutrient pools dominate growth, the multiple linear regression will show a coefficient for the x variable (K_R) and the regression line shows a negative linear relationship between y and dilution. Natural populations are likely intermediate between these three conditions and their functions should be weighted towards the most significant pool. The extent to which each pool affects the growth rate of the phytoplankton differently with dilution will influence the magnitude of the coefficients predicted by the multiple linear regression. Thus, for example, the K_R predicted for a system which is highly dependent on recycled nutrients for phytoplankton growth would be greater than the K_R for a population less dependent on the recycled pool. In this case the regression line for the highly dependent population will be steeper than that for the less dependent one. The multiple linear regression technique used should be able to distinguish between significant and non-significant coefficients in order to more accurately estimate the growth parameters of that population.

Objectives

1. To evaluate the appropriateness of the dilution technique outlined in Andersen et al. (1991) for nutrient limitation studies in an estuarine river.
2. To monitor changes in N and P-limitation and dominant pools in the the tidal freshwater and estuarine regions of the York River system during the 1990 summer to winter seasonal transition.

Hypotheses

1. During the 1990 summer to winter seasonal transition, the tidal freshwater and estuarine phytoplankton of the York River system will shift from a N-limited (summer) to a P-limited (winter) growth condition.
2. Tidal freshwater phytoplankton, as a result of river flow, experience high new nutrient concentrations and therefore show a dominant growth dependency on new extracellular N and P pools.
3. Estuarine phytoplankton, as a result of lower new nutrient input and higher phytoplankton growth rates, experience low ambient nutrient concentrations and rely heavily on recycled N and P pools for growth.

Materials and Methods

The York River system, Virginia, encompasses three rivers, the York, Pamunkey, and Mattaponi. Its streams flow southeasterly from the foothills of the Blue Ridge Mountains in Virginia to the Chesapeake Bay near Yorktown, Virginia. The York river itself is formed by the confluence of the Pamunkey and Mattaponi rivers 48 kilometers from its mouth at West Point, Virginia (Hyer et al. 1972, Haas 1977). The Pamunkey is tidal 90 km upstream of West Point (Division of Water Resources, 1970) and drains an area of 1,005 km^2 (361 mi^2). The non-tidal river, the upper 47 km, drains an additional 2,776 km^2 area (999 mi^2). The surface area of the tidal Pamunkey is 29.28 km^2 and the water volume is 1.098×10^8 m^3 at mean low water (Brooks 1983b). The average tidal range varies from 0.90 m at West Point to 1.18 m 87 km upstream (Brooks 1983b). The 39 year average discharge for the Pamunkey near Hanover, Virginia is 28.74 $m^3 s^{-1}$, ranging from 0.34 to 1,140 $m^3 s^{-1}$ over the 39 years (USGS 1982).

The York River is tidal for its entire length which extends 48 km from the river mouth near Yorktown, Virginia to its bifurcation at West Point. The York basin alone drains an area of 598 km^2 (215 mi^2) The water surface area of the York is 210.92 km^2 (127 mi^2) and its water volume is 9.09 x 10^8 m^3 at mean low water (Brooks 1983a). The tidal range for the York varies form 0.70 m at the mouth to 0.90 m at West Point (Brooks 1983a). The 39 year average discharge for the York is 45.82 m^3 s^{-1}, ranging from 0.51 to 1,619 m^3 s^{-1} over the 39 years (USGS 1982).

This study compared a tidal freshwater site (salinity < 1.0 psu) and an estuarine site (~20.0 psu) on the York River system (Fig. 1). The freshwater site (Pamunkey) was located off a Virginia public pier on the Pamunkey River approximately 81 kilometers from the mouth of the York River. The estuarine site (York) was located at the old Gloucester Point ferry pier approximately 10 kilometers from the mouth of the York River.

Eight dilution experiments (4 per site) were performed during the 1990 summer-winter seasonal transition using a modification of the design described by Anderson et al (1991). The Pamunkey experiments commenced on 27 August, 1 October, 6 November, and 16 December. The York experiments commenced on 30 August, 4 October, 8 November, and 19 December. One other dilution experiment was conducted with York water from 27-30 November to assess growth lag periods at lower ambient water temperatures. During sampling, surface water temperature and salinity were measured using a mercury thermometer and a refractometer. Water column light attenuation data was collected using a 4 π subaqueous light meter interfaced with a LICOR model LI-1000 data logger (August, October and November) or a Secchi disk (December). Sufficient surface water was collected at each station to fill three 20 liter carboys using a clean and rinsed plastic bucket. Sampling occurred in the mid-afternoon and in the case of the Pamunkey sampling, water was immediately transported to the Virginia Institute of Marine Science (VIMS).

After collection, 20 liters of sample water was filtered by gravity through a 73 μm Nitex® mesh filter to remove large zooplankton; this water was the whole water (WW) used in making the dilutions. Thirty liters of sample water was filtered through Whatman® GF/A™ (August) or sequentially through GF/A™ and GF/F™ (October, November and December) glass fiber filters by vacuum (<100 mm Hg) to produce dilution water (DW) free of phytoplankton and grazers. Approximately 125 ml of DW was collected and stored frozen for inorganic N and P analysis (no samples taken in August) using Technicon Autoanalyzer. During the August and October experiments dilutions were immediately mixed following filtering of the sample water and incubations began that evening. For the November and December experiments WW and DW were stored seperately overnight in 20 liter carboys at near ambient temperatures in the dark and incubations commencing before 09:00 the next morning.

Dilutions were mixed to provide the following fractions of WW: 0.05, 0.10, 0.20, 0.40, 0.60, 1.00. Replicate one liter polycarbonate bottles were filled with the mixed dilutions. Each dilution series received either: 1) NH_4^+ and PO_4^{3-} (+NP), 2) NH_4^+ (+N), or 3) PO_4^{3-} (+P) to achieve N and P concentrations of 25 μM and 5 μM, respectively. Trace minerals and other nutrients were assumed not to be limiting to phytoplankton growth over the incubation period. Replicate bottles containing only DW and +NP were incubated as controls.

Bottles were incubated for 24 h (August, October and November) or 48 h (December) in a 1.2 x 3.0 m flow-through water bath on the VIMS ferry pier (except August). Window screening was used to mimic average ambient light intensity. Incident and incubator light levels were monitored using 2π deck cell and 4π subaqueous sensors, respectively, with hourly averages stored on the data logger. During the August experiments, as the result of water pump failure, bottles were incubated in situ off the end

of the VIMS ferry pier at a depth equivalent to average light levels. The depth of the bottles was periodically adjusted for changes in tide height.

The phytoplankton community in the WW at 0 h was assessed by epifluorescence microscopy. For eukaryote enumeration, 2-8 ml of sample were fixed with 0.3% glutaraldehyde (final concentration) and stained with 7.5 $\mu g\ ml^{-1}$ (final concentration) 4',6'-diamidino-2-phenyl-indole (DAPI) (Porter & Feig 1980) and then with 3.0 $\mu g\ ml^{-1}$ (final concentration) 3,6-diaminoacridine (Proflavin) (Haas 1982). For bacterial enumeration in the October, November and December experiments, 2 ml of sample were stained with 0.01% (final concentration) acridine orange (Hobbie 1977). The stained samples were filtered under vacuum (<150 mm Hg) onto 0.2 or 0.8 μm pore black stained Nuclepore® filters. The 0.2 μm pore filters were used in the August experiments and for all of the acridine orange stained samples. The 0.8 μm filters were used for DAPI/Proflavin slides in the October, November and December experiments. The filters were mounted onto breath-dampened slides, with a drop of immersion oil and a cover slip placed on the slide-mounted filter, and stored frozen until analysis. Slides were analyzed for cell types, concentrations, and biovolume on the computer assisted image analysis system developed by Sieracki et al. (1989) and Sieracki and Webb (1991). Biomass was estimated for eukaryotes using the biovolume to biomass conversion factor of 220 $\mu g\ l^{-1}$ (Bratback and Dundas 1984) and, for prokaryotes, the conversion factor of 300 $\mu g\ l^{-1}$ (Fry 1988).

Chl-a samples were taken in triplicate at 0 h from each prepared dilution and in duplicate at 24 h from each sample bottle (also at 48 h for the December experiment). Five to 15 ml of sample were filtered onto 25 mm Whatman® GF/F™ glass fiber filters. The filters were placed in dark test tubes pre-filled with 8 ml dimethyl sulfoxide and acetone extraction solution (Webb & Hayward unpubl.). Fluorescence was read the following day on a Turner Designs Fluorometer, calibrated with a spinach standard (Ray et al. 1989). Replicate Chl-a readings were reduced using simple descriptive statistics; mean, standard

deviation and coefficient of variance. Since Chl-a was measured in the control DW bottles, Chl-a concentrations measured in the experimental bottles were corrected for Chl-a added with the DW as follows:

$$P_C = P_M - (1 - x)P_{DW}, \tag{13}$$

where P_C represents the corrected Chl-a concentration, P_M is the measured (uncorrected) Chl-a concentration, P_{DW} is the mean DW Chl-a concentration, x is fraction of WW present in the dilution. Corrections done for initial Chl-a measurements used the mean of the initial DW Chl-a values and corrections done for final Chl-a measurements used the mean of the final DW Chl-a values obtained from the incubated control bottles (DW + NP).

Because it was believed that the volume fraction WW to the total volume of each dilution did not accurately reflect the dilution of the photo- and heterotrophic micro-, nano- and picoplankton, the dilution series was measured as the fraction of WW Chl-a. It was assumed that the initial proportions of photo- to heterotrophs in the dilutions did not change with respect to the WW and, therefore, the dilution of WW Chl-a directly corresponded to the dilution of WW heterotrophs. In order for this assumption to hold true, only corrected Chl-a values for each dilution were used. Uncorrected values would underestimate the dilution of heterotrophs by measuring Chl-a added with the DW during mixing (see Discussion for further consideration of the effects of DW Chl-a on the effectiveness of this technique). Dilutions were calculated using the following equation:

$$x_a = \frac{P_{Cx}}{P_1}. \tag{14}$$

In the above equation, dilutions were measured by the fraction of initial WW Chl-a (x_a) present at time 0 in the WW/DW mixture. The mean of the initial P_C values for each dilution (x) was divided by the mean of initial Chl-a values of the WW (P_1).

In November an additional dilution experiment was conducted to investigate if falling water temperatures were increasing the time lag between the nutrient addition and growth response of the phytoplankton. In this experiment the dilution series was only enriched with +NP. Consequently only 14 bottles were incubated (two per dilution plus two DW's). The incubation lasted a total of 72 h and Chl-a samples were taken at time 0, 24, 48, and 72 h and apparent growth was calculated for each 24 h interval. The Chl-a concentrations and r's for each dilution were plotted against time to determine growth lags.

