

The Role of Picoplankton in Phytoplankton Dynamics
of a Temperate Coastal Plain Estuary

A Thesis

Presented to

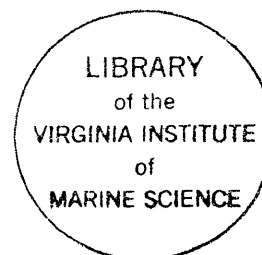
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of
Master of Arts

by:


Robert Triau Ray



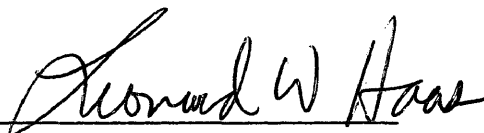
APPROVAL SHEET

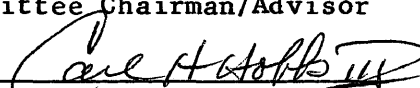
This thesis is submitted in partial fulfillment of
the requirements for the degree of

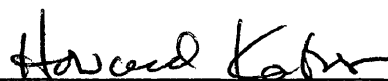
Master of Arts



Robert Triau Ray


Approved, August 22, 1986


Leonard W. Haas
Committee Chairman/Advisor


Carl H. Hobbs, III


Howard I. Kator


Gene M. Silberhorn


Kenneth L. Webb



Richard L. Wetzel

TABLE OF CONTENTS

	<u>PAGE</u>
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	viii
INTRODUCTION	2
MATERIALS AND METHODS	7
RESULTS	17
Physical Parameters	17
Standing Stock	19
Carbon Fixation	41
Diffusion Chambers	50
DISCUSSION	58
The Role of Picoplankton in Primary Production	58
Standing Stock	58
Carbon Fixation Rates	61
Diffusion Chamber Estimates	66
Plankton Dynamics in a Variable Vertical Salinity	
Structure Estuary	67
Physical Parameters	67
Standing Stock	68
Primary Production	74
CONCLUSION	77
LITERATURE CITED	79
VITA	85

ACKNOWLEDGMENTS

I gratefully acknowledge, first and foremost, the help, guidance and humor of Larry Haas. From the very beginning, this research would not have been possible without his assistance. I also wish to thank Ken Webb for his support as well as the other members of my committee.

Successful completion of this project was facilitated by Bob Lukens, Gary Anderson and Pat Hall in the Computer Center who were always there to help, even at a moment's notice.

I wish to thank all those others: students, staff and faculty who gave their support; especially John Zeigler and Hilary Neckles who served as inspiration and advisers. Finally, I thank Fran who had to live with a student for so long. She is always there with encouragement, support and understanding (not to mention her editing).

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Mean organism densities determined by epifluorescence microscopy	21
2. Values used for calculating cellular carbon	34
3. Mean carbon biomass determined from mean cell volumes and literature volume to carbon values	37
4. Assimilation values for greater than 3 um and less than 3 um fractions by fraction, day and intensity	46
5. Carbon specific growth rates of greater than 3 um and less than 3 um autotrophs based on ¹⁴ C uptake values and a 12 hour photo-period	49
6. Photosynthesis versus irradiance slopes (alpha) for greater than 3 um and less than 3 um fractions ...	51
7. Bacterial numbers and coefficients of variation in replicate chambers after 24 in situ incubation ...	52
8. Carbon : Chlorophyll values from growth chamber experiments after 24 hour incubation	54
9. Autotrophic growth rates derived from chlorophyll and carbon changes over a 24 hour incubation period in diffusion chambers	55
10. Bacterial intrinsic rate of increase, mean growth and grazing coefficients and coefficient of variation from growth chamber experiments	56

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Location of sampling site	8
2. Diffusion chamber design	14
3. Physical parameters during 1985 sampling season	18
4. Surface chlorophyll <i>a</i> values	20
5. Red-fluorescing cyanobacteria abundances	22
6. Orange-fluorescing cyanobacteria abundances	24
7. Diatom abundances	25
8. Autotrophic Flagellate abundances	26
9. Dinoflagellate abundances	27
10. Other autotroph abundances	29
11. Heterotrophic bacteria abundances	30
12. Heterotrophic flagellate abundances	31
13. Other heterotroph abundances	33
14. Plankton carbon biomass, total and less than 3 μm fraction	35
15. Phytoplankton carbon biomass, total and less than 3 μm fraction	36
16. Picoplankton contribution to total autotrophic carbon biomass	38
17. Cyanobacteria biomass contribution to picophytoplankton community	40
18. Photosynthetic carbon fixation	42
19. Picophytoplankton contribution to carbon fixation	44
20. Chlorophyll specific carbon fixation at 293 and 54 $\mu\text{E m}^{-2} \text{s}^{-1}$	47

<u>Figure</u>	<u>Page</u>
21. Chlorophyll specific carbon fixation at 43 and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$	48
22. Surface chlorophyll and tidal height by day	69
23. Cyanobacteria abundances and tidal height by day	72

ABSTRACT

The contribution of picoplankton ($< 3.0 \mu\text{m}$, $> 0.2 \mu\text{m}$) to phytoplankton production was examined in the lower York River estuary during the summer of 1985. This estuarine system oscillates between a homogeneously mixed and stratified water column in conjunction with the neap-spring tidal cycle and permitted observation of the effect of mixing on phytoplankton.

Picoplankton made up 7% of the seasonal autotrophic biomass. Absolute and relative estimated autotrophic carbon contribution by the picoplankton was greatest in the first half of July, when 10 - 16% of the $750 - 1050 \mu\text{g C L}^{-1}$ was in less than 3 μm fraction. Phycocyanin containing chroococcoid cyanobacteria were the major carbon biomass contributors to the autotrophic picoplankton. Seasonally, phycoerythrin containing cyanobacteria were approximately 1/4 as abundant as the phycocyanin containing forms. Together they represented 60% of the estimated carbon biomass in the autotrophic picoplankton.

At approximately 15% surface light irradiance autotrophic picoplankton were responsible for 9% of the total autotrophic carbon fixed. At 1 - 2% of surface light irradiance the autotrophic picoplankton increased their contribution to 13% of the total carbon fixed. At 15% surface light irradiance, larger phytoplankton had significantly higher chlorophyll specific carbon uptake rates than picophytoplankton, throughout the season (seasonal means of 5.17 and $2.50 \mu\text{g C (ug Chl)}^{-1} \text{ hr}^{-1}$, respectively). At ca. 1 - 2% surface light irradiance, chlorophyll specific carbon uptake rates of the 2 groups was not significantly different (means ranged 1.13 - 2.16). Carbon specific growth rates (day^{-1}) of the picophytoplankton (seasonal mean of 1.15) were significantly higher than the larger phytoplankton (seasonal mean of 1.02). The slope of the chlorophyll specific carbon uptake versus irradiance line at light limited photosynthetic levels for the greater than 3 μm phytoplankton was not significantly different ($P < .05$) than the picophytoplankton slope (mean seasonal alpha values of 0.06 and 0.05, respectively).

Picoplankton and larger autotrophs decreased in abundance in surface waters as destratification occurred (Δ salinity less than 1.0 ppt) both autotrophic. As the water column restratified, the phytoplankton increased in number, with increases of 3 - 154% in the picoplankton and increases in the larger phytoplankton of 10 - 664%. Chlorophyll specific carbon uptake rates indicated a trend towards higher rates at the end of the destratification cycle and lower rates during periods of stratification and initial destratification.

THE ROLE OF PICOPLANKTON IN PHYTOPLANKTON DYNAMICS
OF A TEMPERATE COASTAL PLAIN ESTUARY

INTRODUCTION

Estuaries are highly productive environments, harboring many commercially important animals. Atlantic coast estuaries serve as nursery and juvenile feeding grounds for the majority of Atlantic coast commercially exploited fish species (Levinton 1982). Primary production by phytoplankton is of major importance in temperate estuarine environments because of its role in the the base of the food web (Haines 1979, Thayer et al. 1978, Kemp and Boynton 1981).

Despite the extensive studies on temperate estuarine phytoplankton (see review by Boynton et al. 1982), the community is not comprehensively understood. Tidal dynamics, salinity and light gradients, basin morphology, changing nutrient conditions, vertical mixing and varying fresh water inputs all contribute to a complex physical-chemical system. This complexity is reflected in the phytoplankton community which is also affected by biological factors (e.g. grazing and mortality). To enumerate all species-species and species-environment interactions is not currently possible. One approach to understanding the dynamics of such a system is to identify those components of the system that play a major role in terms of overall energy flow. Thus, rates of energy flow between components need to be examined, and some assessment made of their importance to the system.

Size groupings are a useful approach for describing different components of the plankton community. Takahashi and Bienfang (1983) found that size fractionation of subtropical Hawaiian waters separated phyletic groups which were quite invariant. Li et al. (1983) suggested analysis based on size has benefits in that: (1) it is measurable, (2) it has value as a physiological scale, and (3) it has been used as the basis of a hypothesis of pelagic ecosystem structure. Size generally correlates inversely with growth and metabolic rates of organisms (Ikeda 1970, Fenchel 1974, Sheldon 1984). Trophic relationships are also indicated as the size of an organism limits the size of available prey or potential for osmotrophy.

Recently, investigators have suggested that picophytoplankton (i.e. < 2.0 μm) make a substantial contribution to primary production in oceanic waters (Johnson and Sieburth 1979, Waterbury et al. 1979, Li et al. 1983, Platt et al. 1983, Murphy and Haugen 1985). Available evidence to date suggests that the importance of picophytoplankton increases in community primary production in oligotrophic offshore waters relative to coastal waters and at low light intensities. These investigators have reported percent contribution to total chlorophyll *a* by the < 2.0 μm size fraction in oceanic environments ranges from 25 to 90 percent. Primary production by the picophytoplankton has been reported to range from 20 to 80 percent of the total carbon fixation.

The phytoplankton of Chesapeake Bay have been studied for at least 60 years (Wolfe et al. 1926, Cowles 1930), but the emphasis in the past has been on net plankton, or larger planktonic organisms.

Only in the last 25 years have the smaller phytoplankton been suspected of being an important component of the community (Patten et al. 1963). Studies in the last 15 years have demonstrated the importance of estuarine nanoplankton (i.e. smaller than 20 μm) (Van Valkenburg and Flemmer 1974, McCarthy et al. 1974, Sellner 1983). McCarthy et al. (1974) reported that plankton $< 10 \mu\text{m}$ contributed 81% of the phytoplankton biomass as measured by chlorophyll *a*, and 94% of the bay primary production during one summer cruise. These values are representative of typical late spring and summer conditions when total phytoplankton biomass is relatively low (i.e. chlorophyll *a* values of 2-15 $\mu\text{g Chl}_a \text{ L}^{-1}$).

Most Chesapeake Bay phytoplankton studies have been conducted on a seasonal basis, but significant hydrological changes can occur over a relatively short time period (Haas et al. 1981a). The spring-neap tidal cycle in the James, York, and Rappahannock Rivers has been observed to affect the vertical water column salinity structure, with greatest mixing and thus vertical salinity homogeneity about 4 days after spring tides (Haas 1977). Top to bottom salinity difference was observed to be a good indication of vertical water column stability. Haas (1975) noted a fortnightly periodicity in phytoplankton biomass and productivity in the York River coincident with the spring-neap tidal cycle.

The significance of picophytoplankton in estuarine systems has not been established, but picophytoplankton in general, and Synechococcus type cyanobacteria in particular, have been observed in Atlantic coast estuaries (Waterbury et al. 1979, Campbell et al.

1983). Perkins et al. (1981) observed orange fluorescing (under blue light excitation) unicellular cyanobacteria in a study of the Chesapeake Bay picoplankton between 1978 and 1980. Near the mouth of the bay, numbers ranged from 2×10^3 cells ml^{-1} in midwinter to 3.5×10^5 cells ml^{-1} in late summer-early fall. At the mouth of the York River, Virginia, concentrations ranged from 1×10^2 (midwinter) to 6×10^4 cells ml^{-1} (late summer-early fall). Two isolates were established in unialgal cultures and were determined to be species of Synechococcus.

Cyanobacterial cells obtained in the middle of the Chesapeake Bay and observed with epifluorescence microscopy fluoresce an orange color which is also typical of oceanic forms (Haas, personal communication). A 1983 transect of the James River revealed a decrease in orange fluorescing forms and a concomittant increase in red fluorescing forms with decreasing salinity (Haas, personal communication). Red-fluorescing (phycocyanin containing) Synechococcus forms have been isolated (WH5701 and WH8101) and observed in the Carmens River estuary (Long Island Sound) and New York Harbor, in abundances ranging $0.1-6 \times 10^3$ cells ml^{-1} during spring and summer (Campbell et al. 1983) but were always a minor portion of the total cyanobacteria population.

It is possible that picophytoplankton production constitutes a significant portion of the total plankton primary productivity at times of high abundance. The production of the picoplankton has implications relating to the estuarine food web, as nanoplankton grazing allows for the transfer of this energy to larger zooplankton (Haas and Webb 1979), while larger phytoplankton are probably

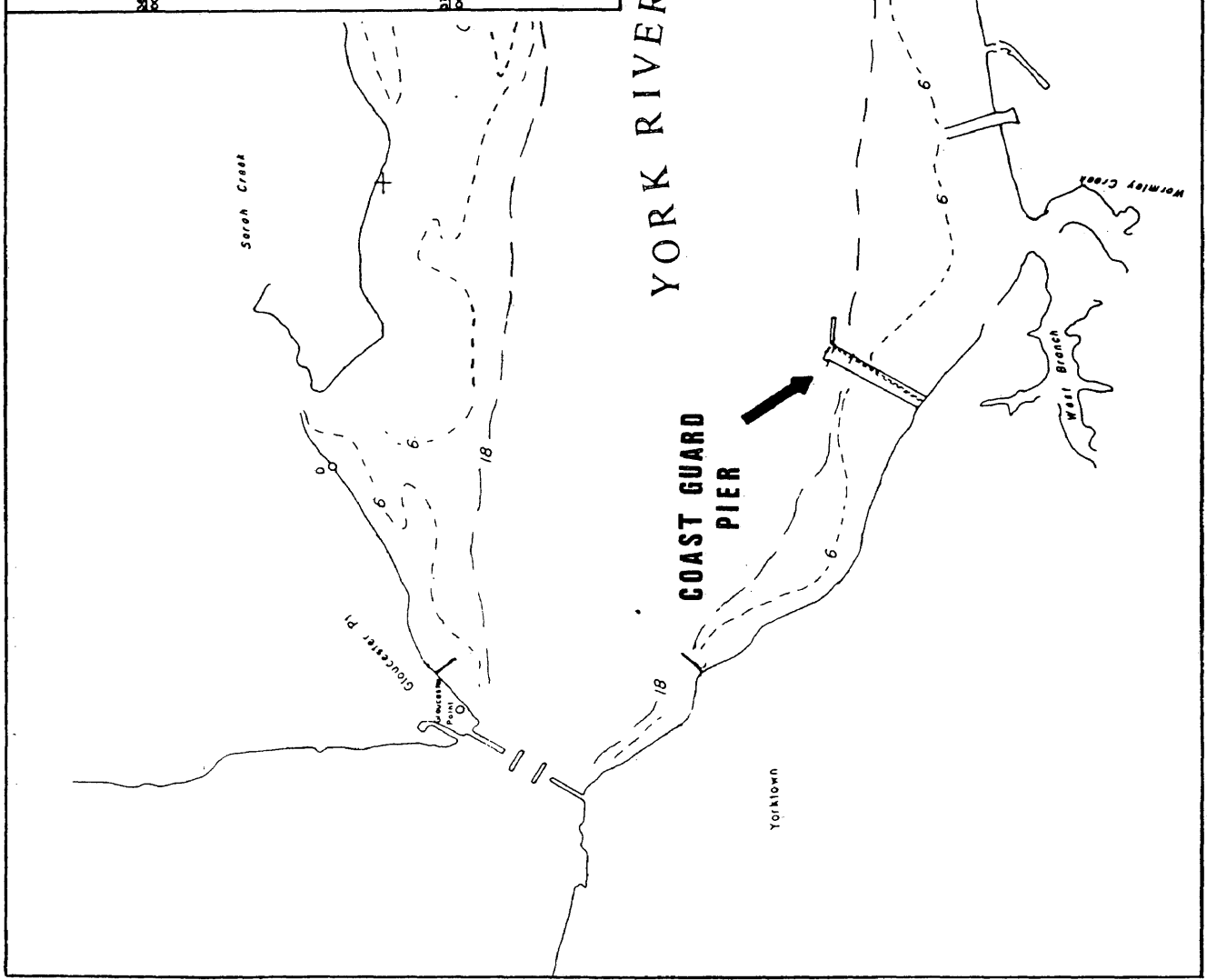
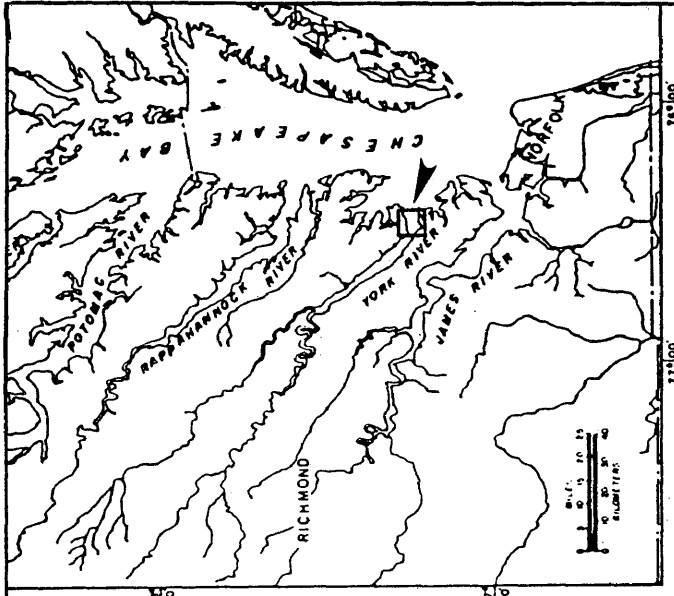
unavailable to these organisms. Grazing by nanoheterotrophs may also control cyanobacterial populations, decreasing the likelihood of cyanobacterial blooms.