Apparent growth rates (r) were derived from changes in corrected Chl-a over 24 h time intervals (t = 1 d). Because both December experiments showed a significant growth lag during the first 24 h, the 24 h sample was considered the initial and the 48 h, the final Chl-a concentration. In all incubations r was calculated using the following equation:

$$r = \frac{1}{t} \ln\left(\frac{P_{Cf}}{P_{C0}}\right) \tag{15}$$

In the above equation t is the time (1 d for all incubations), P_{Cf} is the final, and P_{C0} is the initial, corrected Chl-a value. The +NP treatments were assumed to follow Landry and Hassett's parameters (1982) and r was regressed against the fraction of WW Chl-a (x) as a simple linear function to provide estimates of the gross growth rate (k, the y-intercept) and the WW grazing rate (g, the negative of the slope) for the phytoplankton (Andersen et al. 1991). Grazing was considered significant at $p \leq 0.15$ and when not significant it was assumed to equal zero. When g = 0, k was assumed to equal the mean of the r for the dilution series. Using initial WW Chl-a concentrations and the calculated values of k and g, estimates were made for the 24 h gross and real production of the phytoplankton community at each site for every experiment. The two productions were compared to provide estimates on the percentage of potential production and standing stock grazed over the 24 h incubation.

Grazing was assumed to be the same linear function of dilution for all three treatments. Grazing-corrected growth rates (μ) were calculated for the +N and +P selectively treated dilution series by using +NP grazing rate (see Equation 2). Growth coefficients for intracellular (K_R), recycled (K_I) and new (K_E) extracellular pools of the limiting nutrient were estimated by using a multiple regression of values for μ in the +N and +P selectively nutrient treated dilution series against x and x^{-1} (see Equation 12). Coefficients below the F-value significant at $p = 0.05$ were removed from the solution and significant negative coefficients were ignored (Andersen et al. 1991). Daily production estimates for intracellular, new and old extracellular N and P were calculated by multiplying the initial WW Chl-a by the corresponding pool coefficient.

Results

The ambient physical conditions, chlorophyll and nutrients at the Pamunkey and York sampling sites from July 1990 to January 1991 are given in Table 1 and Figure 2. Surface water temperature was similar at the two sites, falling from 30 to 5°C during the summer-winter seasonal transition (Fig. 2A). Pamunkey surface salinity was consistently below detection (< 1 psu) while York salinity averaged 20 (± 3) psu. Light attenuation at the two sites also differed, averaging 3.8 (± 1.3) m and 1.7 (± 1.0) m at the Pamunkey and York, respectively (Table 1).

The York generally had greater concentrations of Chl-a than the Pamunkey (Fig. 2A). Both sites showed a generally decreasing Chl-a with time. Pamunkey Chl-a fell from a July high of 15 $\mu g\ l^{-1}$ to a December low of 3.1 $\mu g\ l^{-1}$ (Fig. 2A). York Chl-a fell from a July high of 35 $\mu g\ l^{-1}$ to a December low of 4.5 $\mu g\ l^{-1}$, but rebounded in January to 17 $\mu g\ l^{-1}$ (Fig. 2A). On all sampling dates Chl-a was present in the DW and ranged from <1 (November and December) to 10% (August and October) of WW Chl-a concentration. The level of DW Chl-a was similar in both the Pamunkey and York waters.

Phosphate concentrations varied little between sites and between samplings (Table 1). The average Pamunkey and York phosphate concentrations were 0.14 (± 0.02) and 0.17 (± 0.05) μM, respectively. Dissolved inorganic N concentrations varied much more with the transition of the seasons and between sites (Table 1). Since P concentrations varied little, inorganic N to P supply ratios show a rise in N concentration after a small initial decrease in the Pamunkey, and two peaks for N in the York (Fig. 2B). Nitrate was the dominant form of dissolved inorganic N (DIN) in the Pamunkey, comprising 69.5 (± 12.2) % of the sum of ammonium and nitrate, while ammonium was the dominant species in the York, comprising 87.5 (± 7.6) % of the DIN. At both sites nitrite was present in only trace amounts. Changes in the ratio of dissolved ammonium to phosphate concentrations (Fig. 2C) show a similar pattern for the York, owing to the overriding dominance of ammonium species there. For the Pamunkey, however, the pattern for the DIN:P ratio (Fig. 2B) is distinctly different than the NH_4^+:P ratio (Fig. 2C), where 6 of the 8 samples have supply ratios below 16:1.

Table 2 shows the phototrophic and heterotrophic abundances of micro-, nano-, and picoplankton sampled at the two sites. In the Pamunkey, cyanobacteria and phototrophic dinoflagellate abundances decreased dramatically during the study period while phototrophic flagellate, cryptophyte, and diatom abundances fluctuated greatly between samplings. The Pamunkey's heterotrophic plankton abundances were relatively constant between the August and November experiments but in December the bacterial population decreased and the flagellate population increased. In the York water cyanobacteria, phototrophic flagellates, and diatom abundances sharply declined from August to December while phototrophic dinoflagellate and cryptophyte abundances varied widely between samplings. After an initial drop between August and October, bacterial abundance remained constant. The heterotrophic flagellate abundance declined after October. Trends in biomass (Table 3) mirror the trends for cell abundances. The heterotroph to phototroph

biomass ratio (Table 3) shows that the Pamunkey community was generally more heterotrophic and heterotrophy generally increased from summer to winter at the York site.

Figure 3 shows results of the 72 h dilution experiment done with York water in late November. In situ water temperature was 11°C and salinity was 18 psu. A delayed growth response to the added N and P was observed in the low dilutions (0.24 fraction of WW Chl-a and greater) and negative growth occurred in the highest dilution during the first 24 h. Chl-a in all dilutions increased logarithmically after 24 h. Based on these results, the December incubations were extended to 48 h for both sites.

The apparent growth rate vs. dilution plots for the +NP treatments for each of the eight experiments are shown in Figure 4. Generally there was more variation between replicate bottles in the Pamunkey experiments than in the York. Simple linear regressions results (Table 4) indicate that both growth and grazing rates of phytoplankton at both sites generally declined from August to December. Because the model requires an estimate of the grazing impact on the phytoplankton community if it exists, I reduced my type II error (β) by allowing my type I error (α) to equal up to 0.15. Consequently the goodness of fits (R^2) for the Pamunkey regressions are rather weak, but all four Pamunkey regressions do describe a trend (slope > 0) at a $p < 0.15$.

Table 5 displays initial whole water Chl-a concentrations and the rates of potential and real primary production based on the +NP growth and grazing results for the eight experiments. Both sites showed a decline in potential and real production from August to December. At the Pamunkey site grazing reduced potential production by more than 50% in August and October, 30% in November and 77% in December. The amount of standing stock grazed showed a similar trend with a decline August to November and a resurgence in December. Grazing impact was also reflected by changes in the growth efficiency. The York water showed a decrease in potential and real production between August and October

as well as a decrease in grazing pressure and an increase in growth efficiency. Only potential production could be estimated for the November and December experiments since no grazing was detected.

The observed values of μ, grazing corrected growth rates, for the +N and +P treatments are shown in Figures 5 and 6 respectively along with the curve resulting from the multiple regression model. Generally the responses of the +N and +P treatments were very similar at each site but differed between sites. The sites also showed different trends over the seasonal transition (Figures 5 and 6). Table 6 shows the numeric results of the model calculations for growth coefficients (d^{-1}) as determined by backwards multiple regression. The multiple regression gave significant but negative values for the intracellular pool coefficients for both N and P in the Pamunkey December and York August experiments. These occurred when there was a pronounced shift from recycled to new extracellular pool dependency at the high dilutions (Figures 5 and 6). Since negative values cannot translate into real rates they were ignored and were set equal to 0 in Figure 7. Andersen et al. (1990) treated negative values similarly. In the August York experiment, the highest dilution is omitted because the Chl-a for the dilution was not different than the Chl-a for the controls at both time 0 and time 24 and, consequently, was corrected to equal zero and was ignored (see Material and Methods). In the November Pamunkey experiment, the highest dilution series for both +N and +P were lost and the next highest dilution is shown in Figures 5 and 6 but due to its extremely low value it was not used in the multiple regression. In the December Pamunkey experiment, two replicates in the +N and +P treatments showed negative growth throughout the 48h incubation and were considered lost, but all dilutions were represented.

The Pamunkey community showed an exclusive growth dependency on intracellular pools for both N and P in the August, October, and November experiments (Figures 7A and C). In these three experiments the P pool growth coefficient was greater than the N

coefficient, though the difference declined to a point in November where both were basically equal (Table 6). In December , the Pamunkey community shifted its reliance from intracellular to extracellular pools for both N and P (Figures 7A and C). In the extracellular pool both new and recycled N and P were used with more growth occurring from recycled nutrients (Table 6). The combined growth from the new and recycled nutrients showed slightly greater growth in the N pool (Table 7).

The York community, by contrast, relied exclusively on extracellular nutrients in August (Figures 7B and D). In August the recycled pool dominated both N and P growth while the new pool was rather small but more important for N growth (Table 6). In the October experiment the intracellular pool emerged to dominate both N and P growth (Table 6). The extracellular pool was also present with recycled dominating new nutrients for growth (Table 6). In November the community shifted to exclusively intracellular pools for growth for both N and P (Table 6). For the December experiment intracellular pools dominated, exclusively in the P pool and with a small input from new extracellular in the N pool (Table 6). In August and November P growth was greatest while in October and December N growth was greatest (Table 7).

In August and October for both P and N the combined growth coefficients in the York exceeded those for the Pamunkey. In November the two site showed nearly even combined growth coefficients while in December they shifted with higher growth coefficients seen in the Pamunkey than in the York for both for N and P. These trends are further highlighted by Chl-a ($\mu g\ l^{-1}$) production estimates for the 24 h incubation compiled in Table 7.

Discussion

I. The Dilution Technique:

Some modifications of Andersen et al.'s (1991) technique were necessary for use on the York River system. The first modification was that dilutions were measured as the fraction of WW Chl-a and not simply the physical mixture of WW and DW. It was assumed that dilution of Chl-a was proportional to dilution of grazers and reflected the true effectiveness of the physical dilution. Unfortunately, DW produced in all eight experiments contained measurable Chl-a. Likely, the ineffectiveness of the filtering to produce Chl-a free DW resulted from the usage of Whatman GF/A and GF/F filters. Taguchi and Laws (1988) found that up to 35% of picoplankton Chl-a present in coastal and open water samples passed through Whatman GF/F filters. Both river sites had high concentrations of cyanobacteria in August and October (Table 2) and a significant percentage of these cyanobacteria and other picoplankton passed through the filters into the DW (10% of WW Chl-a for both sites during both experiments). Therefore, bulk Chl-a measurements of the dilutions did not directly correspond to the concentration of grazers and larger phytoplankton but included picoplankton populations added with the DW. Consequently, bulk Chl-a measurements overestimated the abundance of grazers by underestimating the dilution. The presence of Chl-a in the DW necessitated the second modification which corrected all bulk Chl-a measurements for the Chl-a added with the DW and allowed for accurate calculation of dilutions (see Materials and Methods).

Cyano- and heterotrophic bacteria are significant food sources for heterotrophic nanoplankton and ciliates (Borsheim 1984, Anderson and Fenchel 1985, Sherr and Sherr 1987, Gonzalez et al 1990). In higher dilutions, the addition of picoplankton present in the DW likely cause the initial ratio of prey to grazers to be significantly higher than the ratio in

the WW. The ratio of phytoplankton to grazers ($r_{p:g}$) in a given dilution can be described by the following equation:

$$r_{p:g} = \frac{(xP_{ww} + (1 - x)P_{dw})}{xG_{ww}}. \quad (16)$$

In the above equation the phytoplankton to grazer ratio ($r_{p:g}$) equals the sum of the fraction of WW (x) times the WW concentration of phytoplankton (P_{ww}) and one minus the fraction of WW times the DW concentration of phytoplankton (P_{dw}); the sum is then divided by the fraction of WW times the WW concentration of grazers (G_{ww}). Thus, if the DW is cell-free then the $r_{p:g}$ for each dilution equals the $r_{p:g}$ for the WW, while if the DW contains picoplankton then the ratio will be greater for the dilutions than for the WW. The highest dilutions will have the most significantly greater $r_{p:g}$ than the WW $r_{p:g}$, because in these dilutions the most picoplankton are added and concentration of grazers is the lowest. For example, given: 1) P_{ww} equals 1 x 10^6 cells ml^{-1}, 2) P_{dw} equals 1 x 10^5 cells ml^{-1} (DW contains 10% of the WW Chl-a in the form of phototrophic picoplankton, as was true in the August and October experiments), and 3) H_{ww} equals 1 x 10^5 cells ml^{-1}; a 0.1 fraction WW dilution would increase the $r_{p:g}$ from 10:1 in the WW to 19:1 in the dilution:

$$r_{p:g} = \frac{0.1(1 \times 10^6 \text{ cells ml}^{-1}) + 0.9(1 \times 10^5 \text{ cells ml}^{-1})}{0.1(1 \times 10^5 \text{ cells ml}^{-1})};$$

$$r_{p:g} = 19. \quad (17)$$

This diluting inequity between phytoplankton and grazer violates one basic assumption of the technique which, because both populations should be reduced proportionally with dilution, expects the encounter rate of grazers to phytoplankton to decrease linearly with dilution (Landry and Hassett 1982).

The consequence of this violation leads to a non-linear reduction in the grazing rate with dilution during the incubations (Gallegos 1989), such as saturated grazing noted in dilution

experiments by Gallegos (1989) and Falkenhayn (1990). The grazing rate in the dilution (g_x) may be described as follows (from Equation 7c, Gallegos 1989):

$$g_x = g_1\left(\frac{f_x P_x}{f_1 P_1}\right) \tag{18}$$

In Equation 18, grazing rate in the dilution (x) is the product of the grazing rate in the WW (g_1) and the quotient of the phytoplankton concentration function in the dilution and in the WW $\left(\frac{f_x P_x}{f_1 P_1}\right)$. Using Equation 18 in the above example, it can be seen that the addition of picoplankton in the DW leads to a lower than expected reduction in grazing pressure:

$$g_{0.1} = g_1\left(\frac{f_x(1.9 \times 10^5 \text{ cells ml}^{-1})}{f_1(1 \times 10^6 \text{ cells ml}^{-1})}\right);$$

$$g_{0.1} = g_1\, 0.19\left(\frac{f_x}{f_1}\right);$$

$$g_{0.1} \propto 0.19\, g_1. \tag{19}$$

In the above example, the grazing rate when the grazer concentration is reduced to 10% is actually 19% of the original grazing rate. Thus under conditions of picoplankton enrichment, grazing rate would not be linearly related to the dilution of the grazer. The non-linearity would change if grazing also became saturated or the grazer population grew significantly over the incubation period (Gallegos 1989). Picoplankton enrichment may also favor the growth of a specific grazer which flourishes in the bacteria enriched environment.

With respect to the accuracy of Andersen et al.'s (1991) technique in these experiments, phytoplankton growth dependencies on recycled extracellular and intracellular nutrient pools were likely overestimated while growth dependencies on new extracellular nutrient pools were underestimated. The estimate errors occurred because the total phytoplankton abundances were not proportionally reduced with physical fraction of WW, leading to

smaller than predicted reduction in phytoplankton competition for extracellular dissolved nutrients and subsequently, lower growth rates in the highest dilutions. In order to minimize the estimate errors, final Chl-a measurements were corrected for the amount of Chl-a resulting from the Chl-a originally added with the DW. The amount of Chl-a in each measurement resulting from the DW was obtained from the incubated control bottles. Since the incubated control bottles lacked grazers and the picoplankton present grew uncropped and picoplankton added with the DW to the dilution bottles did experience grazing over the incubation period; it followed that Chl-a values for DW obtained from the controls would be higher than the corresponding Chl-a values for DW in the dilution bottles. Consequently, subtracting out the control Chl-a values from each dilution bottle's Chl-a measurement, proportional to the original volume of DW added, removed the maximum possible Chl-a due to DW picoplankton growth and, therefore, over-corrected for the actual DW Chl-a present in each bottle. In this way, any changes which occurred to the DW Chl-a in the experimental bottles were translated into changes in the corrected Chl-a. It was assumed that changes in the DW Chl-a concentration would reflect changes which would normally occurred to the diluted WW Chl-a had no DW Chl-a been present. Thus, reasonable grazing rate estimates in the +NP treatments and pool dependency and nutrient limitation predictions in the +N and +P treatments could be made using the corrected values.

Because cyanobacteria are known to be ubiquitous in aquatic systems (Johnson and Sieburth 1979, Waterbury et al. 1979), GF/A, C, F filtered water used in dilution experiments must be assumed to contain viable Chl-a (Taguchi and Laws 1988). In systems such as the Chesapeake Bay and its tributaries cyanobacteria can comprise a considerable percentage of the phototrophic biomass (Table 3, Haas unpubl. data, Ray et al. 1989, Falkenhayn 1990, Webb, Sieracki and Kindler, unpubl. data). Dilution experiments conducted on such systems need to account for and minimize DW Chl-a.

Corrections for the added cells, such as described in this paper, may reduce the estimating errors of the technique. Nevertheless, the best solution to these problems is to filter WW with a smaller pore-sized filter, such as a Nuclepore 0.22 μm membrane. Unfortunately, smaller pore-sized filters may increase the already lengthy time required for producing DW. Andersen et al. (1991) used GF/C filters and consequently, their DW likely contained picoplankton Chl-a. However, by also using GF/C filters for Chl-a measurement it is likely that they never observed Chl-a in their DW and made no provisions for its presence.

Another complication of implementing Andersen et al.'s (1991) technique to the York River system was the high turbidity in the Pamunkey water (Table 1). The turbidity largely resulted from a high particulate load in the form of plant material, silts and clays (personal observations). Much of this material passed the 73 μm screen and quickly settled when place in carboys and incubation bottles. Tranvik (1989) describes the importance of attached bacteria and flagellates to production, grazing, and nutrient remineralization in a humic lake. It is possible that the settling of these particles and their diluting may have impacted growth and grazing in these bottles in a non-linear fashion and lead to the dispersion of data points. As discussed previously, Gallegos (1989) shows non-linear grazing to particularly confound a linear relationship between apparent growth rates and dilution. The Pamunkey regressions of +NP treatment apparent growth rates against dilution proved to be highly scattered and showed low correlation coefficients (Fig. 4 and Table 4). Slopes, however, were significant in all four experiments at $p \leq 0.15$ (Table 4). Since the model required an estimate of a maximal grazing rate if any rate was detectable (Andersen et al. 1991), I allowed a larger chance of a type I error in order to reduce the likelihood of committing a type II error. My intent was not to arrive at some highly precise estimate of grazing but, rather, to be sensitive to any trend which showed grazing and, thereby, achieve a rough approximation of grazing impact on the Pamunkey community.

Filtering also removed the suspended particles from the DW. Dilutions of Pamunkey water not only reduced the concentrations of cells but also changed the environment by greatly reducing the surface area for biological and chemical reactions to occur. The removal of physical forces in the polycarbonate bottles also compounded the reduction of surface area by allowing particles to settle. This complicates interpretation of the results because the technique is built on the assumption that only grazing and nutrients ultimately affect the growth rate. Unfortunately, my experimental design allowed me no avenue by which to address or quantify the impact of these suspended particle complications. Future dilution experiments using turbid waters should estimate the total surface area of the suspended particles in the WW and each dilution to determine the actual dilution of suspended particles. By doing so, changes in growth rates between the dilutions could be related to the reduction in surface area to see if the reduction significantly affected growth in the system. Gentle agitation or rotation of the batch culture bottles might supply sufficient energy to maintain the particles in suspension during the incubation of the samples.

The final complication resulted from declining ambient temperatures during the summer-winter seasonal transition. Duarte (1990) discussed the effect of time lags on growth estimates in algal cultures. Low temperatures, by slowing growth rates, increased lag responses to nutrient treatments enough to require longer incubations in order to observe real growth responses to the nutrient addition (Duarte 1990). Results from the November lag growth dilution experiment (Fig. 3) demonstrated long growth lags, especially pronounced in the two highest dilutions, over the first 24 h of incubation. Although some growth lag probably occurred in all experiments, they were not large enough to adversely affect the growth estimates in the first three (August, October, and November). However, to ensure accurate growth estimates in the December experiments, the incubations were extend for both the Pamunkey and York from 24 to 48 h. Because

both December incubations showed negative or no growth during the first 24 h, growth estimates were made from Chl-a changes over the final 24 h.

II. Phytoplankton Growth in the York River system:

The major conclusion from this study supports one theme Hecky and Kilham (1988) built with their discussion on supply ratios: biological conditions rather than physical or chemical conditions directly affect growth. This is not meant to dismiss the important roles which physical and chemical environmental parameters, such as light, temperature, and nutrient concentrations, play in setting upper limits to growth. Rather, it is to show that the manner by which an environment is used by phytoplankton is more clearly the result of the physiological traits of the population and the ecological processes within the system. For example, the results for the Pamunkey site showed decreasing short term N-limitation and a switch to P-limitation while results for the York showed alternating N- and non-limitation from August to December (Table 7). A concurrent study (Webb, Sieracki and Kindler, unpubl. data) found no P-limitation of long term growth in the tidal fresh and estuarine phytoplankton of the York River system during the autumn and winter of 1990-91. However, the supply ratios (Table 1) predict P-limitation in the York and Pamunkey sites in all but the September (York) and October (Pamunkey) sampling during the 1990 autumn. These results again confirm the conclusion of Hecky and Kilham (1988): supply ratios often do not predict nutrient limitation.