The role of picophytoplankton (in this study defined as the 0.2 to 3.0 μm autotrophic plankton) in temperate estuaries has not been well documented. The purpose of this study was to determine the relative contribution of the picoplankton to overall phytoplankton dynamics in the York River Estuary at a time when they have been observed in the past to increase in numerical abundance. Observations included abundances and biomass estimates of the picoplankton and total plankton community, carbon fixation rates, and growth rates and grazing rates upon the less than 3.0 μm size fraction. The biweekly oscillation of the York River Estuary between a vertically stratified and homogeneous water column allowed for observations of the effects of water column mixing upon the summer phytoplankton community.

MATERIALS AND METHODS

The study area, the lower York River, is a sub-estuary of the Chesapeake Bay, a temperate coastal plain estuary (Pritchard 1965). Sampling was conducted at the Yorktown Coast Guard Pier (Fig. 1) during July, August, and September 1985. Water depth at the end of the pier (ca. 13 m) permitted sampling below the pycnocline. Water samples were collected at 1, 3, 5, and 12 m depths 3 times per week at slack water before ebb tide. The top 3 samples were obtained using a Van Doren bottle. The 12 meter sample was obtained using flexible plastic tubing with an intake 1 meter above the bottom, a vacuum flask and hand vacuum pump. Temperatures of the 1,3, and 5 m samples were measured to the nearest .05 °C with a stem thermometer. Sub-samples were taken for laboratory salinity determination with a Beckman Induction Salinometer model RS-7B. Downwelling irradiance was measured at the time of sampling using a Li Cor LI 188 Integrating Photometer and a Lambda Instrument Corporation Quantum submersible flat cosine sensor. The mean light attenuation coefficient (k) was calculated (using a linear regression and 6 light measurements between 0.5 and 5 meters below the surface) where $k = -\ln(I_z/I_0) / z$, and I_z and I_0 are irradiance at depths z and 0,. The 1% light level depth was calculated substituting k into the regression. Equal volumes of water from the 1, 3, and 5 meter depth samples were combined as a composite surface sample and returned to the lab for analysis.

Figure 1: Location of sampling site: Yorktown Coast Guard Pier,
Yorktown, Virginia, U.S.A.



The composite surface sample was partitioned into 3 size fractions: total (unfiltered), less than 15 μm (using a 15 μm Nitex sieve), and less than 3 μm (using 47 mm diameter, 3 μm pore size Nuclepore filters and vacuum filtration of < 10 cm of mercury). Chlorophyll *a* concentrations of each of the surface size fractions and the 12 m sample were determined using a DMSO/acetone extraction procedure (Hayward and Webb, unpublished). Water samples (5 ml) were filtered through 25 mm Whatman glass fiber filters (GF/F) and the filters placed in light-proof, capped 10 ml vials containing 8 ml of extraction fluid (45% DMSO, 45% acetone, 10% distilled H_2O , with 0.1 % DEA). The samples were kept in the dark at room temperature for 5 days before being read on a Turner Designs fluorometer.

Identification, enumeration, and biomass estimates of the plankton community were performed using epifluorescence microscopy (Haas 1982). Samples (2 ml) of each fraction were stained with proflavin (0.033% w/v in distilled water, 0.2 μm filtered, 25 $\mu\text{l ml}^{-1}$) and fixed with gluteraldehyde (6.0% v/v in 0.2 μm filtered river water, 50 $\mu\text{l ml}^{-1}$). Samples were filtered on to 25 mm diameter, 0.2 μm pore size Nuclepore filters prestained in Irgalin Black (Watson et al. 1977). Filters were placed on glass microscope slides with low fluorescence immersion oil (Cargill type LF) above and below the filter. Number 1 1/2 coverslips were applied to the filters and the slides were stored at -12°C prior to counting. Counts were made with a Zeiss Standard microscope equipped with a Planapochromat 100X, 1.25NA oil immersion objective, 10X calibrated ocular (100 μm X 100 μm grid with 5 μm divisions) and a HBO 50 W high pressure mercury lamp. Two filter sets were used for epifluorescent observation. The first

had an excitation range in the blue wavelengths- exciter filter BP 450-490, chromatic beam splitter FT 510, and barrier filter LP 520 (Cat. no. 48 77 09). With this filter set heterotrophic organisms (eucaryotes and procaryotes) fluoresced green due to the proflavin stain. Autotrophic and heterotrophic eucaryotes were differentiated on the basis of the red autofluorescence of the chlorophyll *a* of the autotrophs. The second filter set had an excitation range in the green wavelengths- exciter filter BP 510-560, beam splitter FT 580, and barrier filter LP 590 (Zeiss Cat. no. 48 77 14). This filter set allowed for easy visualization of the red-fluorescing cyanobacteria, which were often not readily apparent under the first filter set. Orange autofluorescence was an indication of phycoerythrin containing cyanobacterial strains while red autofluorescence indicated phycocyanin containing strains (Campbell et al. 1983).

Organisms were counted and classified according to 9 types: heterotrophic bacteria, heterotrophic flagellates, other heterotrophs, orange fluorescing cyanobacteria, red fluorescing cyanobacteria, diatoms, autotrophic flagellates, autotrophic dinoflagellates and other autotrophs. For heterotrophic bacteria, between 10 and 40 lines (100 μm X 5 μm area per line) were scanned. For cyanobacteria, between 10 and 40 grids (1 X 10^4 μm^2 area per grid) per slide were counted depending on the concentration of organisms. For eucaryotic organisms, between 20 and 100 fields per slide were counted. A micro-computer (Panasonic Sr. Partner) with remote keypad (Computerwise model no. TT5-001) was used for entering counts and calculating cell densities using software written in Pascal and developed in conjunction with Bob Lukens. Biomass estimates were based on cell

densities, average cell volume calculations, and literature values of carbon : volume ratios. The appropriate dimensions of at least 100 randomly selected cells of each category counted were measured, the volume of each cell calculated and the mean cell volume derived (Sournia 1978). Organic carbon content for each category was calculated based on the estimates of Eppley et al. (1970) for non-diatom eucaryotes, Taguchi (1976) for diatoms, and Bratbak and Dundas (1984) for bacteria.

Productivity studies utilizing ^{14}C were performed weekly on composite total and composite less than 3 μm fraction samples. In an effort to minimize diel effects on productivities, samples were collected between 05:30 and 06:30 AM, Eastern Daylight Savings Time (not necessarily slack water) and incubations begun between 07:30 and 08:30 AM. Chlorophyll determinations and slide counts on the samples were performed as described above. The surface composite total fraction was filtered through a 73 μm Nitex sieve to remove larger heterotrophic grazers. Glass liquid scintillation vials (ca. 23 ml) were filled two thirds full with sample, inoculated with 100 μl of $\text{NaH}^{14}\text{CO}_3$ (2.5 μCi activity, Lot no. 2645135, Cat. no. 17441H, ICN) in distilled water with 2.0% NaCl and NaOH (pH 11.4), then filled full with sample and capped. Samples were then immediately incubated at in situ temperature (controlled by a flowing water bath system) under 4 light intensities (Durotest Ultra High Output Vita-Lights, attenuated with neutral density screening) and dark control vials were included. Light intensities in the 4 incubator compartments were measured with a surface flat cosine sensor and Li Cor Integrating Photometer prior to the experiments. Measurements were taken without water in the

incubator and indicated light intensities of 293, 54, 43, and 28 $\mu\text{E m}^{-2} \text{sec}^{-1}$ in the different compartments. After 4 hours the vials were removed from the incubator, 5 ml of sample were withdrawn and 4 ml of methanol:glacial acetic acid (95:5 v/v) added. The vials were placed in a drying oven at 60°C and contents allowed to evaporate. When dry, the material was resuspended in 10 ml of distilled water and 10 ml of Aquasol-2 (New England Nuclear). After 36 hours, the samples were counted in a Beckman LS-150 Liquid Scintillation counter. The external standard channels ratio (ESCR) method was used to establish a quench curve (Hendee, 1973), and ^{14}C uptake rates ($\mu\text{g C L}^{-1} \text{hr}^{-1}$) were calculated using the equation of Parsons et al. (1984). Total CO_2 was determined using method 7.2 in Parsons et al. (1984).

Chlorophyll specific uptake rates ($\mu\text{g C } (\mu\text{g Chl}_a)^{-1} \text{hr}^{-1}$) were determined for the greater than 3 μm fraction by subtracting the mean carbon uptake in the less than 3 μm fraction from the total fraction uptake, subtracting the mean chlorophyll value for the less than 3 μm fraction from the total fraction chlorophyll value, and dividing the greater than 3 μm uptake value by the greater than 3 μm chlorophyll value. Analysis of variance was performed on logarithmically transformed uptake and assimilation values (which helped normalize the data and created homogeneous variance) using SPSS-X. The T method (Tukey's Honest Significant Difference) was used to establish the minimum significant range for unplanned comparisons (Sokal and Rohlf, 1981). Unless otherwise noted, the alpha level was set at .05.

Estimates of phytoplankton growth rates were made using the ^{14}C uptake and slide count data. Assuming a 12 hour light period and linear uptake of CO_2 over time, carbon uptake per autotrophic carbon

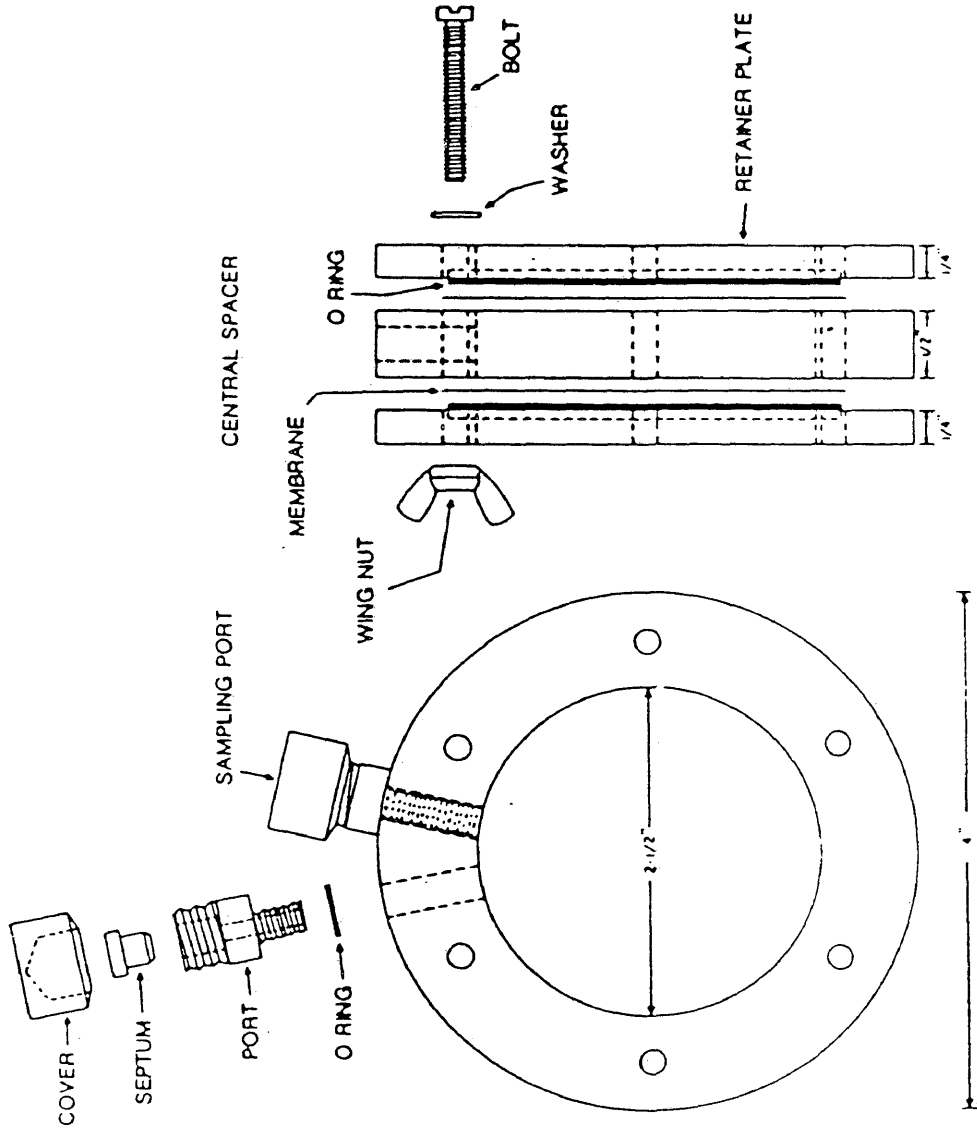
biomass was calculated for each incubated light intensity. The Kruskal-Wallis non-parametric test for differences between means (Sokal and Rohlf 1981) and the Rank Sum non-parametric test for difference between 2 means (White modification of the Wilcoxon Rank Sum test, Ambrose and Ambrose 1978) were used to examine significant differences in growth rates.

Chlorophyll specific carbon uptake rates at the 3 lower light levels were used to calculate alpha values, the initial slope of the photosynthesis versus irradiance curve. Alpha values were obtained for the greater than 3 μm and less than 3 μm phytoplankton. Linear regression (Least Squares Method, using Lotus 1-2-3 version 2.0 spread sheet software) was used to calculate alpha, and the line was forced through the origin.

Growth and grazing mortality rate estimates of the heterotrophic and autotrophic bacteria were made using dilution chamber experiments (Landry et al. 1984). Diffusion chambers (Rhodes and Kator 1983; Fritz-Thomson 1986), were constructed of Lexan polycarbonate, with an inside diameter of approximately 55 mm and a width of approximately 18 mm, containing a water sample of approximately 40 ml (Fig. 2). The sides were fitted with 0.2 μm Nuclepore filters to allow exchange of dissolved substances with the ambient environment. Chambers were incubated in situ for 24 hours at a depth of approximately 0.5 m using a floating chamber-holding apparatus. Duplicate chambers were filled with either a surface sample, a 3 μm filtered surface sample, or a 50% unfiltered and 50% 3 μm filtered surface sample. Growth and grazing rates were determined using the size fractionation technique of Landry et al. (1984). With the assumption that the density of

Figure 2: Diffusion chamber design. Approximate holding capacity 40 mls. (dimensions in inches)

DIFFUSION CHAMBER



phytoplankton, P , at time, t , can be described by the exponential equation $P_{(t)} = P_{(0)} e^{(k-g)t}$, where k = growth rate and g = grazing mortality rate, one can algebraically manipulate the equation to isolate k and g :

$$e^{(k-g)t} = P_{(t)} / P_{(0)}$$

$$(k-g)t = \ln (P_{(t)} / P_{(0)})$$

$$k - g = \ln (P_{(t)} / P_{(0)}) / t$$

r , the intrinsic rate of increase or net growth rate is equal to $(k - g)$. $P_{(t)}$ and $P_{(0)}$ were determined with epifluorescence microscopy. Growth and grazing mortality rates were obtained by solving two equations simultaneously using $P_{(t)}$ values from chambers with different degrees of grazing pressure in each equation. Differential grazing pressure was achieved by 3 μm filtration which removed a portion of the grazing community as determined by epifluorescence counts of heterotrophic flagellates. Three assumptions are implicit in the calculation of the coefficients: (1) that prey growth rates are independent of grazing pressure or prey density; (2), that grazing rates are linearly related to prey density; and (3), that the density of phytoplankton, P , at time, t , can be described by the exponential equation $P_{(t)} = P_{(0)} e^{(k-g)t}$. The equation:

$$r = \ln (N_t / N_0) / t$$

where N_t and N_0 are carbon or chlorophyll values in the chambers at time t and time 0, respectively, was also used to calculate less than 3 μm and greater than 3 μm autotrophic intrinsic rates of increase.