The patterns of dominance for the three nutrient pools over the study period helps to explain the conditions which affected phytoplankton growth. Intracellular nutrient pools dominated growth when physiological and ecological processes prevented phytoplankton cells from using either new or recycled extracellular nutrients or both. Intracellular pools for both N and P dominated supply to phytoplankton growth in the Pamunkey during the August, October and November experiments. During December pool dependence switched

so that new and recycled extracellular pools supplied the nutrients for growth. In the lowest salinity regions of estuaries and tidal freshwater regions of rivers, nutrient supplies are generally considered to be under hydrodynamic control (Filardo and Dunstan 1985, Schuchardt and Schirmer 1991). New nutrients resulting from land runoff dominate the dissolved pool (Anderson 1986, Fisher et al. 1988). Generally, river flow increases during late winter and spring months and decreases during summer months. River discharge was lowest during September and steadily increased from October through December 1990 (Fig. 8). The expectation is that during higher discharge in the winter and spring months, phytoplankton growth in the tidal freshwater regions should be dependent primarily on new extracellular nutrient pools. During lower discharge periods (summer and early fall), lower new nutrient supplies and higher temperatures should lead to a shifting of phytoplankton growth dependency from new to recycled extracellular nutrient pools as increased growth rates deplete the poorly replenished extracellular supplies. This pattern was not seen in the Pamunkey, during the low discharge period of the late summer intracellular, instead of recycled extracellular, pools dominated growth and as discharge increased during the seasonal transition growth shifted to recycled, and not new, extracellular nutrients. Possible explanations for these discrepancies are examined below.

Experiments with cultured dinoflagellates and diatoms have demonstrated that uncoupling of uptake and assimilation leads to the formation of intracellular nutrient pools (Collos 1980, Collos 1982, McCarthy 1980, Nalewajko and Lean 1980, Droop 1983). During uncoupling, assimilation is the rate limiting step for growth. The environmental impetus for this uncoupling is related to nutrient deprivation or starvation of the phytoplankton. Cultured diatoms, for example, first subjected to nutrient starvation, have shown "luxury uptake" and formation of intracellular pools when placed in nutrient enriched media (Demanche et al 1979, Dortch 1982, Dortch et al. 1982, Dortch et al. 1984). It is speculated that in natural systems growth reliance by phytoplankton on

intracellular nutrient pools reflects an environment where extracellular nutrient supply is sporadic on temporal and spatial scales significant to the phytoplankton (Dortch et al. 1985, Miyata et al. 1986, Dortch and Postel 1989).

The Pamunkey phytoplankton, upon first inspection, appear to be neither nutrient deprived nor starved, the dissolved ambient nutrient concentrations appear high (Table 1), but growth is dependent on intracellular pools. However, the phytoplankton experience NH_4^+ concentrations < 1μM, which is considered low (McCarthy 1980), in all four Pamunkey experiments (Table 1). Ammonium and urea are normally the preferred forms of dissolved N for natural phytoplankton. Many phytoplankton populations will take up NH_4^+ even at very low concentrations instead of nitrate which may be present at much higher concentrations (McCarthy 1980). Garside (1981) found that N-uptake was limited by light during the fall and winter in the New York Bight, and the type of N-uptake was controlled by the presence or absence of NH_4^+. High NH_4^+ concentrations inhibited phytoplankton uptake of nitrate, while nitrate was taken up when NH_4^+ levels were low (Garside 1981). Webb and Haas (1976) found that urea, as well as NH_4^+, is an important N source for phytoplankton growth in the York River. McCarthy et al. (1977) also found NH_4^+ and urea to be the major N sources for Chesapeake Bay phytoplankton and that NO_3^- uptake only occurred when NH_4^+ levels were low. Berman et al. (1984) observed the same N uptake preference in lake phytoplankton. They also noted that when NO_3^- use did occur, its uptake and assimilation were slower than for NH_4^+ (Berman et al. 1984). If the Pamunkey phytoplankton depended on NH_4^+ for growth, then the supply ration of NH_4^+:P, which was below Redfield's N:P ratio of 16:1, would have explained the occurrence of N-limitation even though the combined N concentrations were high enough to predict P-limitation in the August, October, and November experiments (see Figures 2B + C). However, if NH_4^+ was primarily used by the Pamunkey phytoplankton, then their growth dependency should have been on the recycled extracellular pool. If the NH_4^+

supply was not sufficient for phytoplankton growth, then the NO_3^- which was present in higher concentrations should have been utilized and the pool dominance would have been for the new extracellular pool and no N-limitation should have been observed.

Examination of the cell abundances in the Pamunkey (Table 2) shows that the concentration of grazers (h-flags) remained stable in the first three experiments but doubled in the December experiment while abundances of the other cell types declined in December, presumably due to declining ambient water temperatures (Table 1). Also the biomass of the grazers remained constant in all four experiments while the biomass of all other cell types declined (Table 3). It, therefore, makes sense that the Pamunkey phytoplankton did not depend on a recycled extracellular nutrient pool in August, October, and November because the grazing community was not large enough to provide sufficient remineralization of N and P. In fact, production and grazing estimations for the Pamunkey (Table 5) show that the percentage of potential primary production grazed does not increase until December. Owens (1986) concluded that high turbidity regions of an estuary near the freshwater-saltwater interface provide the low light levels necessary for enhanced nitrification by chemoautotrophic bacteria. This may serve to further reduce NH_4^+ available for phytoplankton use. Add to this the high abundances and biomass of heterotrophic bacteria in the Pamunkey (Tables 2 and 3) and phytoplankton are subjected to increased competition for low available NH_4^+. In December, when the grazer population doubles (Table 2) and its biomass increases nine times in proportion to the phototrophic biomass (Table 3), the concentration and biomass of heterotrophic bacteria decline (Tables 2 and 3), the concentration of NH_4^+ doubles (Table 1), and recycled extracellular N and P pools dominate growth (Table 7 and Fig. 7). The increased river discharge in December (Fig. 8) explains the increase in NO_3^- concentration and the occurrence of extracellular pools (Table 7). Why available new extracellular pools of NO_3^- were not used by the Pamunkey phytoplankton in August, October, and November is less clear.

A possible explanation relates to the recent work of Martinez (1991), who observed the formation of N intracellular pools in the diatom *Skeletonema costatum* when an N starved culture was placed in a nitrate enriched medium and subjected to low irradiance. She postulated that the intracellular pools resulted as excess nitrate built up within the cells (Martinez 1991). The nitrate was in excess because NO_3^- reduction requires light and under the low irradiance conditions, the diatoms were unable to reduce the NO_3^- (Martinez 1991). Under conditions of light limitation the diatoms might also release nitrite, a product of incomplete reduction (Martinez 1991). In these experiments, the diatoms responded to recent N deprivation by rapidly taking up the newly abundant N in the form of NO_3^-; however, the cells were unable to fully reduce and use much of the NO_3^-, and the NO_3^- accrued within the cell (Martinez 1991). Under such light limiting conditions in a natural setting, phytoplankton whose growth ultimately depends on an oxidized new nutrient source, such as NO_3^-, will develop and depend on intracellular pools for growth over short periods of time. Since the Pamunkey river tends to be very turbid (see Secchi depths in Table 1), phytoplankton growth reliance on intracellular pools and the development of N-limited growth is explainable by this theory. However, it is important not to mistakenly consider the August, October, November phytoplankton to be highly nutrient stressed. The gross growth rate of the Pamunkey phytoplankton was highest in August and declined through November, stabilizing in December (Table 4). Likely, the late summer and early fall Pamunkey phytoplankton, due to: lower discharge (Fig. 8), greater proportional size of phytoplankton to grazer populations (Tables 2 and 3), low ambient NH_4^+ levels, high turbidity, and high temperatures (Table 1), took up NO_3^- faster than they were able to internally reduce and assimilate it, leading to the intracellular pool dependence and N-limitation.

As Figure 7 demonstrates, the pool dominance pattern for P was similar to that of N at each site. At the Pamunkey site, P concentrations remained constant throughout the study

(Table 1). As with N, P uptake largely follows Michaelis-Menten kinetics and increases with temperature (Nalewajko and Lean 1980). During the August, October, and November experiments, when N was limiting, P could have been taken up by the cells in excess of their need because insufficient N was available. Excess P is stored in intracellular pools as orthophosphate (Miyata and Hattori 1986). Eldridge (1989) demonstrated that phytoplankton will store P intracellularly under conditions of N-limitation. By December when river discharge increased (Fig. 8), increased levels of both NH_4^+ and NO_3^-, along with the other ecological changes described previously, led to P-limited growth. This observation is consistent with past seasonal observations made by Webb and Eldridge (1988) and Eldridge (1989) for the York river. Since P was limiting in December, intracellular stores depleted and growth became dependent on recycled and new extracellular P pools.

In contrast to the Pamunkey, grazing in the York consumed nearly all of the potential primary production (> 90%, compared to 50% in the Pamunkey) during August (Table 5). It follows that in the York, during the late summer, ammonium dominated the extracellular inorganic N (Table 1) and growth relied on recycled pools (Figure 7). Phosphorus recycles in an aquatic system more quickly than N, largely through remineralization by grazers (Lehman 1980, Lehman 1984, Eldridge 1989, Bloem et al. 1990, Moegenburg and Vanni 1991). King (1987) predicted that in steady-state ocean systems N released through grazing remineralization does not balance the actual algal losses incurred as a result of grazing. Consequently, in a stable community the growth needs for N must be augmented by periodic increases in algal nitrate uptake (Eppley and Peterson 1979, Eppley et al. 1983). Also, phytoplankton normally take up phosphate more quickly than they do N species (McCarthy 1980, Nalewajko and Lean 1980). In fact, a strong grazing community, by supplying more P than N, can relax P-limitation (Moegenburg and Vanni 1991). In systems which normally deplete dissolved N and P stores, a close coupling of

growth and grazing develops allowing for the transfer of N and P back into the dissolved pool (Eppley and Person 1979, Azam et al 1983, Caron et al. 1985, Gude 1985).

In the York, growth was N-limited during August, supporting King's (1987) conclusions that grazing does not return sufficient N to the system to meet growth needs. Also supporting this contention was that N dependent growth in August used new extracellular pools to a greater extent than did phosphorus dependent growth (Table 7). In the York, phototrophic biomass (Table 3) and growth rates (Table 4) in the late summer allowed an active grazing community to exist which severely cropped primary production and supplied the majority of N and P necessary to maintain the system. York River undergoes regular periods of stratification and destratification coupled with spring-neap tidal cycle (Haas 1977). In the warm summer months periods of destratification allow for the mixing of sub-pycnocline water and provides nutrients necessary to maintain the recycling dominated system (Webb and D'Elia 1980). However, in contrast to the Pamunkey, intracellular P pools did not develop concurrent with the N-limitation in August (Fig. 7). Likely, the temperature and light regimes were better for uptake and assimilation of both N and P and no detectable transient intracellular pools developed. That is, all nutrients available to the phytoplankton were taken up and assimilated into biomass, which may explain the high Chl-a concentrations (Table 5) and biomass (Table 3) in the York during August. It is also possible that the high grazing pressure made growth on intracellular nutrient pools unnecessary or quickly converted the intracellular nutrients to recycled ones through rapid biomass turnover.

Beginning in October experiment in the York, phytoplankton shifted their dominant nutrient pool dependency for both N and P from recycled extracellular to intracellular pools (Fig. 7). Decreases in temperature and light during October, November, and December caused declines in growth and grazing rates (Table 4) along with phytoplankton and grazer abundances and biomass (Tables 2 and 3). By November, grazing did not crop a

detectable percentage of the primary production (Table 5). The absence of grazing pressure by November meant biomass was not turned over and nutrient residence time within phytoplankton cells increased. Consequently, nutrients were not quickly returned to the dissolved pool but remained within cells forming intracellular pools. In fact, N and P intracellular pools were the sole nutrient supply for phytoplankton growth in November. Dissolved N and P levels in the York, however, remained roughly the same in all four experiments (Table 1) and with the lower growth rates (Table 4) and cell abundances (Table 2) extracellular N and P pools could have adequately supported growth in November and December. This was not seen, however, probably because uptake at lower temperatures was in excess of assimilation and phytoplankton growth, therefore, was buffered from short-term changes in the extracellular nutrient environment. Formation of intracellular pools allows phytoplankton to grow rapidly once the physical environment improves in the late winter or early spring (McCarthy 1980, Nalewajko and Lean 1980, Eldridge 1989). This also helps explain why the York, incontrast to the Pamunkey, never shifted from N-limitation to P-limitation, but rather, N-limitation reduced to a conditon of no limitation in December (Table 7). Because of low temperatures and growth rates, intracellular stores of both N and P were sufficient for balanced growth over the incubation period.

Overall, the results from this study demonstrate the importance of viewing the significant differences which exist between tidal freshwater and estuarine environments in terms of phytoplankton physiology and ecology and not simply in terms of chemical and physical characteristics. The tidal freshwater region of rivers experience complex physical, chemical and biological conditions and the success of phytoplankton in this region is fundamentally related to how the ecology of the system and the physiology of species diminish or enhance their ability to adapt to seasonal and annual changes in their physical and chemical environment. Complex aquatic systems, such as the tidal freshwater region of rivers, are understudied (Schuchardt and Schirmer 1991) but future studies of such

systems and their comparison to simpler systems, such as the lower estuarine region of the York, may serve to yield valuable information as to how ecology and physiology affect conditions of nutrient limitation, blooms, and eutrophication.

Figure Legends

Figure 1. Locations of the Pamunkey (A) and York (B) study sites on the York River System, Virginia, USA.

Figure 2. Changes in A) average temperature (♦) and chlorophyll-a, B) total dissolved inorganic N:P supply ratio, and C) NH_4^+ : PO_4^- supply ratio of surface water collected at the Pamunkey (o) and York (•) sampling sites from July 1990 to January 1991. These data were compiled from the 8 samplings of this study and 5 from a related project (Webb, Sieracki and Kindler, VIMS, ongoing). Curves depict general trends and dashed lines in B and C show the 16:1 N:P ratio.

Figure 3. Chlorophyll-a concentrations and apparent growth rates for surface water collected from the York site plotted against time. Data were compliled from a 72 h dilution incubation in late November 1990 and show a distinct lag period for phytoplankton growth between 0 and 24 h with a maximal growth rate between 24 and 48 h. Note that each growth rate represents the integrated change in cholorphyll-a concentration over the previous 24 h and is not an instantaneous growth rate.

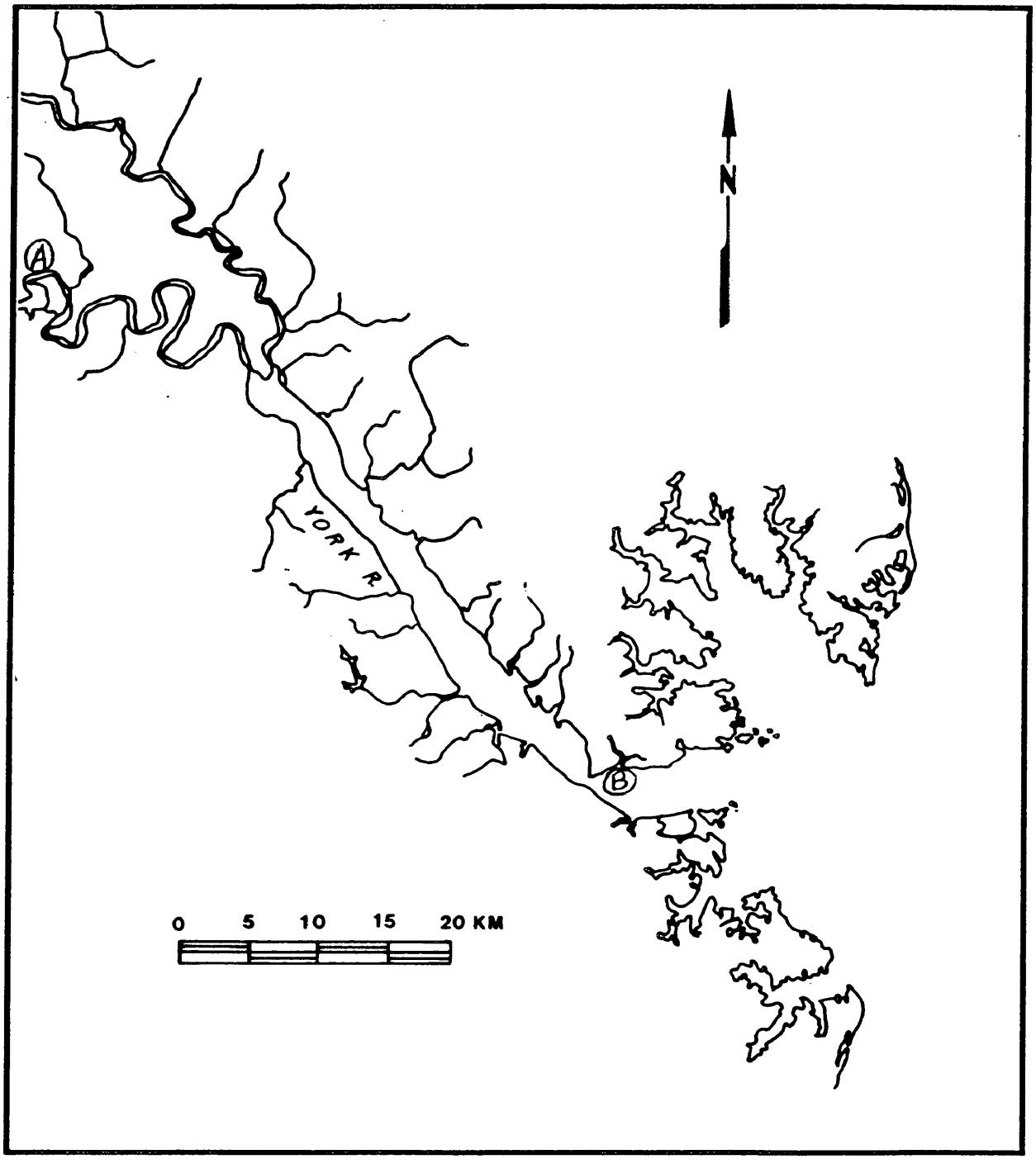
Figure 4. Apparent growth rates as a function of dilution (fraction of whole water chlorophyll-a) for the fully nutrient enriched treatments (+NP) in the Pamunkey (P) and York (Y) water. Lines show simple linear regressions and each box represents one of eight dilution experiments. Horizontal series of four graphs show the seasonal progression from August to December.

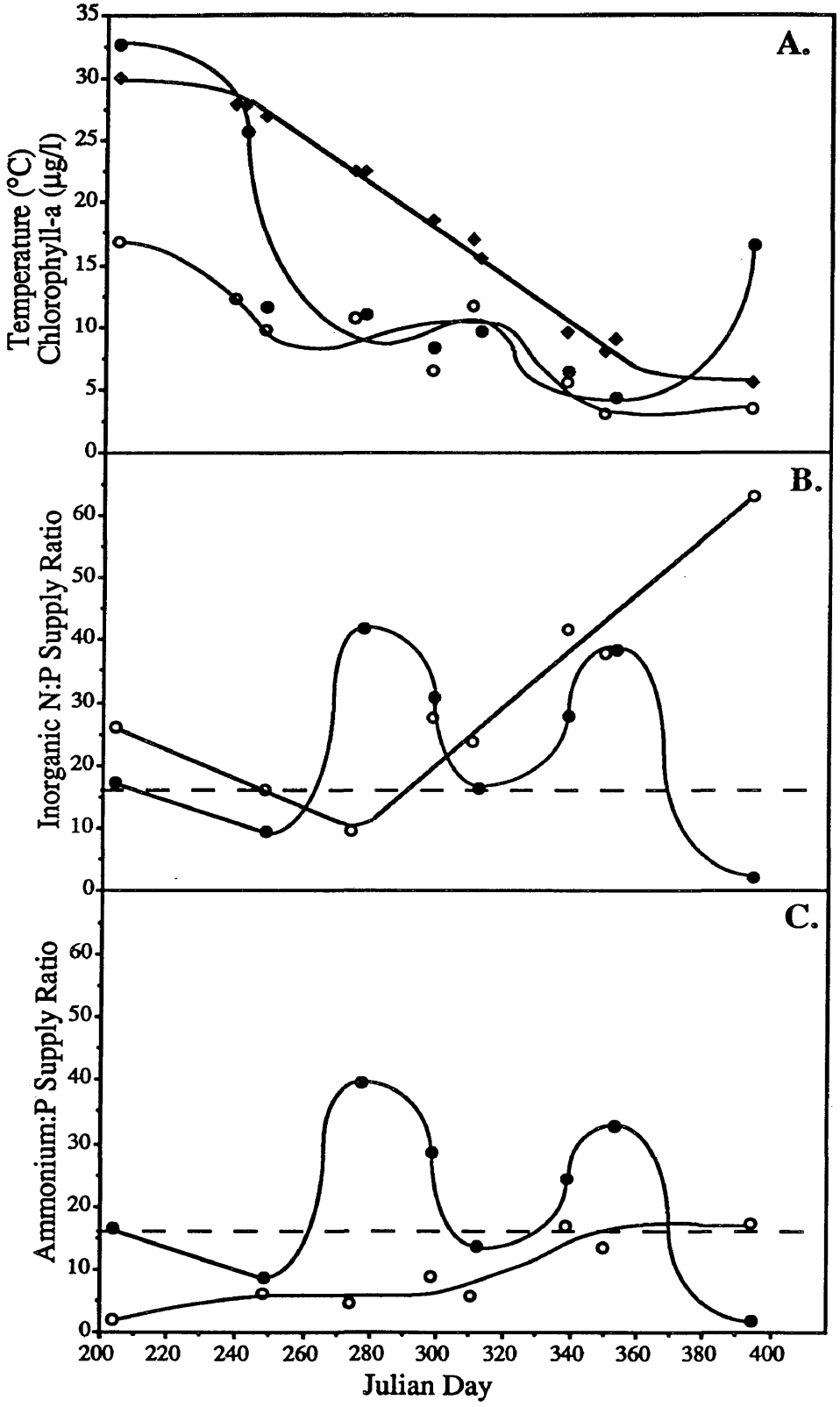
Figure 5. Grazing corrected growth rates as a function of dilution for phosphorus dependent growth (+N) in the Pamunkey (P) and York (Y) water. Lines represent the growth model determined by backwards multiple regression, circled data were not used in the regression. Graphs arranged as in Figure 4.