Four chamber experiments were carried out in late August and September 1985, using samples collected as previously described. Enumeration of the organisms was carried out as previously described.

Chlorophyll *a* concentrations in the chambers after the 24-hour incubation were measured using the DMSO/Acetone extraction procedure. Growth and grazing mortality coefficients were determined for heterotrophic bacteria, orange fluorescing cyanobacteria and red fluorescing cyanobacteria. The Kruskal-Wallace non-parametric test for significant difference between means and the Wilcoxon non-parametric two sample test (Sokal and Rohlf 1981) were used to examine the chamber data and the alpha value was set at .05 unless otherwise noted.

RESULTS

PHYSICAL PARAMETERS

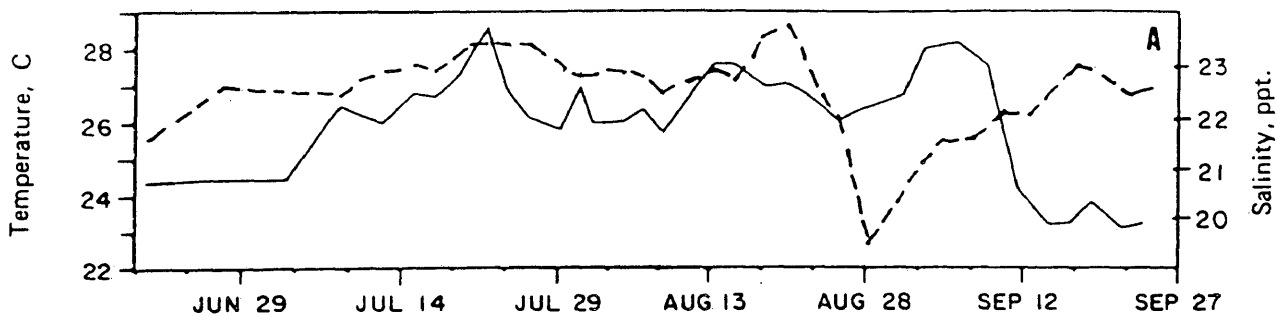
Measured physical parameters during the study are given in figure 3. Mean surface water temperature (Fig.3A) at the beginning of the sampling period rose from 24.5 °C on July 3 to a high of 28.6 on July 22. Water temperature then fluctuated between 25.7 and 28.0°C until September 7, after which the temperature declined to approximately 23 °C .

Mean surface salinity (1,3, and 5 meter values averaged) was 22.54 and ranged from 19.49 to 23.69 ppt., with lowest values occurring August 28 - September 7 (Fig.3A). Delta salinity (bottom salinity minus mean surface salinity) ranged from -0.01 to 5.25 ppt (Fig.3B). Highest delta salinities occurred on the same dates as low mean surface salinities. Daily highest tide height (Fig.3c) data was obtained from the East Coast of North and South America Tide Table 1985 High and Low water predictions for Hampton Roads (Sewells Point).

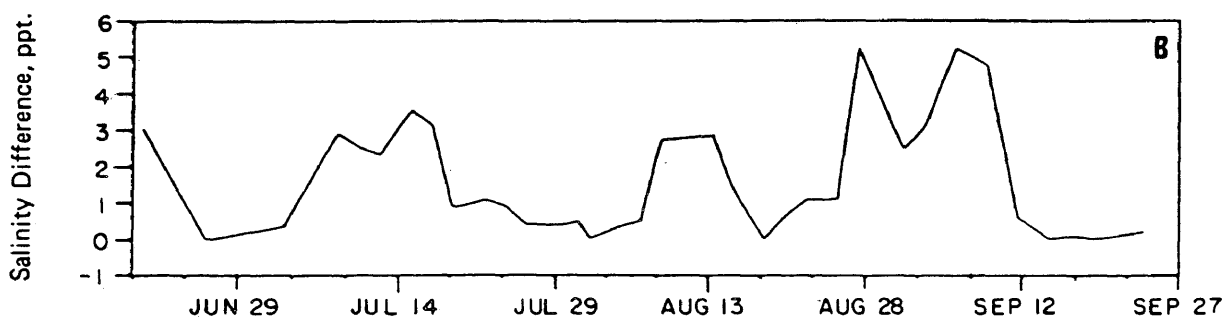
The mean light attenuation during the study was 1.11 (\pm 0.22 1 Standard Deviation). Two periods of relatively high values ($k > 1.25$) occurred: July 26 - August 7; and September 7 - September 13. One period of relatively low light attenuation (k less than 0.85) occurred - July 12 through July 14. The 1% light level depth (Fig.3D) ranged between approximately 2.5 and 6.5 meters.

- Figure 3: Physical parameters, Summer 1985
- A. Mean surface water temperature (degrees centigrade) and surface salinity (parts per thousand)
 - B. Delta salinity (parts per thousand)
 - C. Height of highest daily tide (meters above mean low water)
 - D. One percent light level depth (meters) from calculated light attenuation coefficient

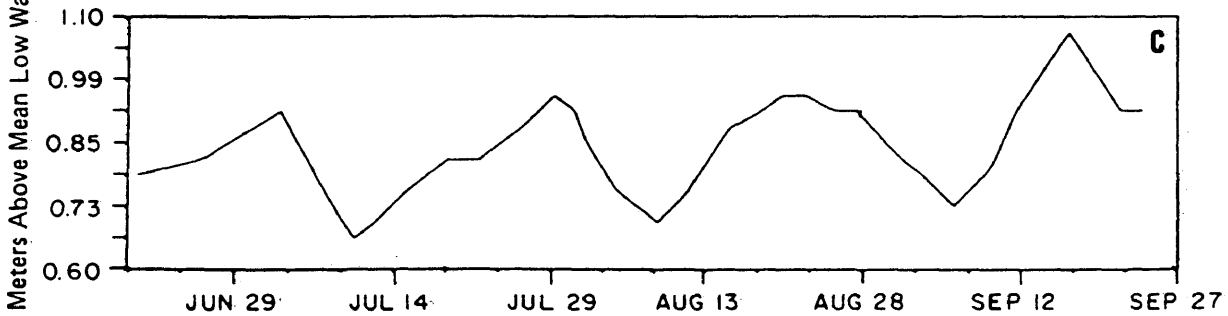
MEAN SURFACE WATER TEMPERATURE AND SALINITY



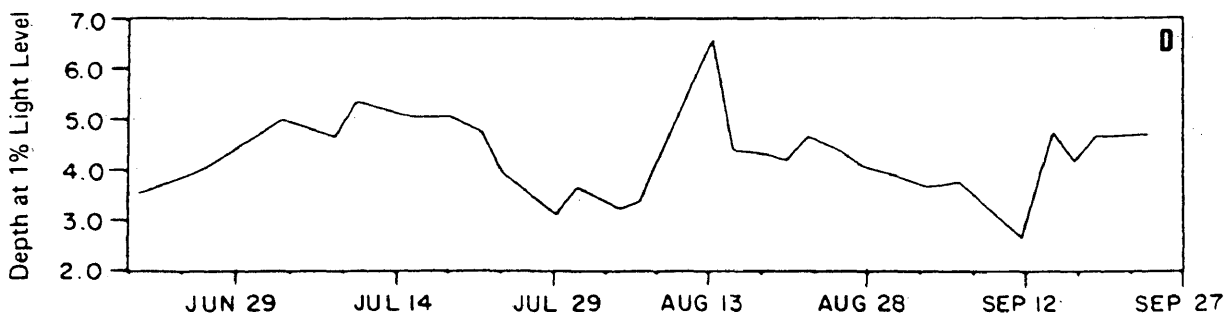
TOP TO BOTTOM SALINITY DIFFERENCE



HEIGHT OF HIGHEST TIDE



DEPTH AT 1% LIGHT LEVEL



Chlorophyll *a* values for the various size fractions are shown in figure 4. Chlorophyll *a* in the unfractionated samples ranged from 4.7 to 46.5 $\mu\text{g L}^{-1}$, with a mean of 14.4 $\mu\text{g L}^{-1}$. Increases and decreases in chlorophyll concentration over time in all three fractions appeared to co-occur. Major peaks in the total chlorophyll *a* values occurred on July 19-24, August 2-7 and September 2-4. Peaks during these periods also occurred in the less than 3 μm fraction chlorophyll values (Fig.4). The seasonal mean of the less than 15 μm fraction chlorophyll was 8.0 $\mu\text{g Chl}_a \text{ L}^{-1}$, or 56% of the mean total chlorophyll *a*. The mean percentage of chlorophyll in the less than 3 μm fraction over the sampling season was 18.03% (\pm 8.115 l Standard Deviation) of the unfiltered samples.

STANDING STOCK

Mean concentrations of autotrophic and heterotrophic plankton are presented in Table 1. Organism densities during the study are shown in Figures 5-13. Red-fluorescing cyanobacteria were the most numerous autotrophic component of the plankton, ranging in densities from 1.7×10^4 to 7.2×10^5 cells ml^{-1} (Fig.5). They were most abundant during 3 periods: July 8-17, August 9-12 and August 28 - September 13. Red-fluorescing cyanobacteria were retained by the 3 μm Nuclepore filter to a higher degree during the latter two periods of relatively high densities.

Orange-fluorescing cyanobacteria attained their maximum densities early in the study (July 8-24 with $5 - 7.5 \times 10^4$ cells ml^{-1}) and generally decreased thereafter with densities ranging between 2-3 X

Figure 4: Chlorophyll a values from surface composite samples.
Total, less than 15 um and less than 3 um fractions.

SURFACE CHLOROPHYLL A VALUES

SUMMER 1985

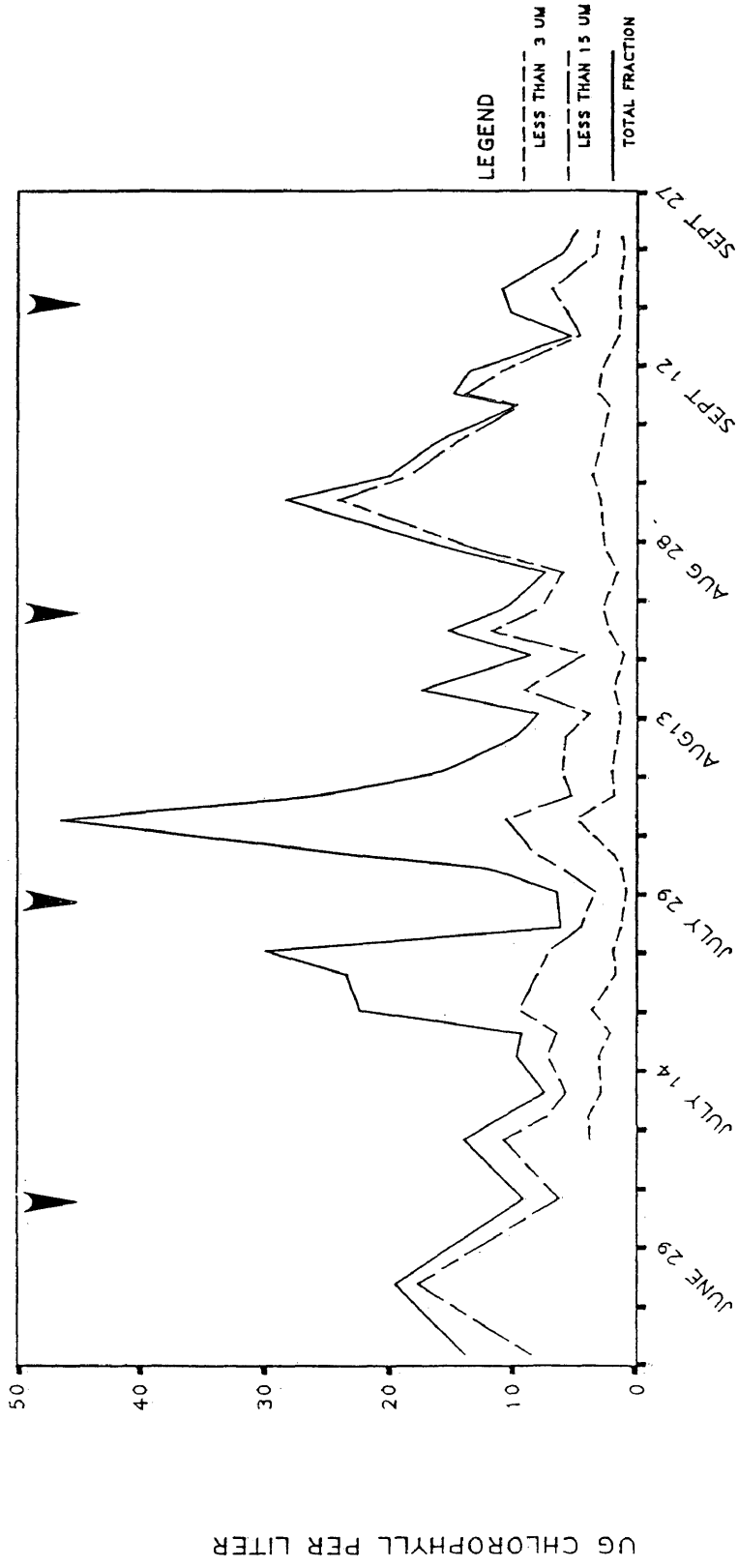


TABLE 1

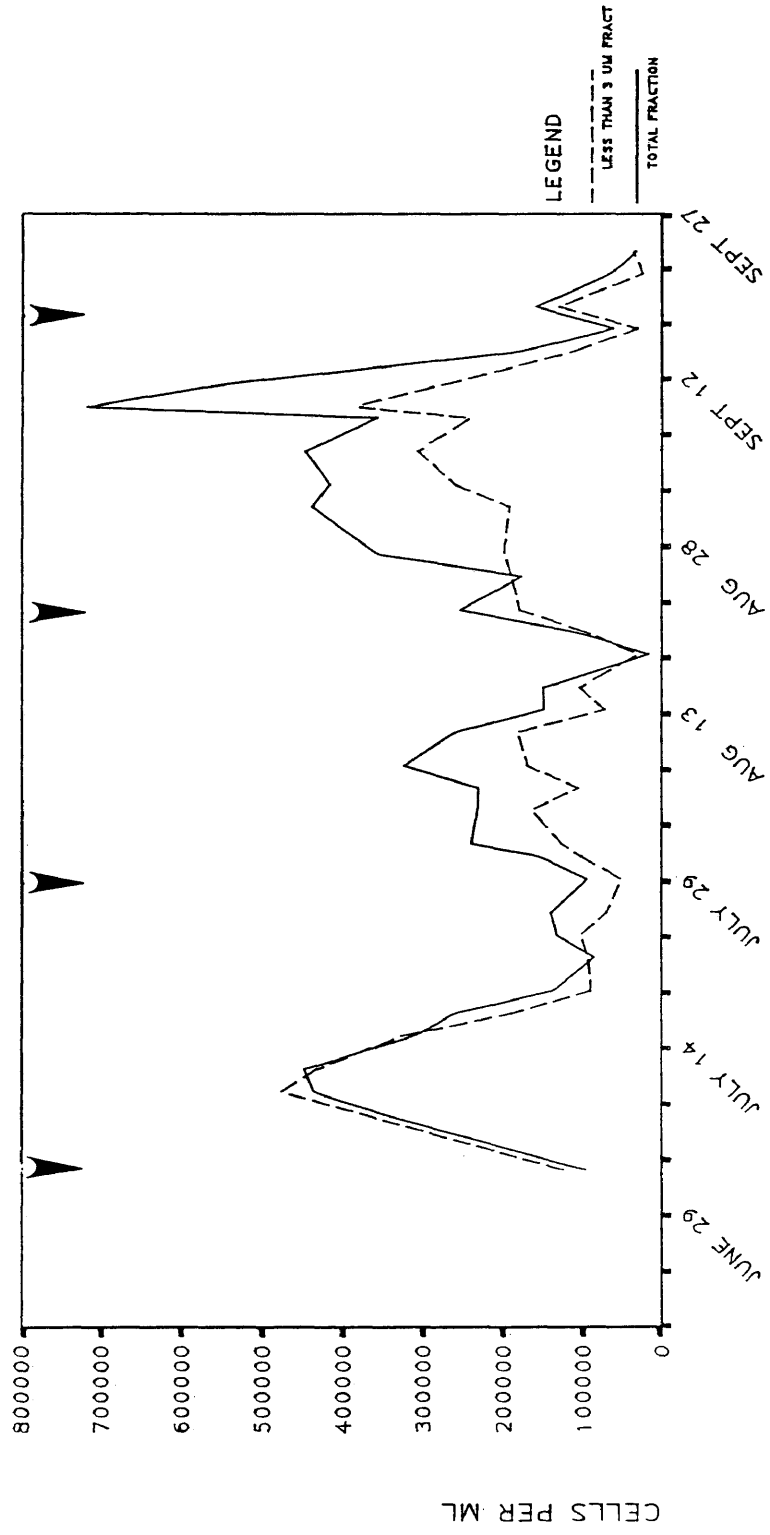
Mean organism densities determined by epifluorescence microscopy
 Summer, 1985 (N = 35)

ORGANISM	MEAN (PER ML)	RANGE	MEAN % IN < 3 UM SAMPLE
Red fluorescing cyanobacteria	244317	16549 - 719746	68
Orange fluorescing cyanobacteria	31523	5361 - 72720	63
Diatoms	12183	1085 - 45275	16
Autotrophic flagellates	4832	964 - 11567	31
Autotrophic dinoflagellates	634	0 - 2610	23
Other autotrophs	706	0 - 2827	38
Heterotrophic bacteria	4482515	1426439 - 8222999	95
Heterotrophic flagellates	2807	771 - 6105	63
Other heterotrophs	679	77 - 2142	46

Figure 5: Red-fluorescing cyanobacteria abundances in surface total and less than 3 um fractions. Arrows at top of figure indicate day of monthly highest tidal height.

RED-FLUORESCING CYANOBACTERIA TOTAL AND LESS THAN 3 UM FRACTIONS

SUMMER 1985



10^4 cells ml^{-1} for August and September (Fig.6). One other major peak occurred August 2-5 with densities of $3.9 - 5.7 \times 10^4$ cells ml^{-1} . The contribution of orange-fluorescing cyanobacteria to the total cyanobacteria averaged 27% with a range of 1 - 40%. The period of lowest contribution to numbers of cyanobacteria by the phycoerythrin containing forms was August 28 to September 13, when the mean percentage was 4.6% .

Diatoms had a substantial peak from August 28 through September 13, with values during that period ranging from 23,260 to 45,275 ml^{-1} (Fig.7). Observed diatoms included Skeletonema, Chaetoceros, Coscinodiscus, Asterionella and numerous other pennate and centric forms. Diatom densities in the less than 3 μm fraction were approximately 10% of the unfiltered fraction, and also showed an increase in densities in late August and early September.

Autotrophic flagellates observed included Chrysophytes, Cryptophytes, Euglenophytes, Prasinophytes and Prymnesiophytes. Densities reached their highest level on September 2 - 11,565 ml^{-1} , with densities above 6,550 ml^{-1} between August 28 and September 7. The less than 3 μm fraction had densities above 2,000 ml^{-1} on August 23-28 (mean seasonal value was 1,505 ml^{-1}). Concentrations of pico-autotrophic flagellates (Fig.8) were also observed July 3-17 with densities greater than 2,000 ml^{-1} .

Autotrophic dinoflagellates occurred in highest densities at the beginning of the study, approximately 2,500 ml^{-1} (Fig.9). Densities decreased over the season, and by the end of August they were relatively rare (less than 250 ml^{-1}). Small autotrophic

Figure 6: Orange-fluorescing cyanobacteria abundances in surface total and less than 3 μm fractions. Arrows at top of figure indicate day of monthly highest tidal height.

ORANGE-FLUORESCING CYANOBACTERIA NUMBERS OF ORGANISMS PER ML

SUMMER 1985

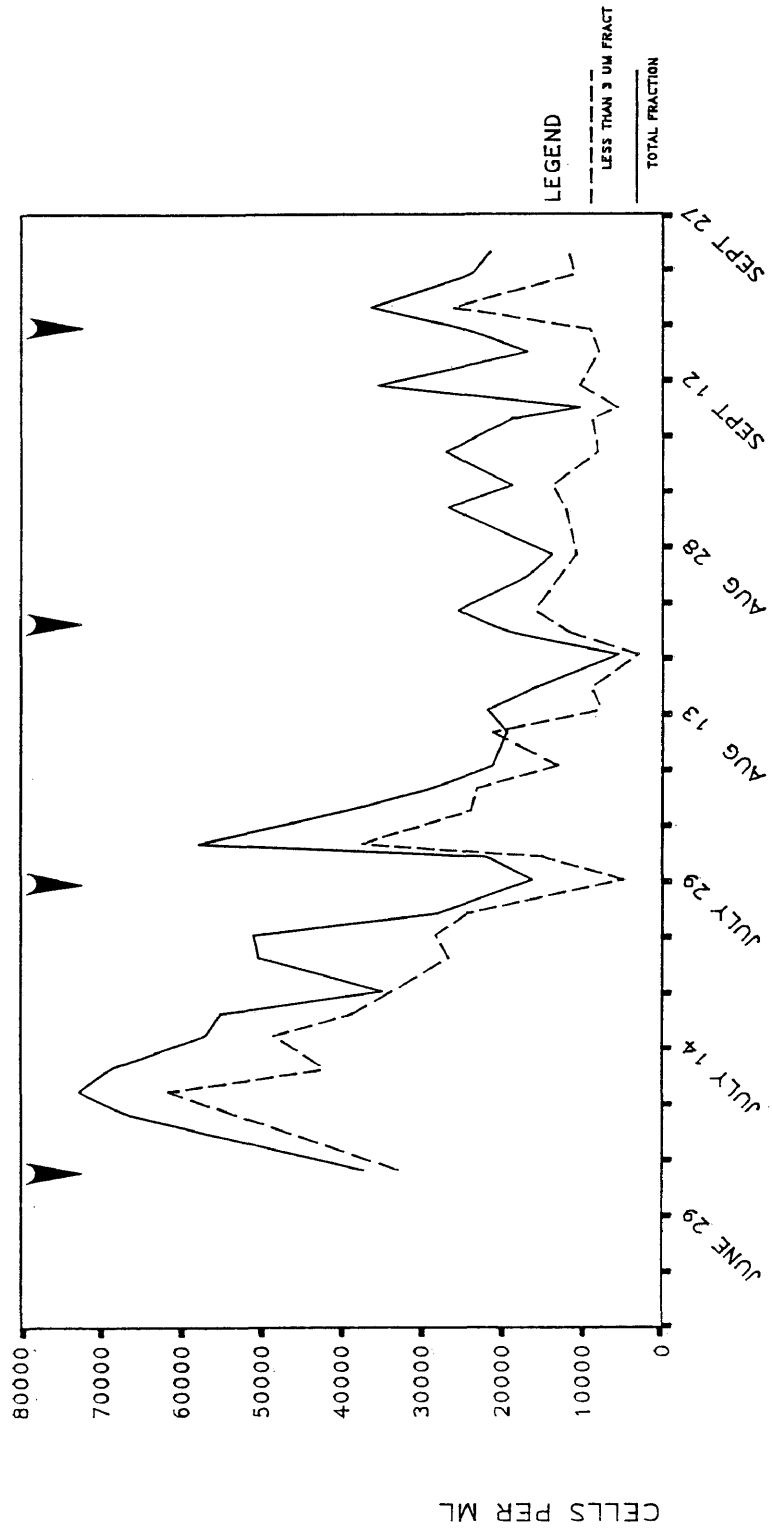


Figure 7: Diatom abundances in surface total and less than 3 μm fractions. Arrows indicate day of monthly highest tidal height.

DIATOM DENSITIES TOTAL AND LESS THAN 3 UM FRACTION

SUMMER 1985

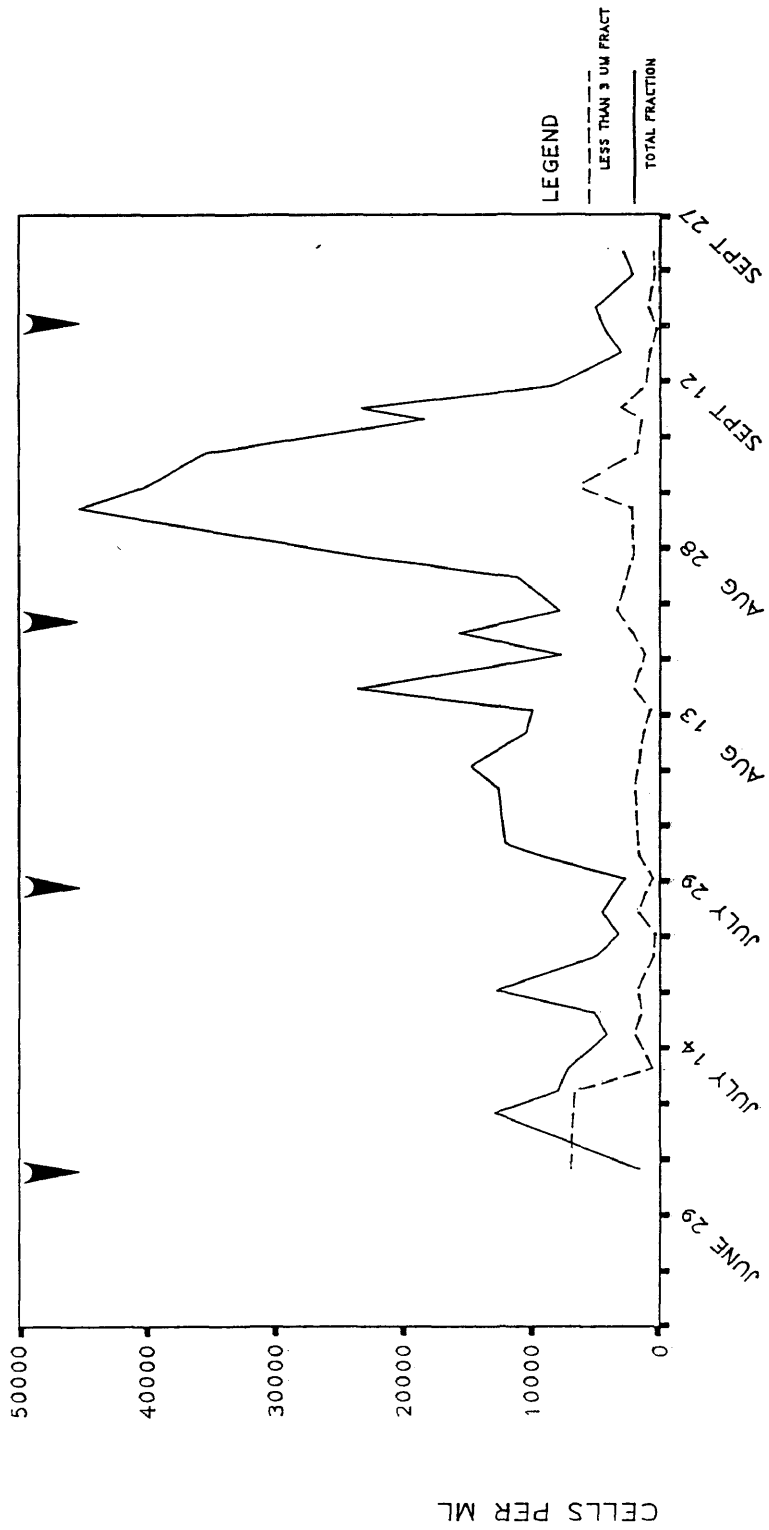


Figure 8: Autotrophic flagellate abundances in surface total and less than 3 μm fractions. Arrows indicate day of monthly highest tidal height.