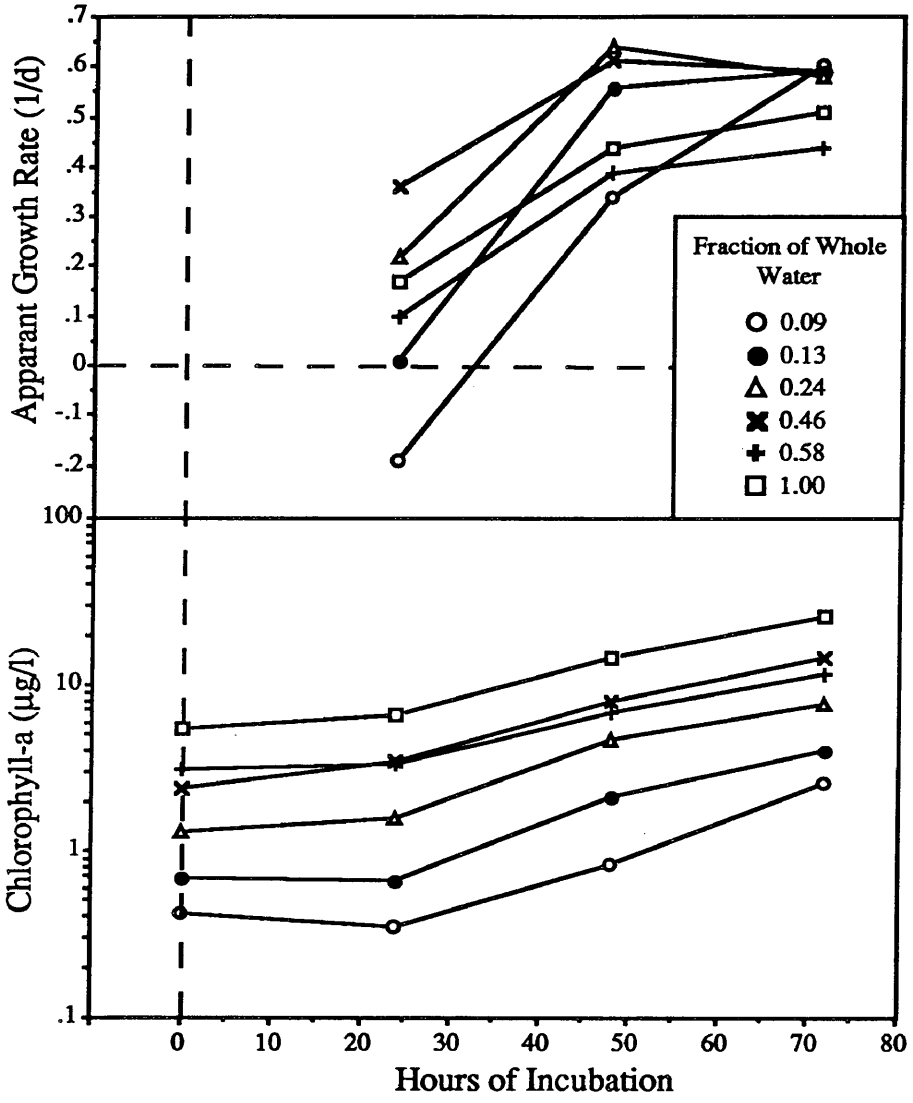
Figure 6. Grazing corrected growth rates as a function of dilution for nitrogen dependent growth (+P) in the Pamunkey (P) and York (Y) water. Lines represent the growth model determined by backwards multiple regression, circled data were not used in the regression. Graphs arranged as in Figure 4.

Figure 7. Proportions of growth as rate coefficients due to intracellular (white), new (black) and and recycled (grey) extracellular pools of phosphorus at the A) Pamunkey and B) York, and nitrogen at C) Pamunkey and D) York study sites.

Figure 8. Pamunkey River discharge data compiled by the U.S. Geological Survey, Richmond, Virginia, for water years 1990 and 1991. Units are in $m^3 s^{-1}$ and data is from March 1990 to February 1991. Data provided by the USGS for October 1990 to February 1991 are provisional and have not been officially released.


N
A
YORK R.
B
0 5 10 15 20 KM


A.
B.
C.
Temperature (°C)
Chlorophyll-a (µg/l)
Inorganic N:P Supply Ratio
Ammonium:P Supply Ratio
Julian Day
0
5
10
15
20
25
30
35
40
50
60
200
220
240
260
280
300
320
340
360
380
400