AUTOTROPHIC FLAGELLATE DENSITIES TOTAL AND LESS THAN 3 UM FRACTION

SUMMER 1985

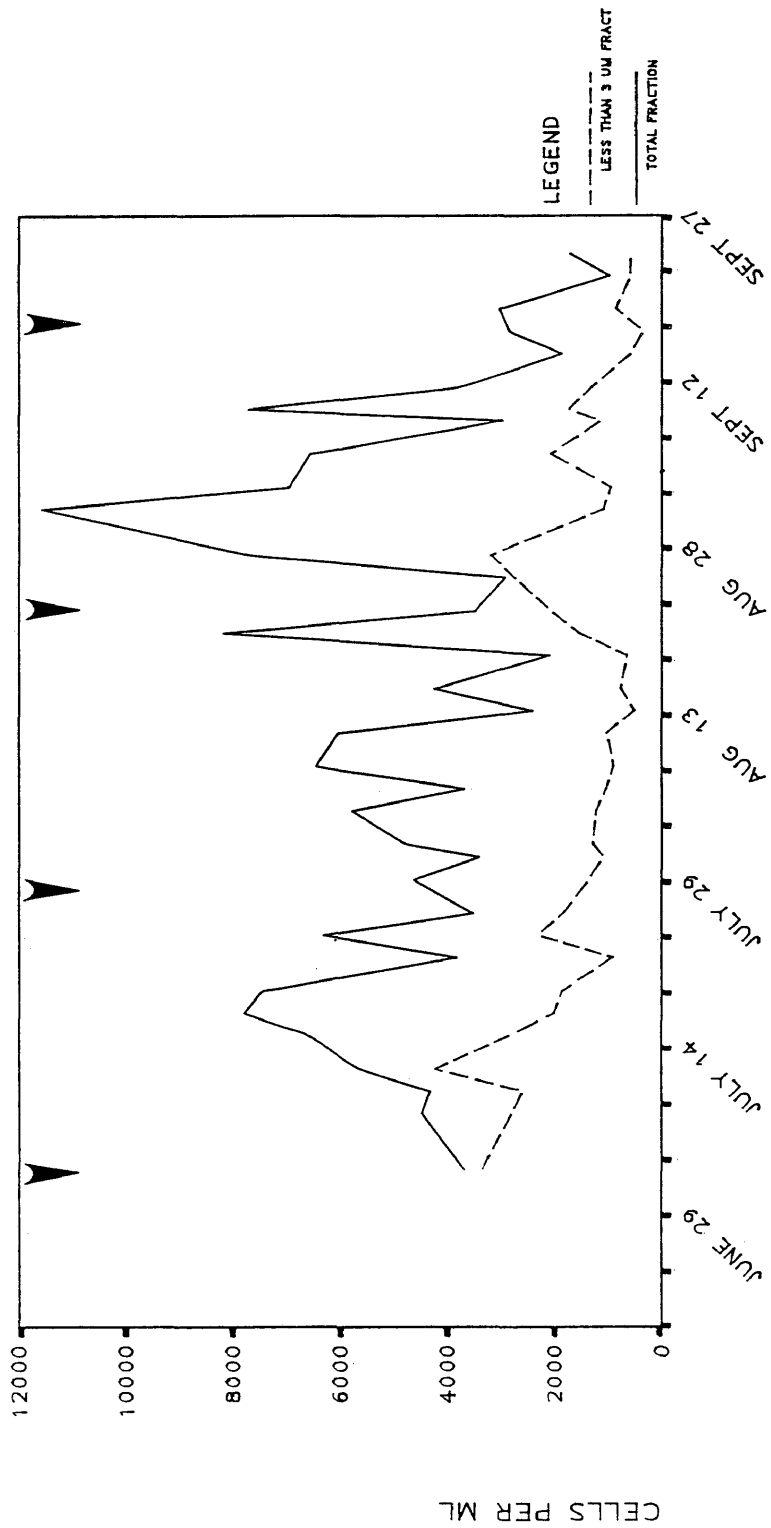
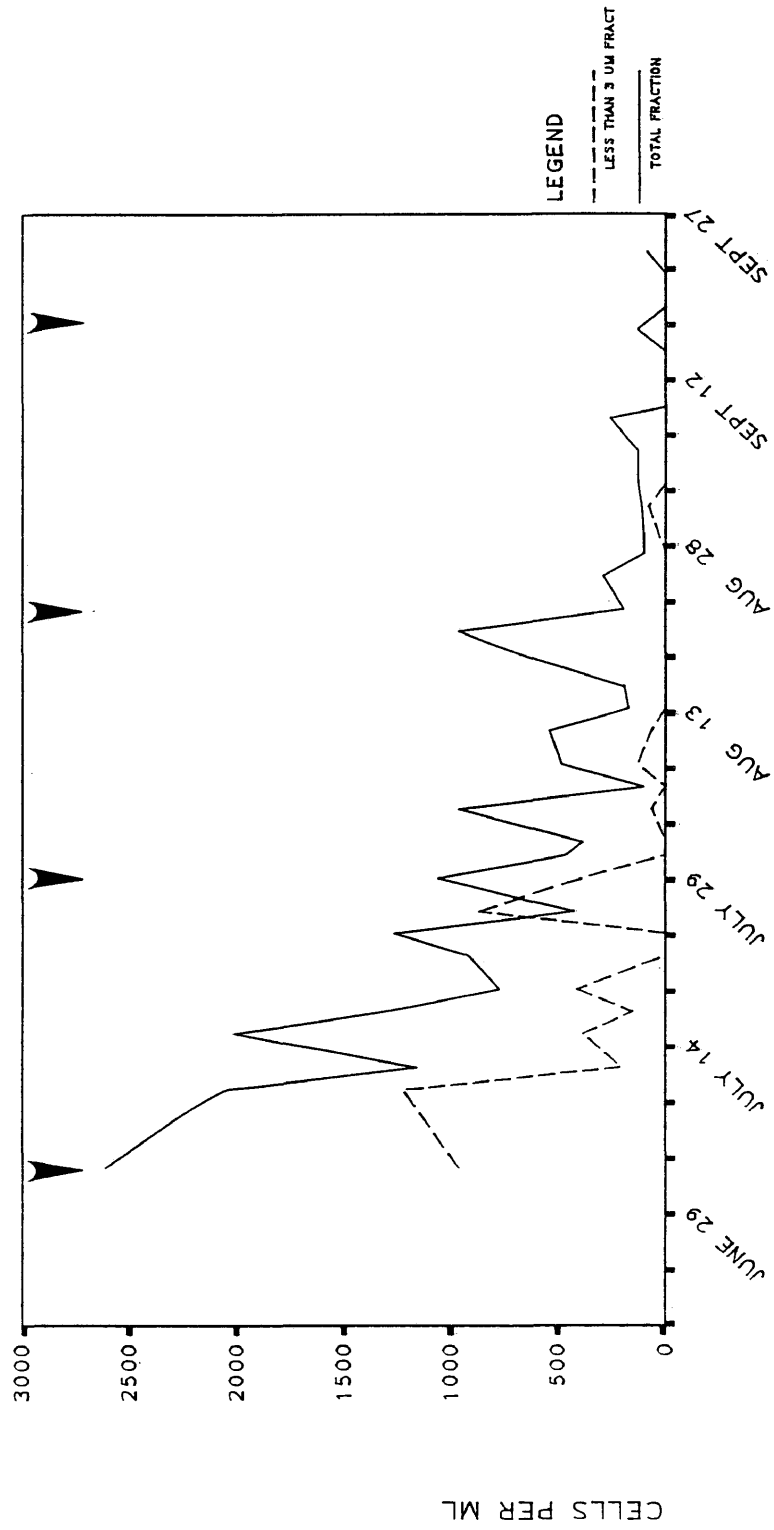


Figure 9: Dinoflagellate abundances in surface total and less than 3 μm fractions. Arrows indicate day of monthly highest tidal height.

DINOFLAGELLATE DENSITIES TOTAL AND LESS THAN 3 UM FRACTION

SUMMER 1985



dinoflagellates were found in the less than 3 μm fraction (up to 1,220 ml^{-1}) in July, but only rarely thereafter.

The category "other autotrophs" was mainly composed of the class Chlorophyceae, including Ulothrix, Scenedesmus, a small unicellular coccoid Chlorophyte (probably of the genus Chlorella), and a large colonial Chlorophyte or Haptophyte 10-40 μm in diameter. The total fraction had two peaks in the "other autotrophs" category, with densities greater than 1,000 ml^{-1} (Fig.10). The first peak occurred August 7-12 and the second occurred August 28 through September 13. During these periods, increases in the less than 3 μm fraction were also apparent, with approximately 1/3 the concentration of the total unfiltered fraction. The percent contribution by the less than 3 μm "other autotrophs" (abundances) during these peaks was variable, ranging from 11-73% of the unfiltered densities.

Heterotrophic bacteria were the most numerous component of the plankton, ranging 1.4 - 8.2 X 10⁶ cells ml^{-1} in the total fraction (Fig.11). Heterotrophic flagellates were found in both the unfiltered and less than 3 μm fractions. This group included choanoflagellates, chrysomonads, dinoflagellates and euglenoids. Mean seasonal densities for the for the unfiltered and less than 3 μm filtered fractions were 2,805 and 1,775 ml^{-1} , respectively (Fig.12). Densities for both fractions were highest July 10-19. The unfiltered fraction densities remained above 1,500 ml^{-1} until September 16, after which they decreased to approximately 1,000 ml^{-1} . Less than 3 μm heterotrophic flagellates were somewhat more variable, but in general remained above

Figure 10: Abundance of other autotrophic forms in surface total and less than 3 μm fractions. Arrows indicate day of monthly highest tidal height.

OTHER AUTOTROPHIC DENSITIES TOTAL AND LESS THAN 3 UM FRACTION

SUMMER 1985

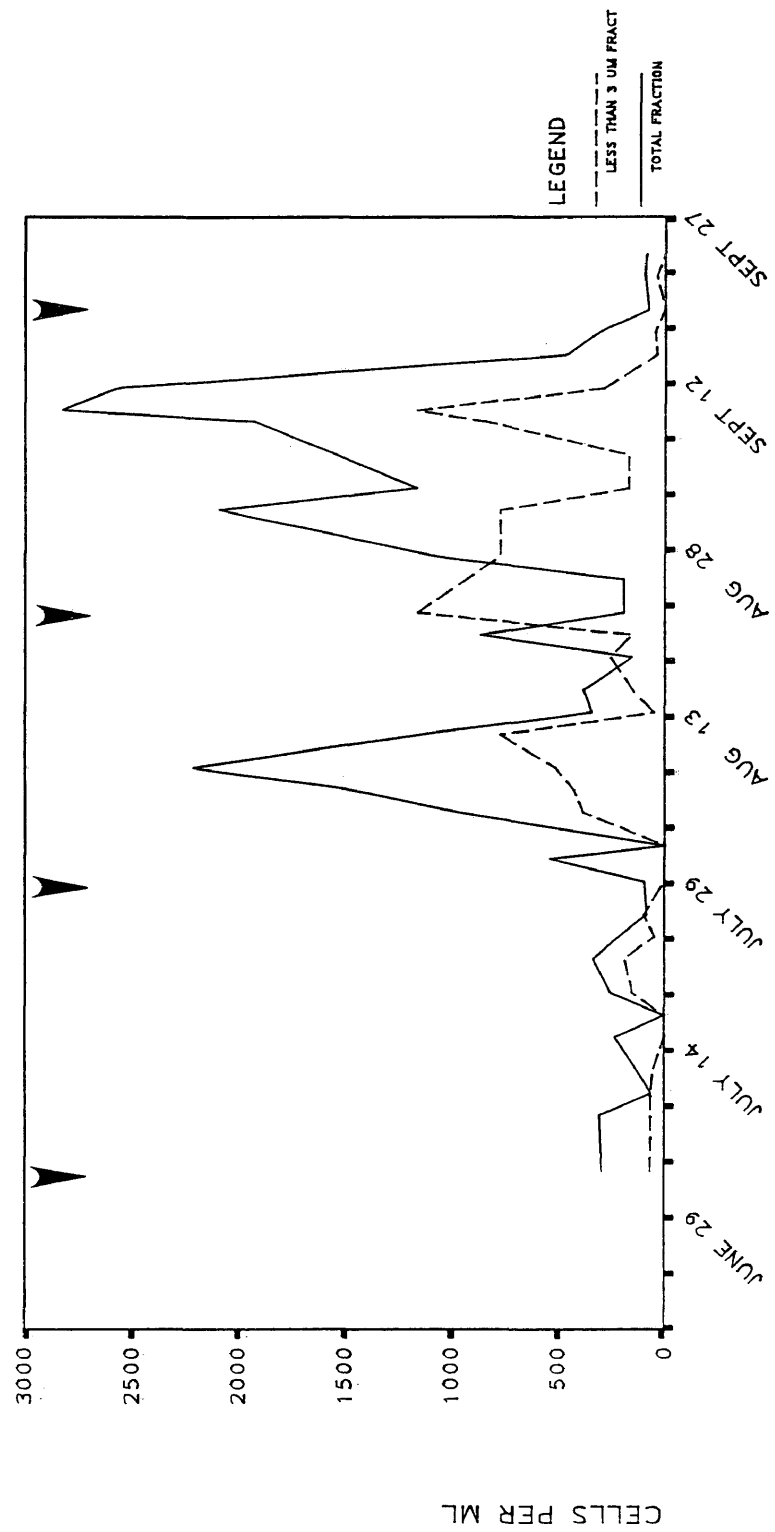


Figure 11: Heterotrophic bacteria abundances in surface total and less than 3 μm fractions. Arrows indicate day of monthly highest tidal height.

HETEROTROPHIC BACTERIA TOTAL AND LESS THAN 3 UM FRACTIONS

SUMMER 1985

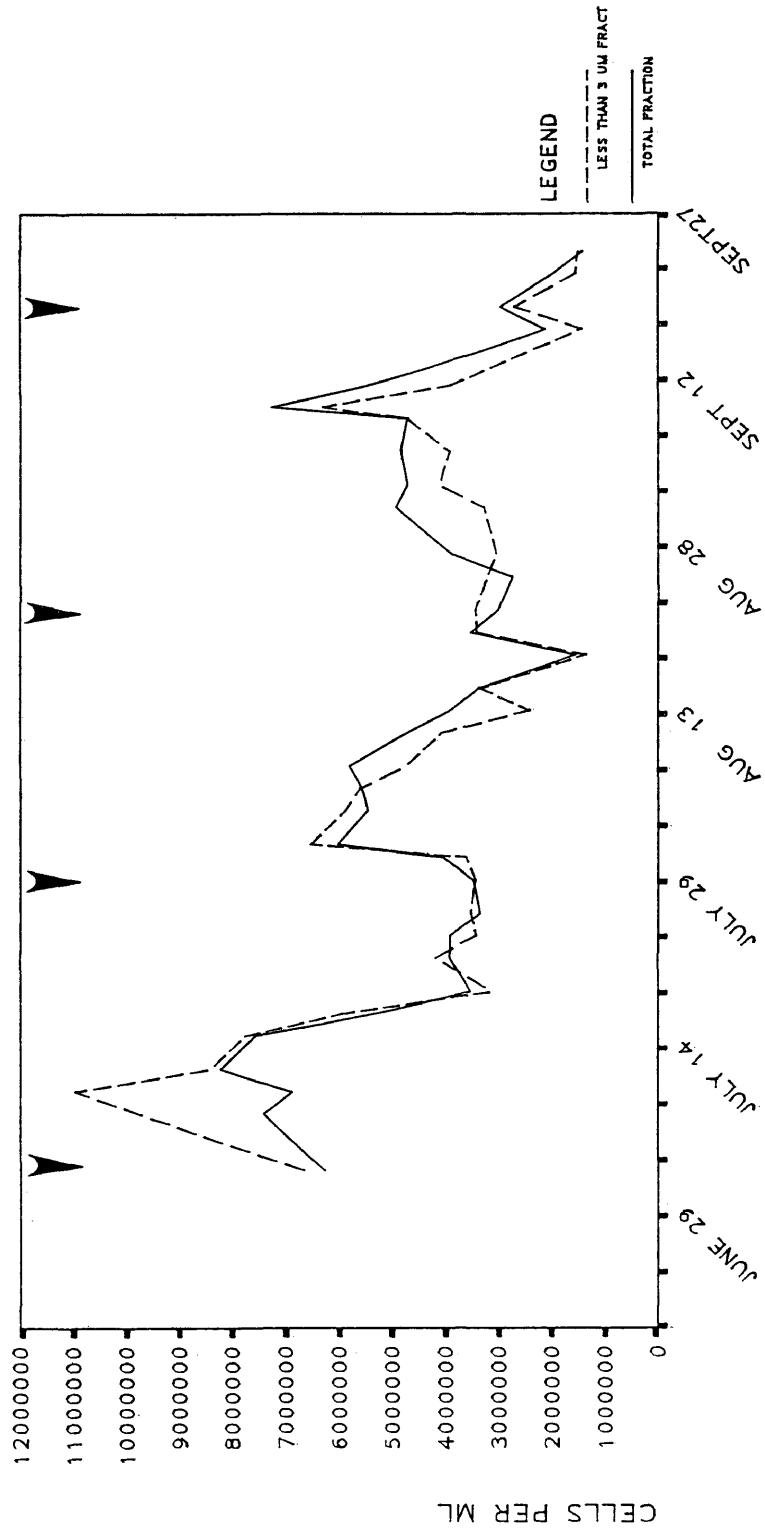
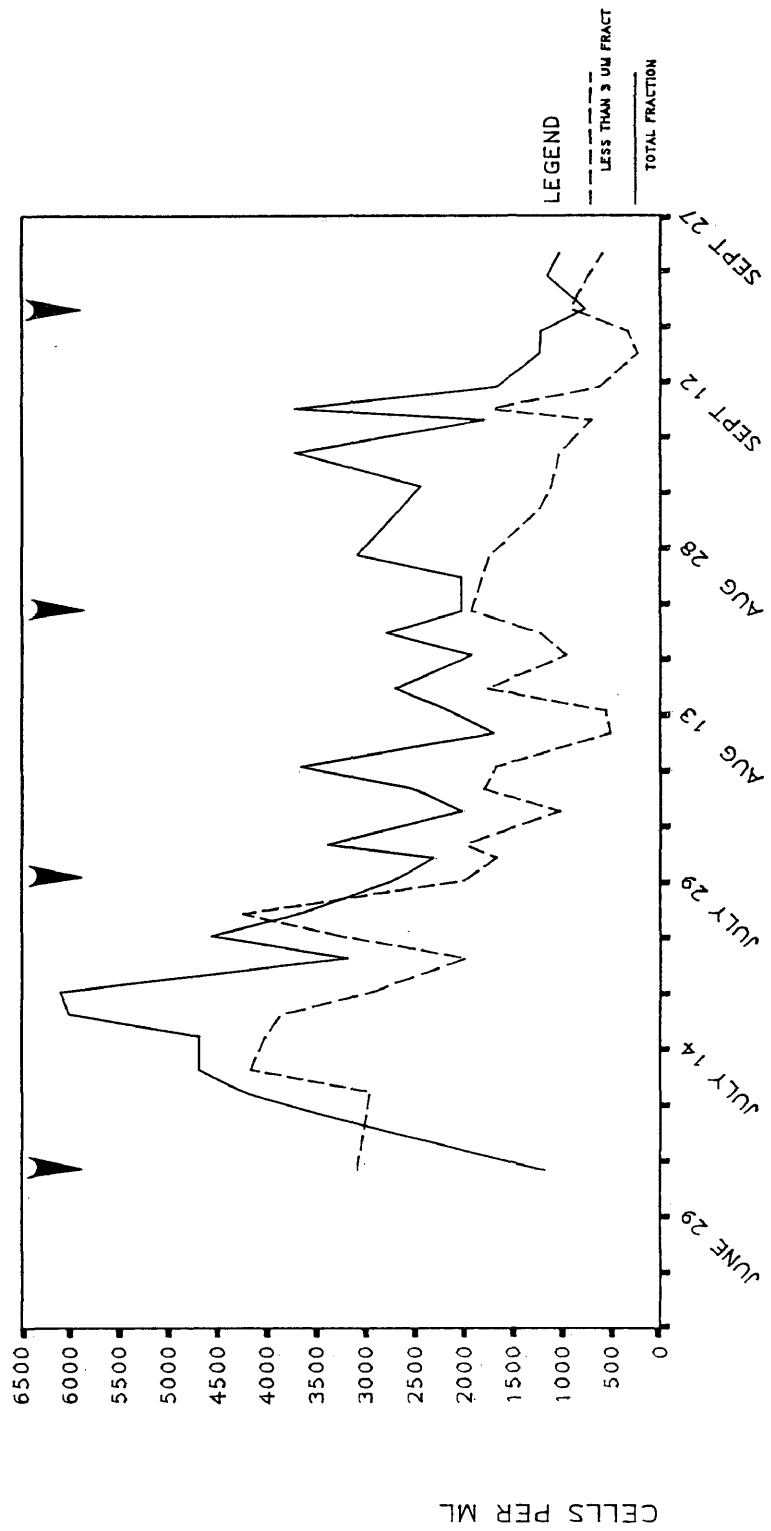


Figure 12: Abundance of heterotrophic flagellates in surface total and less than 3 μm fractions. Arrows indicate day of monthly highest tidal height.

HETEROTROPHIC FLAGELLATES TOTAL AND LESS THAN 3 UM FRACTION

SUMMER 1985



1,000 ml⁻¹ until September 13, when densities decreased to approximately 500 ml⁻¹.

Other heterotrophs included ciliates (including suctorians and tintinids), amoebae, testaceans and foraminifera, as well as an occasional crustacean invertebrate (crab nauplii, copepods, etc.). The densities of these organisms were variable over time with no discernable pattern. One spike occurred on July 26, with 2,140 organisms ml⁻¹ (Fig.13).

Carbon values used for converting cell counts to carbon biomass, as well as mean biovolumes and standard deviations are shown (Table 2). The mean biomass estimate for the unfiltered samples was 1800 ug C L⁻¹ and ranged from 310 to 3500 ug C L⁻¹ during the study. The mean seasonal biomass in the less than 3 um fraction was 145 ug C L⁻¹, and ranged from 30 to 380 ug C L⁻¹. Biomass estimates of the total carbon biomass and autotrophic carbon biomass present in unfiltered and 3 um filtered samples over the sampling period are shown in Figures 14-15. Means for each category of organism over the season are presented in Table 3. The mean contribution to autotrophic carbon by the less than 3 um fraction is 49 ug C L⁻¹, or approximately 7% of the total autotrophic biomass. In contrast, the less than 15 um fraction contributed a mean of 87% to the total estimated autotrophic carbon.

Over the sampling season the picoplankton contributed between 3 and 16% of the total estimated autotrophic carbon biomass (Fig.16). Total autotrophic biomass attained values greater than 1,000 ug C L⁻¹ on two separate occasions. The first was July 8, early in the study,

Figure 13: Abundances of other heterotrophic forms in surface total and less than 3 μm fractions. Arrows indicate day of monthly highest tidal height.

OTHER HETEROTROPHIC DENSITIES TOTAL AND LESS THAN 3 UM FRACTION

SUMMER 1985

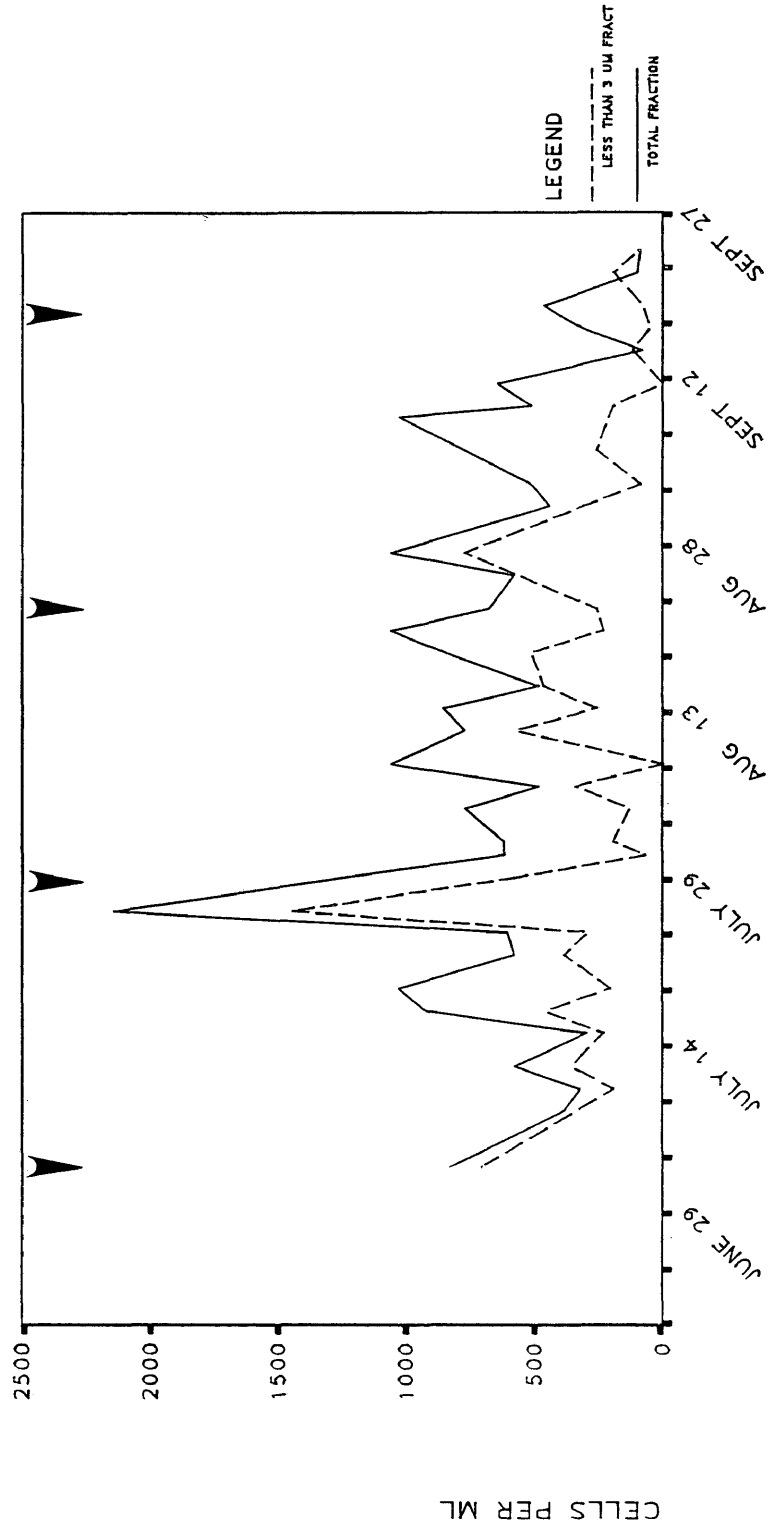


TABLE 2

Values used for calculating cellular carbon

ORGANISM	MEAN VOLUME UM ³	+ 1 SD	EQUATION USED	CARBON PER CELL PICOGRAMS
UNFILTERED COMPOSITE SAMPLE				
Red cyanobacteria	0.5236	(1)	Bratbak and Dundas	0.1519
Orange cyanobacteria	0.5236	(1)	Bratbak and Dundas	0.1519
Diatoms	335.3977	1645.68	Taguchi	19.4498
Auto. flagellates	258.9375	1174.45	Eppley et al.	46.6019
Auto. dinoflagellates	1492.2677	3246.32	Eppley et al.	232.1704
Other autotrophs	327.1677	1655.73	Eppley et al.	58.0610
Heterotrophic bacteria	0.0870	(2)	Bratbak and Dundas	0.0194
Hetero. flagellates	175.9039	525.32	Eppley et al.	32.4010
Other heterotrophs	9420.2103	39414.22	Eppley et al.	1366.5231
LESS THAN 3 UM FRACTION				
Red cyanobacteria	0.5236	(1)	Bratbak and Dundas	0.1519
Orange cyanobacteria	0.5236	(1)	Bratbak and Dundas	0.1519
Diatoms	32.8108	28.72	Taguchi	3.4822
Autotrophic flagellates	27.0239	19.27	Eppley et al.	5.5698
Auto. dinoflagellates	124.6664	58.91	Eppley et al.	23.4423
Other autotrophs	27.3042	41.84	Eppley et al.	8.4013
Heterotrophic bacteria	0.0870	(2)	Bratbak and Dundas	0.0194
Hetero. flagellates	29.1189	11.16	Eppley et al.	5.9748
Other heterotrophs	44.8545	25.37	Eppley et al.	8.9680

(1) Iturriaga and Mitchell 1986 - $1 \mu\text{m}$ diameter for cyanobacteria
 (2) Watson et al. 1977- $0.087 \mu\text{m}$ cell for heterotrophic marine bacteria

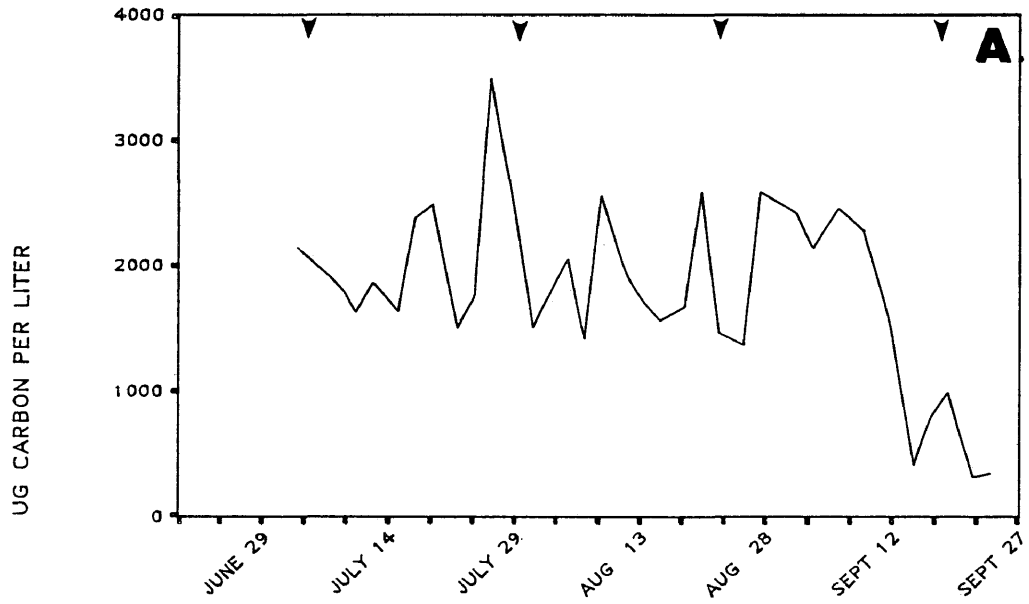
Bratbak and Dundas 1984. 0.22 g C cm^{-3} for marine bacteria.
 Eppley et al. 1974. $\text{Log}_{10} \text{C} = 0.94 (\text{Log}_{10} \text{Volume}) - 0.60.$
 Taguchi 1978. $\text{Log}_{10} \text{C} = 0.74 (\text{Log}_{10} \text{Volume}) - 0.58.$

Figure 14: Plankton carbon biomass (heterotrophic and autotrophic).
Arrows indicate day of monthly highest tidal height.

- A. Total Fraction
- B. Less than 3 μm Fraction.

TOTAL CARBON BIOMASS
TOTAL FRACTION

SUMMER 1985



TOTAL CARBON BIOMASS
LESS THAN 3 UM FRACTION

SUMMER 1985

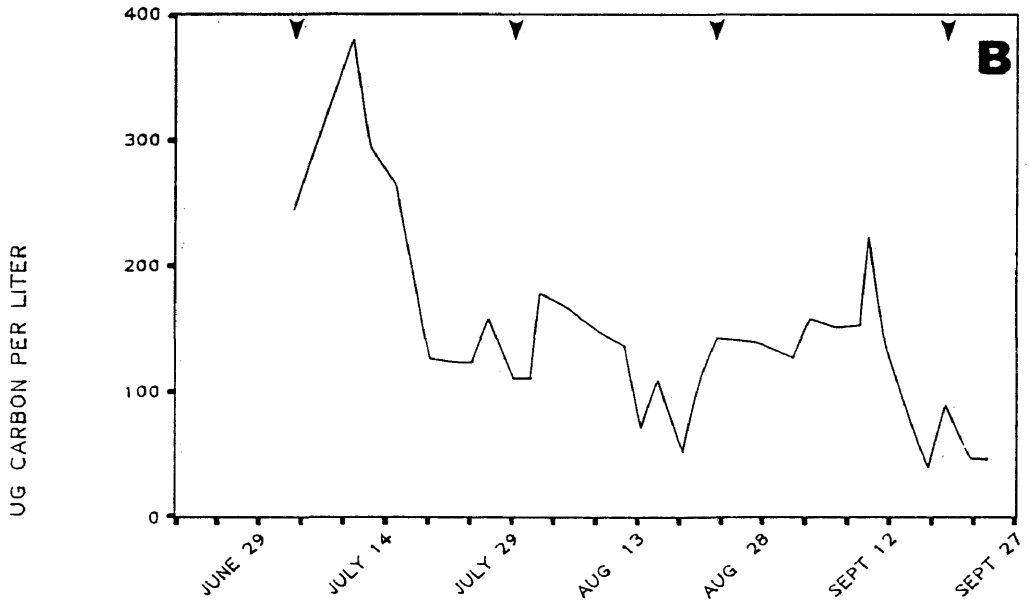


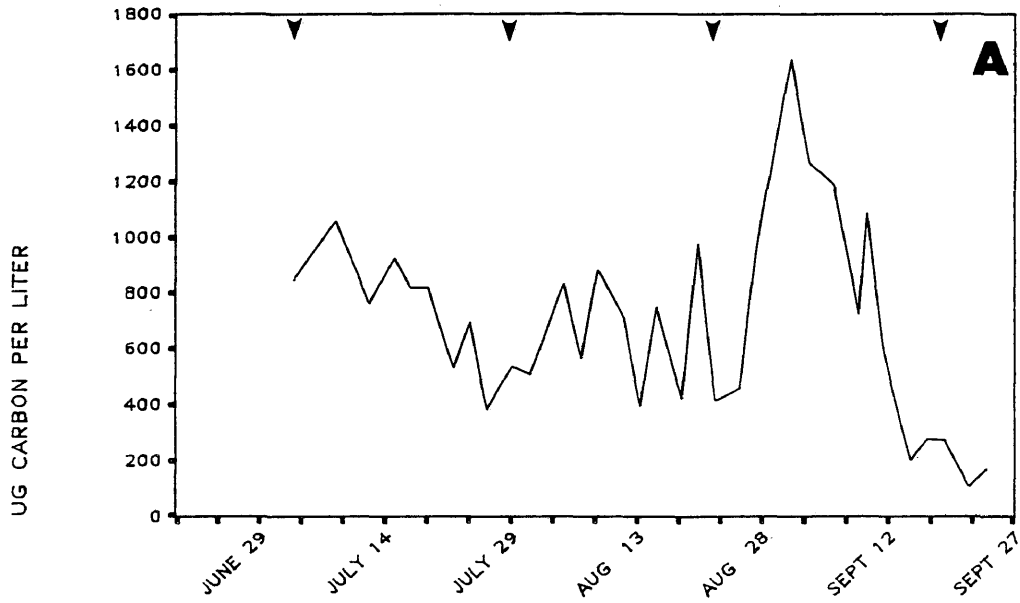
Figure 15: Phytoplankton carbon biomass. Arrows indicate day of monthly highest tidal height.