Apparant Growth Rate (1/d)
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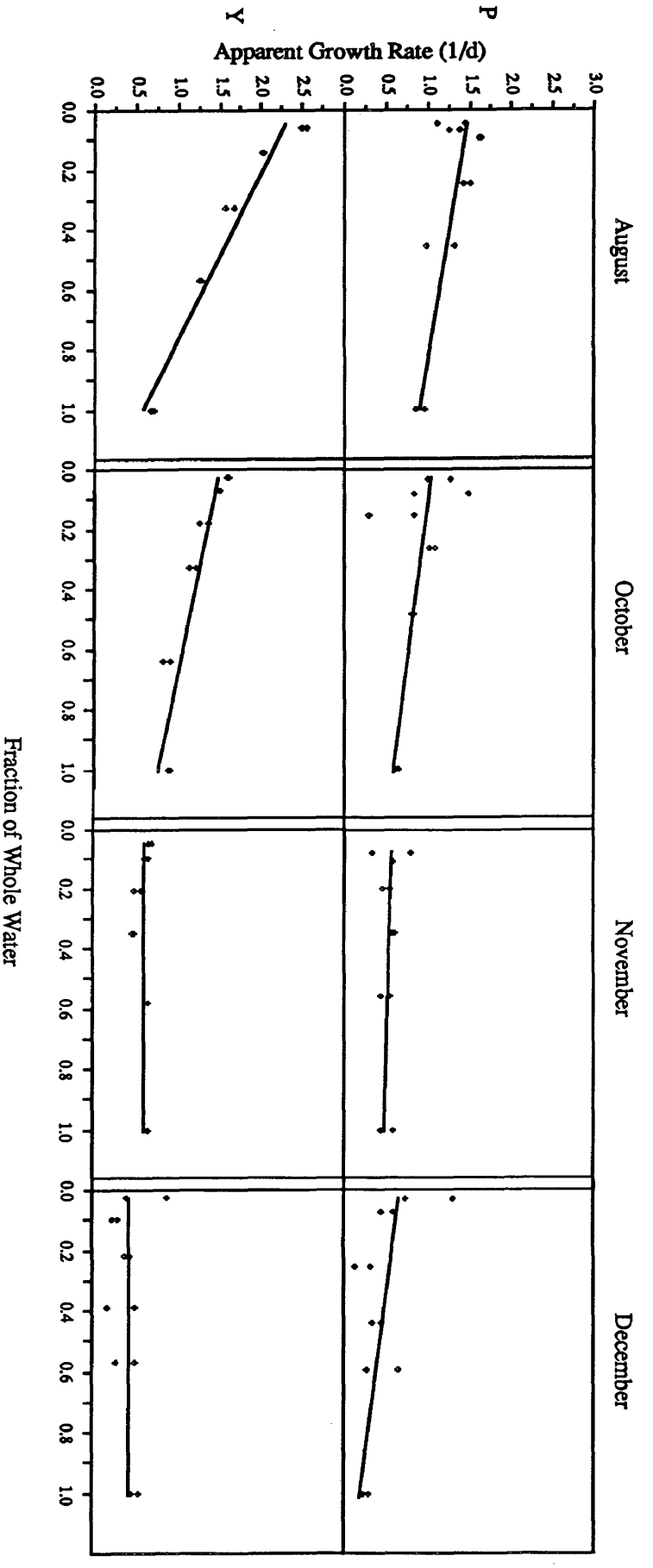
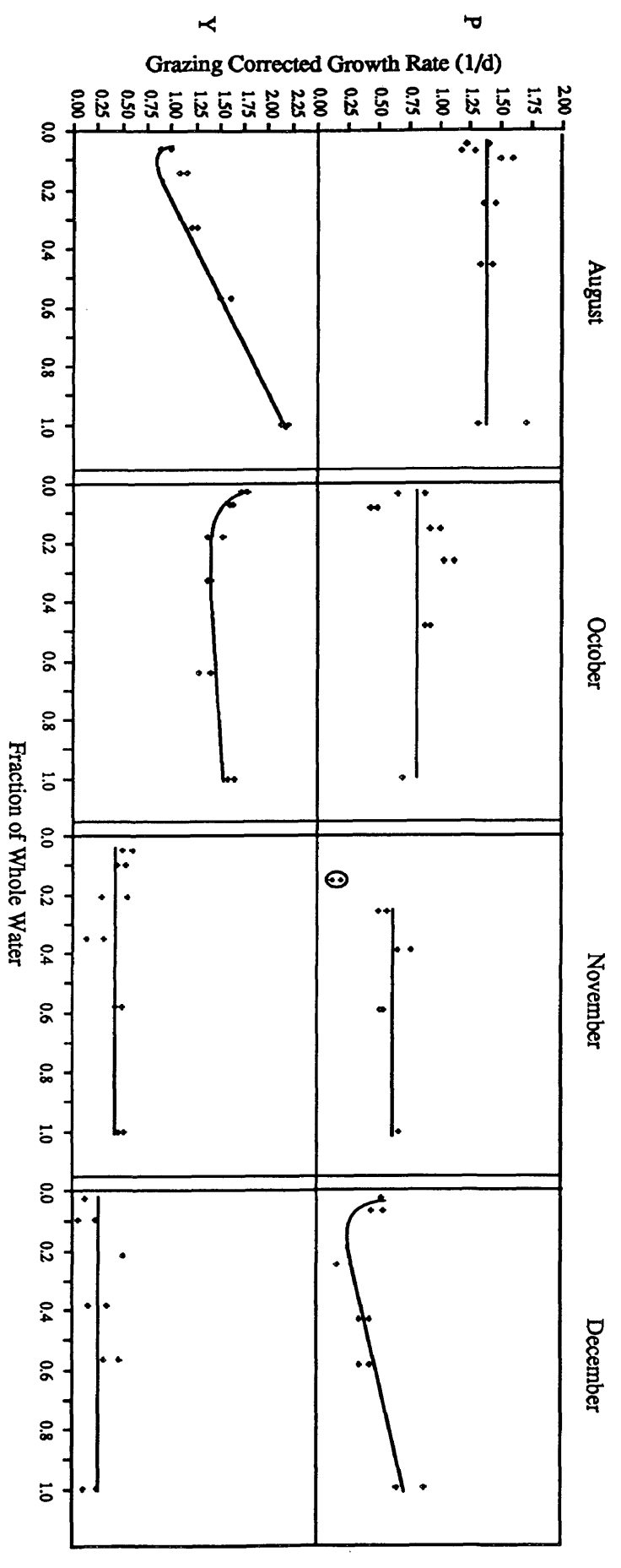
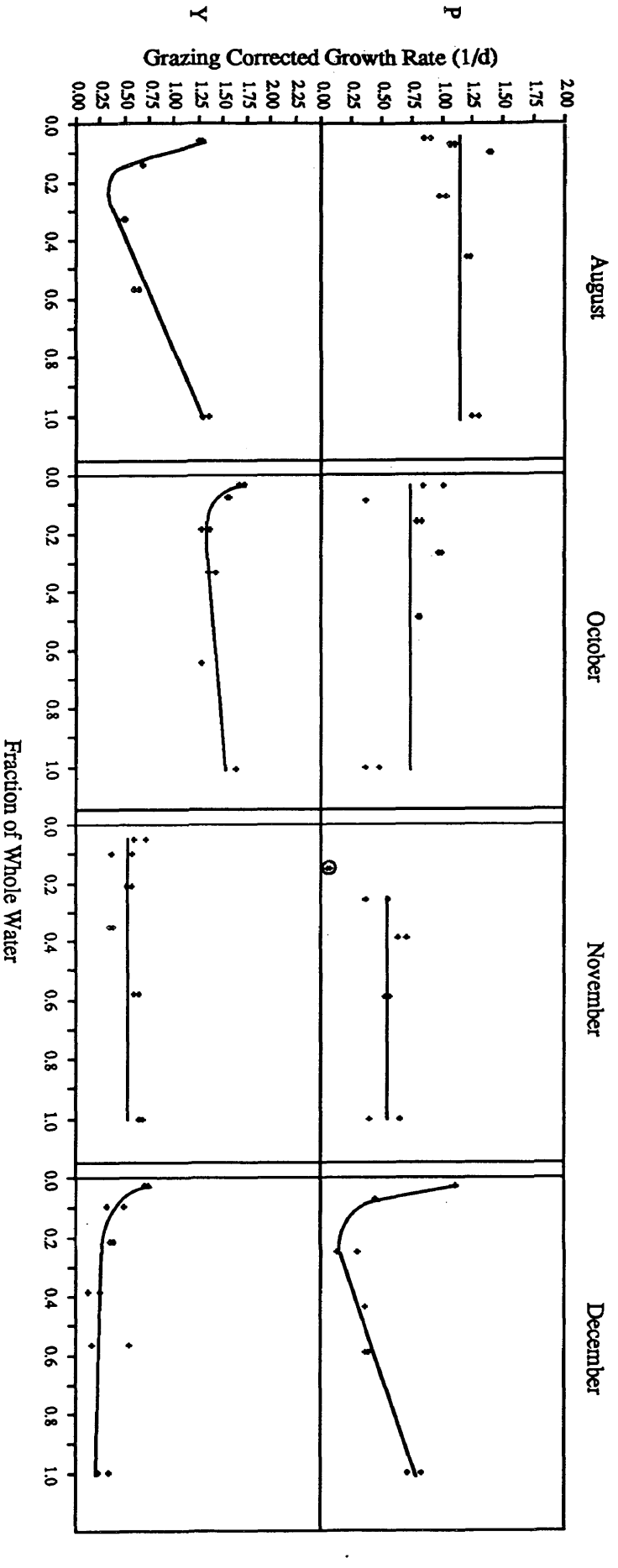
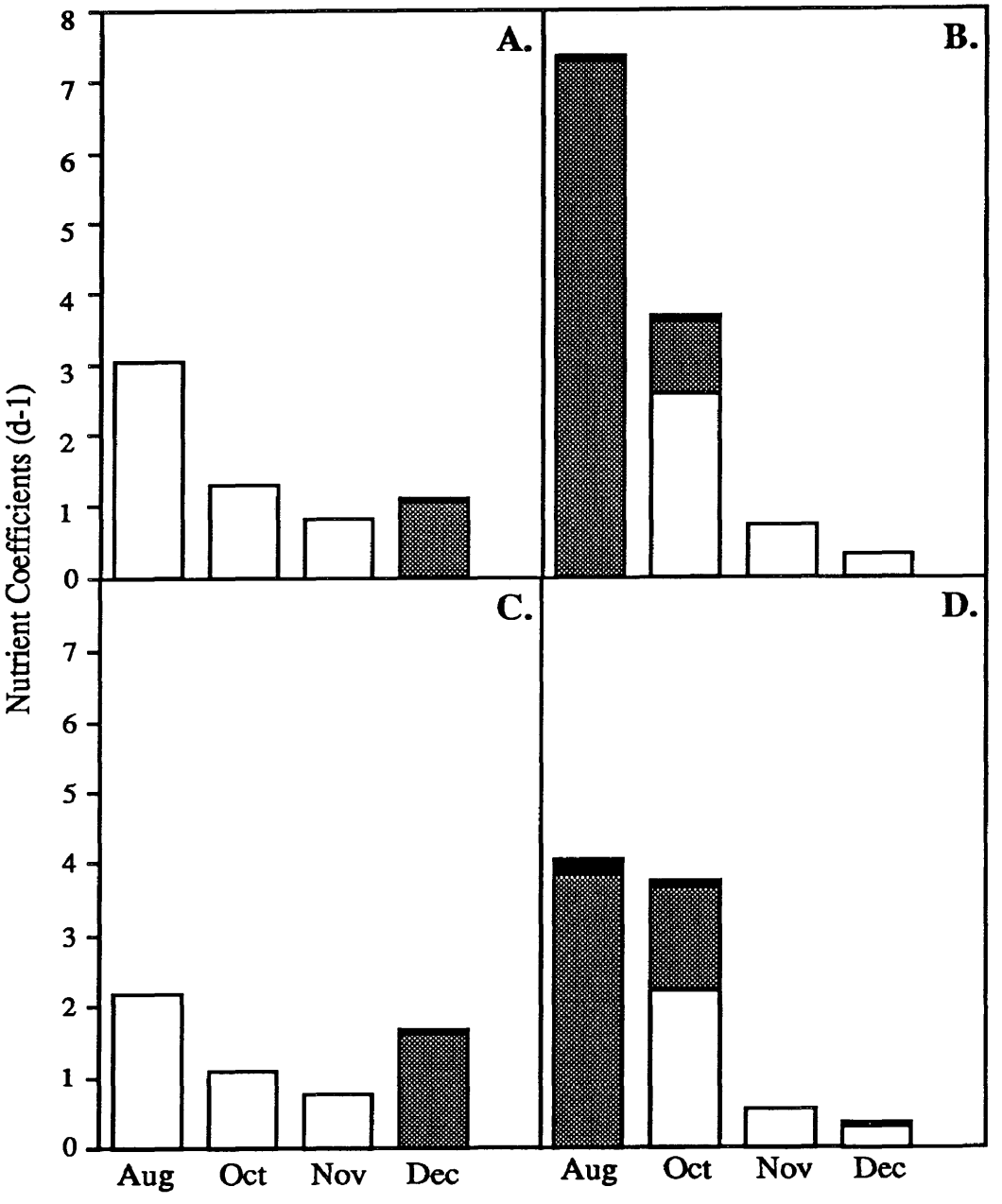
.6
.5
.4
.3
.2
.1
0
-.1
-.2
Fraction of Whole Water
0.09
0.13
0.24
0.46
0.58
1.00
Chlorophyll-a (µg/l)
100
10
1
.1
0
10
20
30
40
50
60
70
80
Hours of Incubation


Y
P
Apparent Growth Rate (1/d)
August
October
November
December
Fraction of Whole Water


P
Y
Grazing Corrected Growth Rate (1/d)
August
October
November
December
Fraction of Whole Water


Y
P
Grazing Corrected Growth Rate (1/d)
August
October
November
December
Fraction of Whole Water


A.
B.
C.
D.
Nutrient Coefficients (d-1)
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2
3
4
5
6
7
8
Aug
Oct
Nov
Dec
Aug
Oct
Nov
Dec

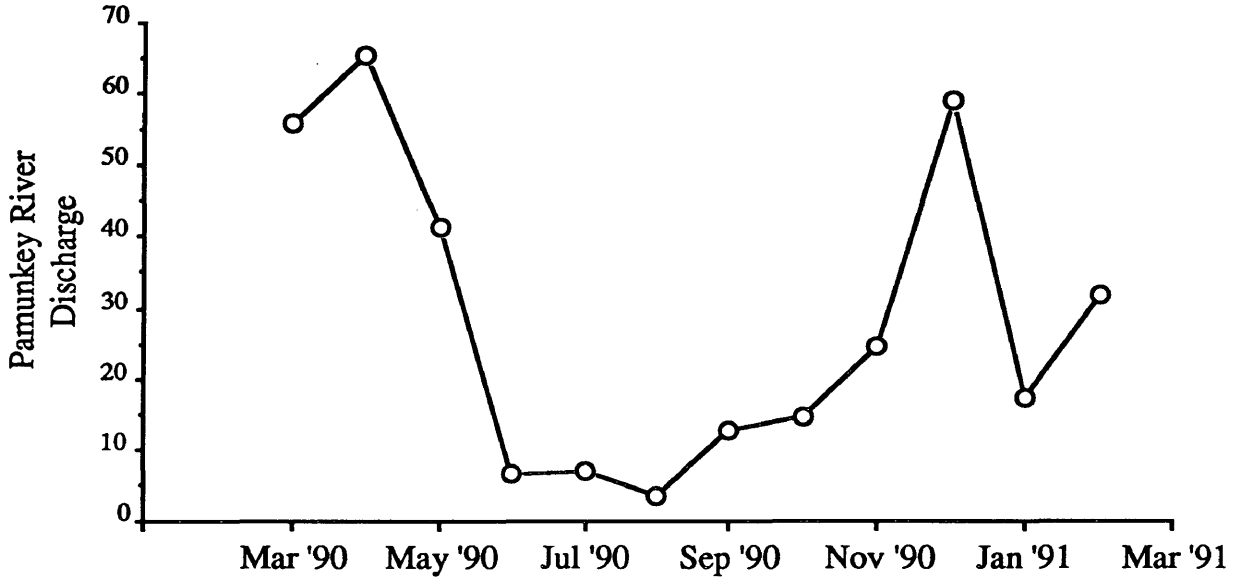
Pamunkey River
Discharge
70
60
50
40
30
20
10
0
Mar '90
May '90
Jul '90
Sep '90
Nov '90
Jan '91
Mar '91

Table 1. Physical and nutrient characteristics of the Pamunkey (Pam) and York sampling sites from July 1990 to January 1991. These data were compiled from the 8 samplings of this study and 5 from a related project (Webb, Sieracki and Kindler, VIMS, ongoing).

Sampling site	Sampling date	Temperature (degrees C)	Salinity (‰)	Light Atten (m^{-1})	PO_4 µM	NO_3 µM	NH_4 µM	%NH_4	Inorganic N:P
Pam	23 Jul	29.8	<1	5.7	0.12	2.76	0.24	7.8	25.9
	27 Aug†	28.0	<1	2.8	*	*	*	*	*
	05 Sept	27	<1	2.8	0.12	1.15	0.72	38.6	16.0
	01 Oct†	22.5	<1	2.4	0.11	0.56	0.51	47.7	9.50
	25 Oct	18.2	<1	2.8	0.15	2.72	1.25	31.5	27.4
	06 Nov†	17.0	<1	3.9	0.15	2.64	0.83	23.9	23.9
	05 Dec	9.5	<1	*	0.17	4.18	2.82	40.3	41.7
	16 Dec†	8.0	<1	4.3	0.18	4.34	2.35	35.1	38.0
	29 Jan	5.2	<1	5.7	0.13	5.99	2.22	27	63.2
York	25 Jul	29.0	*	*	0.14	0.10	2.24	95.8	17.1
	30 Aug†	28.0	22	2.2	*	*	*	*	*
	05 Sept	27.0	*	*	0.19	0.21	1.67	88.8	9.70
	04 Oct†	22.5	21	2.8	0.11	0.26	4.32	94.4	42.0
	25 Oct	19.0	*	*	0.26	0.61	7.35	92.3	31.1
	08 Nov†	15.5	19	*	0.17	0.41	2.35	85.1	16.3
	05 Dec	10.0	21	*	0.14	0.47	3.32	87.5	27.9
	19 Dec†	9.0	23	0.8	0.11	0.68	3.61	84.2	38.6
	29 Jan	5.5	15	1.0	0.16	0.11	0.28	71.8	2.44

*no measurement taken

†experiment dates

Table 2. Phototrophic and heterotrophic plankton cell abundances as determined by image analyzed fluorescence microscopy at the Pamunkey (Pam) and York sampling sites for the eight experiments.

Experiment		cell type classification (# ml^{-1})						
		Phototrophs					Heterotrophs	
site	month	cyano[1]	p-flag[2]	p-dino[3]	p-crypt[4]	diatoms	h-bact[5]	h-flag[6]
Pam	Aug	$2.97x10^5$	9,369	1,036	1,864	<69	$5.57x10^6$	4,833
	Oct	$2.11x10^5$	5,745	<130	370	2,867	$4.44x10^6$	4,624
	Nov	$2.68x10^4$	8,044	<99	1,679	3,259	$5.05x10^6$	3,456
	Dec	$3.02x10^3$	2,226	<32	191	803	$1.01x10^6$	6,164
York	Aug	$1.48x10^6$	21,197	1,839	2,574	18,756	$4.47x10^6$	8,827
	Oct	$6.89x10^4$	18,554	<248	990	4,703	$2.45x10^6$	8,664
	Nov	$1.40x10^4$	11,209	1,931	2,727	<113	$2.61x10^6$	3,181
	Dec	$1.80x10^4$	1,202	234	473	156	$2.09x10^6$	2,322

[1]cyano: chroococcoid cyanobacteria

[2]pflag: phototrophic flagellates

[3]pdino: phototrophic dinoflagellate

[4]pcrypt: phototrophic cryptophyte

[5]hbact: heterotrophic bacteria

[6]hflag: heterotrophic flagellates (includes all counted micro- and nano- sized heterotrophs)

Table 3. Phototrophic and heterotrophic plankton biomass community composition determined by image analyzed fluorescence microscopy at the Pamunkey and York sampling sites for the eight experiments.

Experiment		Biomass (μg C l^{-1})									
		Phototrophs					Heterotrophs		Totals		
site	month	cyano	p-flag	p-dino	p-crypt	diatom	h-bact	h-flag†	Σh	Σp	Σh:Σp
Pam	Aug	29.1	14.9	36.9	69.2	NE[1]	192*	8.67	201*	150	1.34
	Oct	20.8	11.4	NE[1]	6.41	53.0	284	13.2	297	91	3.26
	Nov	5.80	9.84	NE[1]	161	24.7	256	10.7	267	201	1.34
	Dec	0.80	2.49	NE[1]	2.81	14.4	58.3	9.97	68	21	3.23
York	Aug	416	85.0	47.8	15.5	208	157*	31.3	188*	772	0.24
	Oct	15.0	38.9	NE[1]	44.4	23.2	166	31.3	197	121	1.63
	Nov	4.14	12.7	38.1	276	NE[1]	176	58.7	235	331	0.71
	Dec	3.10	2.32	13.7	13.5	0.37	132	5.49	137	33	4.15

*DAPI slide used to size h-bact, all other h-bact measurements are from AO slides

†includes micro- and nano- sized heterotrophs

[1]NE - no cell counted on the slide, consequently, no biovolume or biomass estimates could be made.

Table 4. Phytoplankton growth and grazing rates determined from the fully nutrient enriched dilution bottles (+NP) for the Pamunkey and York sampling sites. Rates were determined by a simple linear regression of apparent growth rates against dilution where gross growth rate (k) is the y - intercept and grazing rate (g) is the negative slope, except where noted. Type one error was less than 15% ($\alpha \leq 0.15$).

Experiment			Growth and grazing parameters					
site	month	n	k (d^{-1})	g (d^{-1})	R2	F-test (p=)	r[1]	CV[2]
Pam	Aug	12	1.47	0.55	0.54	0.01	1.29±0.26	20.2
	Oct	12	1.04	0.42	0.22	0.13	0.90±0.31	34.9
	Nov	11	0.63	0.15	0.25	0.12	0.57±0.10	17.7
	Dec	12	0.64	0.45	0.25	0.10	0.46±0.31	68.5
York	Aug	10	2.38	1.79	0.94	<<0.01	1.63±0.66	40.7
	Oct	12	1.51	0.75	0.83	<<0.01	1.23±0.29	24.0
	Nov[3]	12	-	-	-	-	0.60±0.08	13.2
	Dec[3]	12	-	-	-	-	0.42±0.19	43.8

[1]r = mean apparent (measured) growth rate of all replicates and dilutions.

[2]CV - coefficient of variance

[3]Because of the poor significance for these two regressions (p>0.15), it was assumed that g = 0 and k = r.

Table 5. Bulk chlorophyll-a concentrations and 24 h production estimates (μg Chl-a l^{-1}) based on growth (k) and grazing (g) rates in the +NP treatments for the Pamunkey (Pam) and York sampling sites.

Experiment			Growth Estimates[1]				
site	month	Chl-a ± SD[2]	PP	RP	PG	SG	GE
		μg l^{-1}	μg l^{-1} d^{-1}	μg l^{-1} d^{-1}	%	%	%
Pam	Aug	12.3 ± 0.5	41.2	18.3	55.6	42.8	44.4
	Oct	10.8 ± 1.7	19.8	9.3	53.0	34.3	47.0
	Nov	11.7 ± 0.3	10.3	7.2	30.1	14.1	69.9
	Dec	3.10 ± 0.12	2.78	0.65	76.6	36.2	23.4
York	Aug	25.9 ± 0.5	254	20.8	91.8	83.3	8.18
	Oct	11.0 ± 0.7	38.8	12.5	67.8	52.8	32.2
	Nov[3]	9.80 ± 0.45	7.70	-	-	-	-
	Dec[4]	4.45 ± 0.38	2.32	-	-	-	-

[1]PP = potential production = $P_0 e^k - P_0$, where P_0 is the initial Chl-a concentration.

RP = real production = $P_0 e^{(k-g)} - P_0$.

PG = percent of PP grazed = $\left(\frac{PP - RP}{PP}\right)$ x 100%.

SG = percent of standing stock grazed $\left(\frac{PP - RP}{PP + P_0}\right)$ x 100%.

GE = growth efficiency = $\left(\frac{RP}{PP}\right)$ x 100%.

[2]SD - standard deviation (n = 3).

[3,4]Only PP estimates were calculated because grazing rates for these were below detection.

Table 6. Specific growth coefficients (rates) based on intracellular (K_i), new (K_e) and recycled (K_r) extracellular pools of nitrogen and phosphorus. Coefficients were obtained through backwards multiple regression of grazing corrected growth rates against dilution at a significance level of $\alpha<0.05$.

Experiment		Nitrogen Coefficients (d^{-1})			Phosphorus Coefficients (d^{-1})			
site	month	$K_i \pm SE$	$K_r \pm SE$	$K_e \pm SE$	$K_i \pm SE$	$K_r \pm SE$	$K_e \pm SE$	n
Pam	Aug	2.18 ± 0.16	NS	NS	3.08 ± 0.18	NS	NS	12
	Oct	1.10 ± 0.07	NS	NS	1.30 ± 0.14	NS	NS	12
	Nov	0.76 ± 0.07	NS	NS	0.84 ± 0.06	NS	NS	8
	Dec	NPS	1.60 ± 0.16	0.07 ± 0.01	NPS	1.07 ± 0.31	0.03 ± 0.01	10
York	Aug	NPS	3.84 ± 0.32	0.23 ± 0.02	NPS	7.69 ± 0.76	0.09 ± 0.04	10
	Oct	2.20 ± 0.21	1.44 ± 0.50	0.07 ± 0.20	2.59 ± 0.21	1.02 ± 0.50	0.07 ± 0.20	12
	Nov	0.55 ± 0.06	NS	NS	0.72 ± 0.06	NS	NS	12
	Dec	0.30 ± 0.09	NS	0.02 ± <0.01	0.32 ± 0.06	NS	NS	12

NPS - significant non-positive solution ($\alpha>0.05$, $K_x<0$).

NS - solution not significant ($\alpha>0.05$).

SE - standard error of the estimate.

Table 7. Contributions of total intracellular (I), and new (E) and recycled (R) extracellular nitrogen and phosphorus pools to chlorophyll-a production (μg Chl-a l^{-1} d^{-1}). The ratio of total nitrogen to phosphorus production (N:P) was calculated by summing the production of the three pools for nitrogen and dividing it by the same sum for phosphorus in each experiment.

Experiment		Nitrogen Dependent Growth				Phosphorus Dependent Growth				
site	month	I	R	E	total N	I	R	E	total P	N:P
Pam	Aug	26.8	*	*	26.8	37.8	*	*	37.8	0.71
	Oct	11.9	*	*	11.9	14.0	*	*	14.0	0.85
	Nov	8.89	*	*	8.89	9.83	*	*	9.83	0.90
	Dec	*	4.96	0.22	5.18	*	3.32	0.09	3.41	1.52
York	Aug	*	99.5	5.96	106	*	189	2.33	192	0.56
	Oct	24.2	15.8	0.77	40.8	28.5	11.2	0.77	40.5	1.01
	Nov	5.19	*	*	5.19	6.96	*	*	6.96	0.75
	Dec	1.34	*	0.09	1.43	1.43	*	*	1.43	1.00

*no significant contribution

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