A. Total Fraction

B. Less than 3 μm Fraction.

AUTOTROPHIC BIOMASS
TOTAL FRACTION

SUMMER 1985



AUTOTROPHIC BIOMASS
LESS THAN 3 UM FRACTION

SUMMER 1985

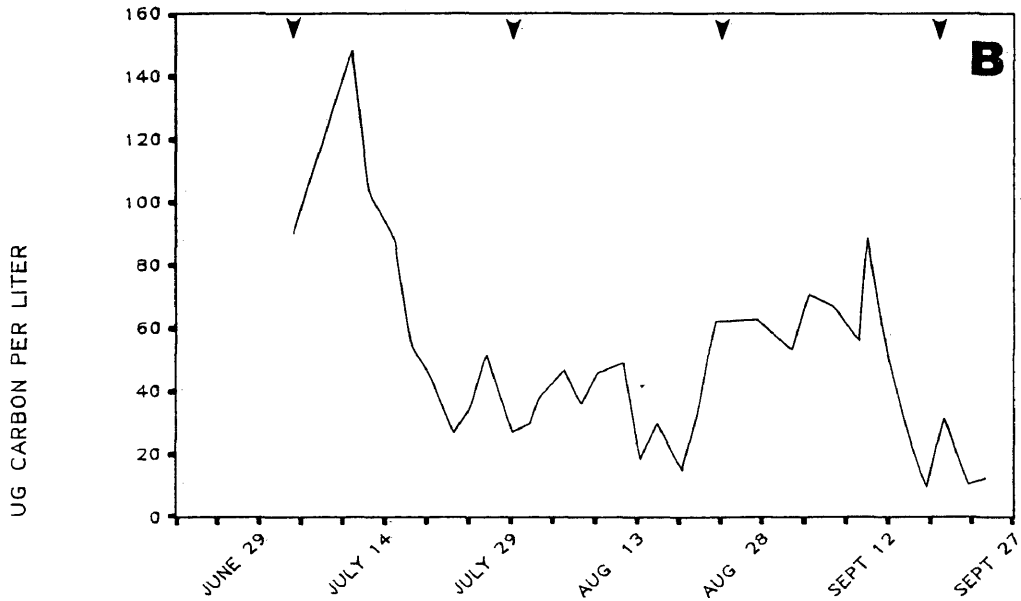


TABLE 3

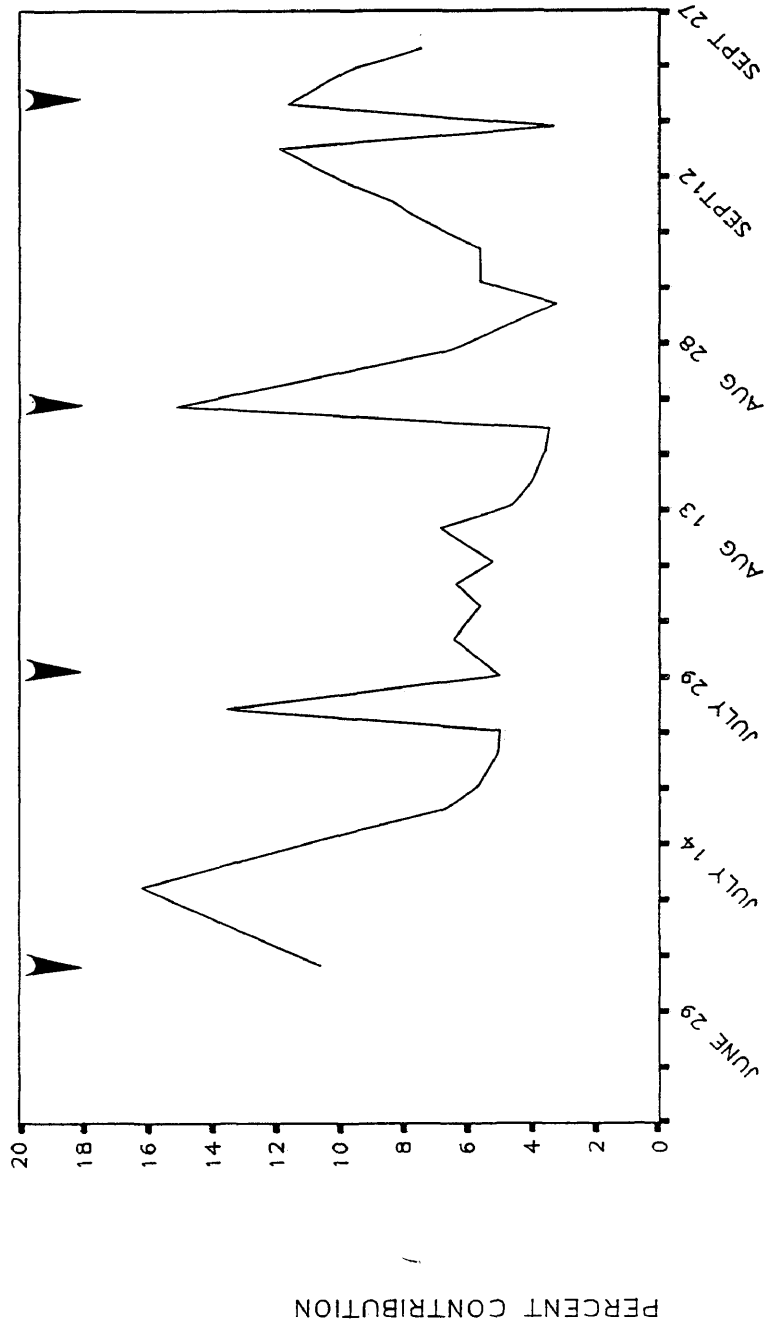
Mean carbon biomass determined from mean cell volumes and
 literature volume to carbon values
 Summer, 1985 (N = 35)

ORGANISM	MEAN ($\mu\text{g C L}^{-1}$)	RANGE	MEAN % IN < 3 μm SAMPLE
Red fluorescing cyanobacteria	37.1	2.5 - 109.3	67
Orange fluorescing cyanobacteria	4.8	0.8 - 11.0	67
Diatoms	237.0	32.3 - 880.6	3
Autotrophic flagellates	225.2	0.0 - 164.2	4
Autotrophic dinoflagellates	147.2	0.0 - 606.0	2
Other autotrophs	41.0	0.0 - 164.2	6
Heterotrophic bacteria	87.0	27.7 - 159.5	95
Heterotrophic flagellates	90.9	25.0 - 197.8	12
Other heterotrophs	928.4	117.1 - 2927.1	0.3

Figure 16: Percent contribution of picoplankton to total autotrophic carbon biomass in surface water. Arrows indicate day of monthly highest tidal height.

LESS THAN 3 UM AUTOTROPHIC CARBON
CONTRIBUTION TO TOTAL FRACTION

SUMMER 1985



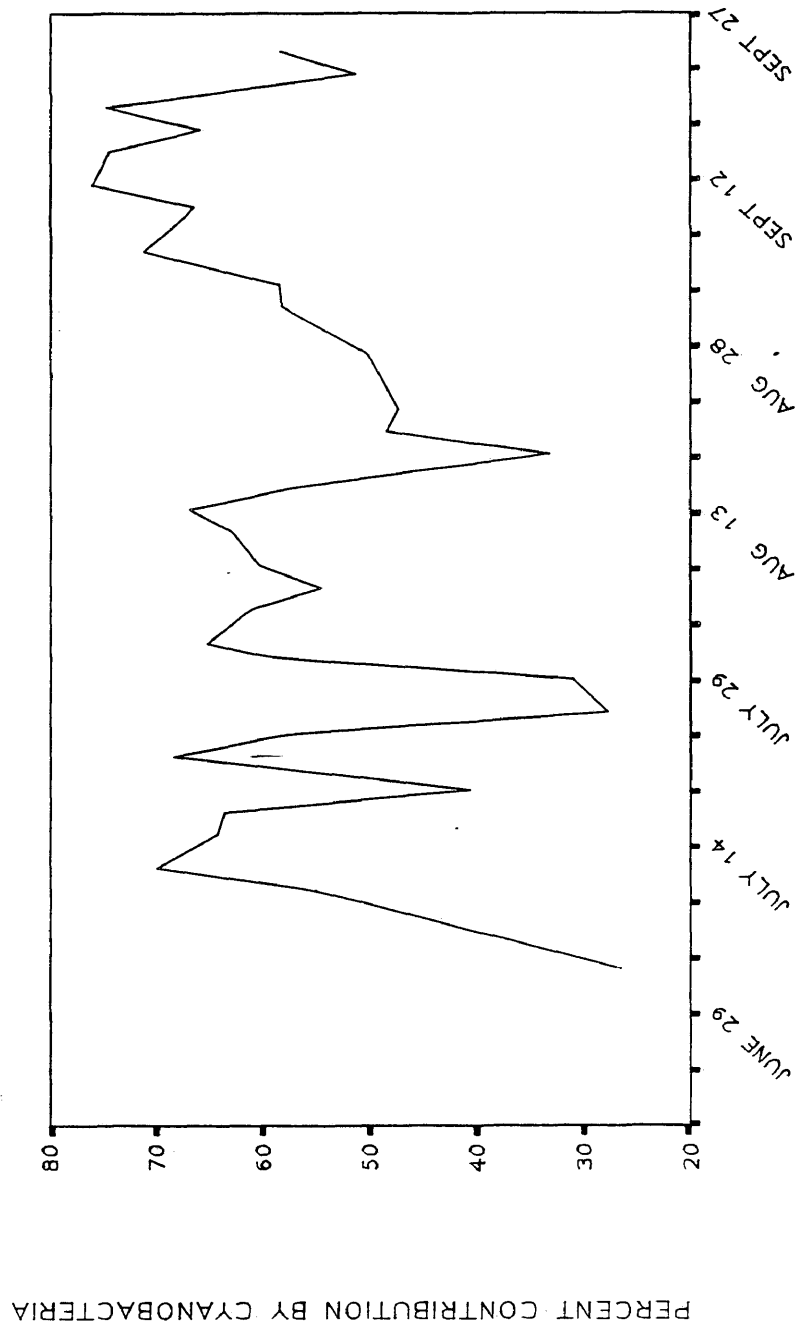
with an estimated concentration of 1060 ug C L^{-1} . Estimated autotrophic biomass (Fig.15A) reached a high of 1637 ug C L^{-1} on September 2, with values above 1,000 but decreasing during September 4-13. The less than 3 μm autotrophic carbon peaked July 10, with a value of 148 ug C L^{-1} . Less than 3 μm autotrophic biomass estimates (Fig.15B) remained above 50 ug C L^{-1} during August 23 - September 13, with a high of 71 ug C L^{-1} on September 4. Autotrophic biomass in the less than 3 μm fraction reached low levels August 13 - 18 and September 16 through the end of the study. The autotrophic carbon in the total and picoplankton size fractions showed the same direction of change in biomass over time. The exceptions occurred July 15, July 26 and August 23. On July 26 and August 23 the total autotrophic biomass decreased while the autotrophic picoplankton biomass increased. On July 15 the less than 3 μm fraction biomass decreased while the total fraction biomass increased.

The mean seasonal contribution of cyanobacteria in the unfiltered samples was 6.5% of the estimated total autotrophic carbon biomass. Cyanobacteria made up approximately 60% of the estimated autotrophic carbon biomass in the less than 3 μm fraction (Fig.17). The remainder of the autotrophic carbon in the less than 3 μm fraction was contributed by diatoms, flagellates and the unicellular coccoid chlorophyte. The seasonal mean contribution to autotrophic flagellate carbon by the less than 3 μm organisms was 4% and less than 15 μm organisms 96%. Heterotrophic flagellate carbon biomass in the less than 15 μm fraction was close to 100% of the total and the less than 3 μm fraction contributed ca. 12%. Approximately 90% of the seasonal

Figure 17: Carbon contribution by cyanobacteria to autotrophic carbon in less than 3 μm fraction.

PERCENT CONTRIBUTION TO AUTOTROPHIC
CARBON BY CYANOBACTERIA

LESS THAN 3 UM FRACTION



diatom biomass was in the less than 15 μm fraction, and ca. 3% in the less than 3 μm fraction.

The mean Carbon : chlorophyll *a* (C:Chl) value for the total fraction over the sampling season was 55 and ranged from 18 to 108 (coefficient of variation was 48.8%). The less than 3 μm fraction had a C:Chl mean of 22 (C.V. = 40.2%). The Rank Sum non-parametric test indicated significant difference ($P < .05$) between C:Chl ratios for the total and less than 3 μm fractions.

CARBON FIXATION

Uptake rates of carbon by the total and less than 3 μm fractions were measured on 10 days during the study (Fig.18). The mean coefficient of variation for replicate uptake values over all days and treatments was 6.6%. Highest rates of CO_2 uptake by both fractions were on August 2, with values of 172.8 and 9.4 $\mu\text{g C L}^{-1} \text{ hr}^{-1}$, for the total and less than 3 μm fractions respectively at 293 $\mu\text{E m}^{-2} \text{ sec}^{-1}$. Lowest carbon uptake values for both fractions at all light intensities except 28 $\mu\text{E m}^{-2} \text{ sec}^{-1}$ occurred July 19. Low carbon fixation rates also occurred on August 23 and September 20, especially in the less than 3 μm fraction. Uptake of carbon by the phytoplankton was significantly different (ANOVA, $P < .015$) with 3 way interactions between fractions, days and light intensities. Uptake rates for the two fractions were significantly different ($P < .05$) for all days and at all light intensities. Carbon uptake at 293 $\mu\text{E m}^{-2} \text{ sec}^{-1}$ for the total fraction was always significantly different than uptake rates at the lower light intensities (54, 43 and 28 $\mu\text{E m}^{-2} \text{ sec}^{-1}$). The less

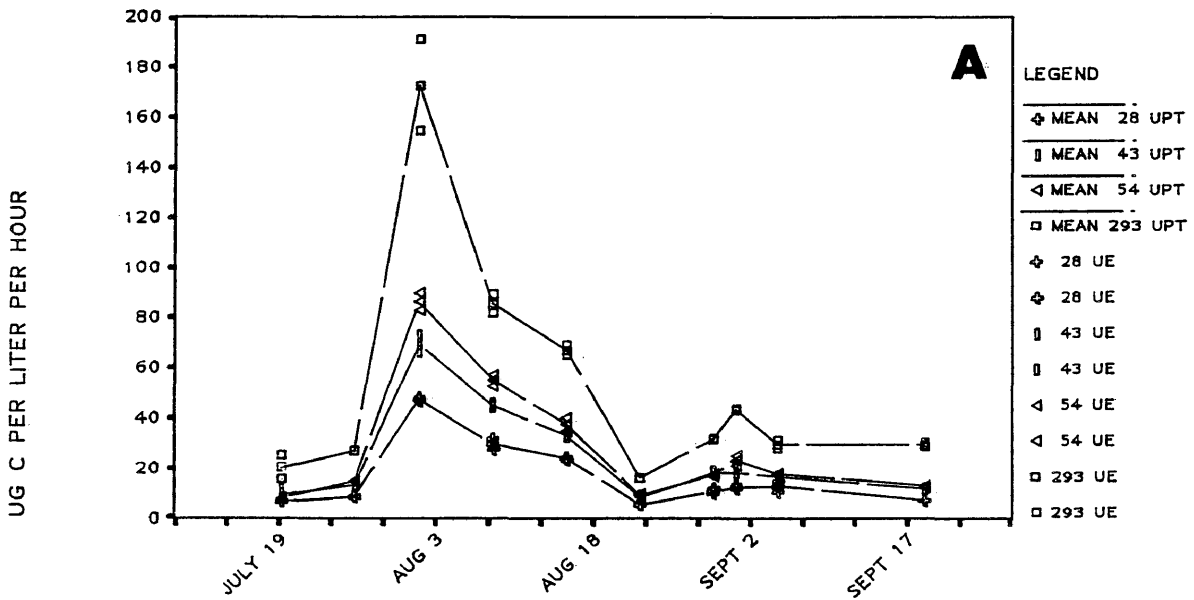
Figure 18: Autotrophic carbon fixation at four light intensities.

A. Total Fraction

B. Less than 3 μm fraction.

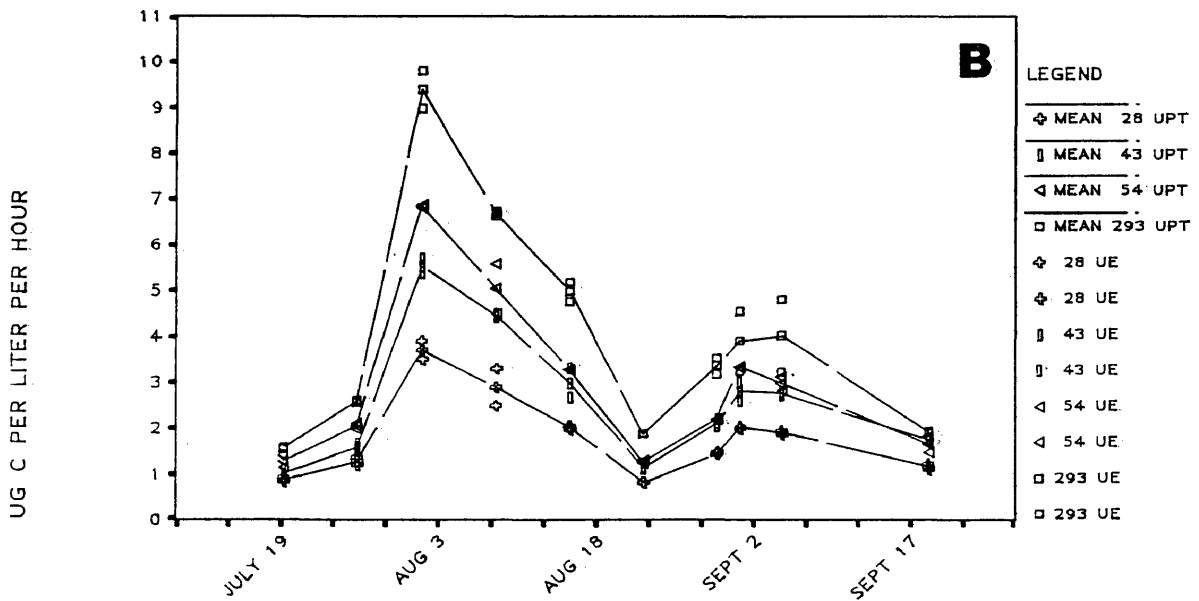
CARBON UPTAKE BY TOTAL FRACTION SUMMER 1985

4 LIGHT INTENSITIES



CARBON UPTAKE BY LT 3UM FRACT SUMMER 1985

4 LIGHT INTENSITIES



than 3 μm fraction had significantly different uptake rates at $293 \text{ uE m}^{-2} \text{ sec}^{-1}$ and $54 \text{ uE m}^{-2} \text{ sec}^{-1}$ only on July 19 and August 30.

Uptake rates of carbon on July 19 and August 23 were not significantly different for any of the examined pairs of treatments. August 23 and September 20 had significantly different carbon uptake rates only at $43 \text{ uE m}^{-2} \text{ sec}^{-1}$ in the less than 3 μm fraction. Uptake of carbon on August 30 and September 6, and uptake on September 2 and 6 were never significantly different for any of the treatments. Between August 30 and September 2 there was a significant difference in carbon uptake for the less than 3 μm fraction at $54 \text{ uE m}^{-2} \text{ sec}^{-1}$ intensity. On August 9 and 16 there was a significant difference in uptake rate only in the less than 3 μm fraction at $54 \text{ uE m}^{-2} \text{ sec}^{-1}$.

The percent contribution of the less than 3 μm fraction to total autotrophic carbon uptake (Fig.19) varied significantly with light intensity over the study (Kruskal-Wallis non-parametric test for significance between means, $P < .025$). Percent contribution to total carbon uptake was significantly different ($P < .05$) at $293 \text{ uE m}^{-2} \text{ sec}^{-1}$ than at the 3 lower light intensities (Wilcoxon Rank Sum test). There was no significant difference in percent contribution to autotrophic carbon uptake between the 3 lower light intensities.

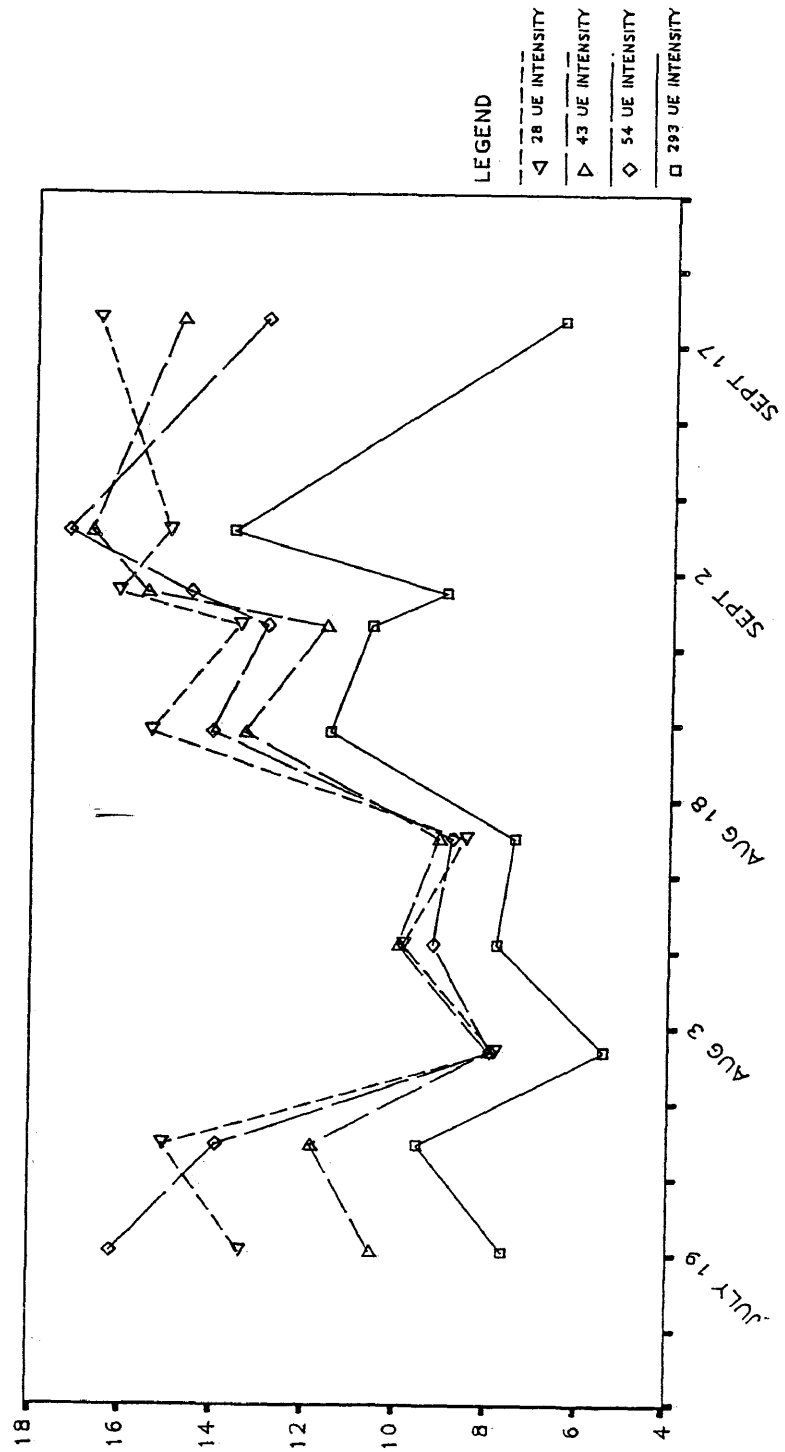
The mean chlorophyll specific carbon uptake rates at $293 \text{ uE m}^{-2} \text{ sec}^{-1}$ (P_{max}) for the greater than 3 μm fraction and less than 3 μm fraction were 5.17 and $2.50 \text{ ug C (ug Chl}_a\text{)}^{-1} \text{ hr}^{-1}$, respectively. Highest P_{max} values for both fractions occurred August 2, 11.68 and $6.89 \text{ ug C (ug Chl}_a\text{)}^{-1} \text{ hr}^{-1}$, for the greater than 3 μm and less than 3 μm fractions respectively (Fig.20). There were significant ($P < .006$)

Figure 19: Contribution to total autotrophic carbon uptake by less than 3 μm fraction at four light intensities.

PERCENT CONTRIBUTION OF 3 UM FRACTION
TO TOTAL CARBON UPTAKE

4 LIGHT INTENSITIES

PERCENT CONTRIBUTION BY LT 3 UM FRACTION



3 way interactions between fractions, days and light intensities on the chlorophyll specific uptake rates. Chlorophyll specific uptake rates are shown in table 4. Chlorophyll specific uptake rates for the two fractions (Fig.20-21) differed the most at the $293 \text{ uE m}^{-2} \text{ sec}^{-1}$ light intensity, where there was significant difference ($P < .05$) on all days except September 6. Greater than 3 μm chlorophyll specific uptake rates at the 293 uE light intensity were always significantly different than the 3 lower light intensities. The less than 3 μm chlorophyll specific uptake rates at 293 and $54 \text{ uE m}^{-2} \text{ sec}^{-1}$ were significantly different only on August 30. On August 9, September 2, 6, and 20, there was no significant difference in the less than 3 μm chlorophyll specific uptake rates at 293, 54 and $43 \text{ uE m}^{-2} \text{ sec}^{-1}$. The less than 3 μm fraction showed no significant difference in chlorophyll specific uptake rates between the 3 lower light intensities on July 19, August 30 and September 20.

Growth rates, calculated from carbon uptake rates and a hypothetical 12 hour photo-period, are presented in table 5. There was significant difference (Rank Sum non-parametric test, $P < .05$) between the growth rates of the less than 3 μm and greater than 3 μm autotrophs over the study period. The less than 3 μm fraction showed consistently higher growth rates than the greater than 3 μm autotrophs or total fraction at all light intensities. Mean growth rates at $293 \text{ uE m}^{-2} \text{ sec}^{-1}$ intensity for the total, the greater than 3 μm and the less than 3 μm autotrophs were 1.021, 1.016 and 1.154, respectively. At $28 \text{ uE m}^{-2} \text{ sec}^{-1}$, mean growth rates for the total, the greater than 3 μm and the less than 3 μm phytoplankton were 0.292, 0.299 and 0.521,

TABLE 4

Chlorophyll specific uptake rates
for the greater than 3 μ m and less than 3 μ m fractions
by fraction, day, and intensity
(μ g C μ g Chl_a⁻¹ hr⁻¹)

DATE	293		54		43		28	
	>3AN	<3AN	>3AN	<3AN	>3AN	<3AN	>3AN	<3AN
19 July	1.66	1.05	.58	.86	.76	.68	.50	.59
26 July	5.03	2.08	2.60	1.65	2.42	1.28	1.48	1.04
02 Aug	11.68	6.90	5.67	5.03	4.55	4.07	3.11	2.72
09 Aug	5.75	3.38	3.63	2.56	2.93	2.26	1.93	1.47
16 Aug	8.50	2.99	4.66	1.97	4.09	1.80	2.96	1.21
23 Aug	2.47	1.55	1.33	1.05	1.27	.95	.78	.68
30 Aug	2.72	1.61	1.43	1.05	1.56	1.02	.90	.70
02 Sept	3.95	1.98	1.96	1.70	1.53	1.42	1.05	1.03
06 Sept	2.47	2.09	1.39	1.55	1.34	1.44	1.05	.99
20 Sept	7.50	1.37	3.00	1.18	2.73	1.25	1.59	.83
\bar{x}	5.17	2.50	2.62	1.86	2.32	1.62	1.56	1.13
C.V.	62.5%	68.0%	62.2%	65.6%	54.3%	59.9%	55.1%	54.9%

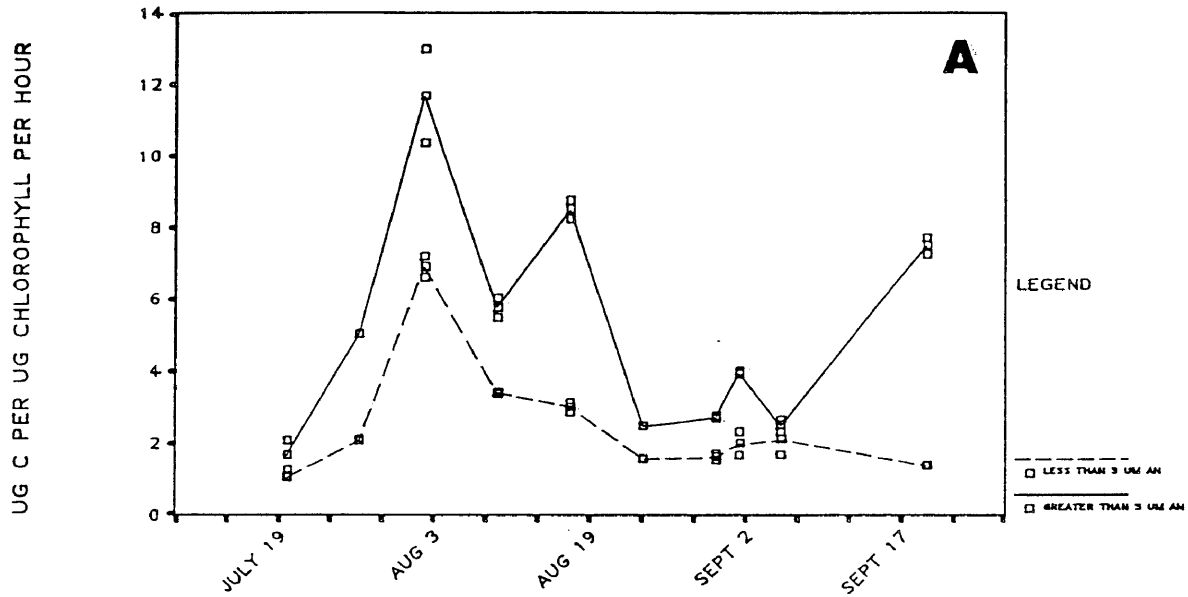
Figure 20: Chlorophyll specific carbon fixation of greater than 3 μm and less than 3 μm autotrophs.

A. 293 $\text{uE m}^{-2} \text{sec}^{-1}$ irradiance

B. 54 $\text{uE m}^{-2} \text{sec}^{-1}$ irradiance.

PHYTOPLANKTON CARBON ASSIMILATION VALUE GREATER AND LESS THAN 3 UM FRACTIONS

293 UE LIGHT INTENSITY



PHYTOPLANKTON CARBON ASSIMILATION VALUE GREATER AND LESS THAN 3 UM FRACTIONS

54 UE LIGHT INTENSITY

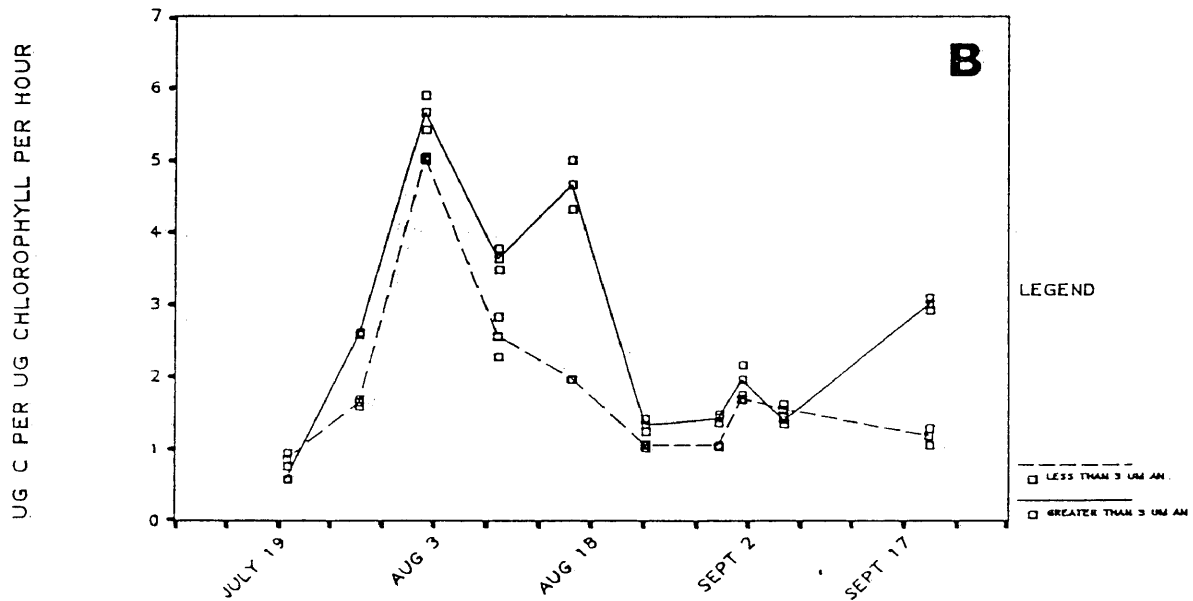
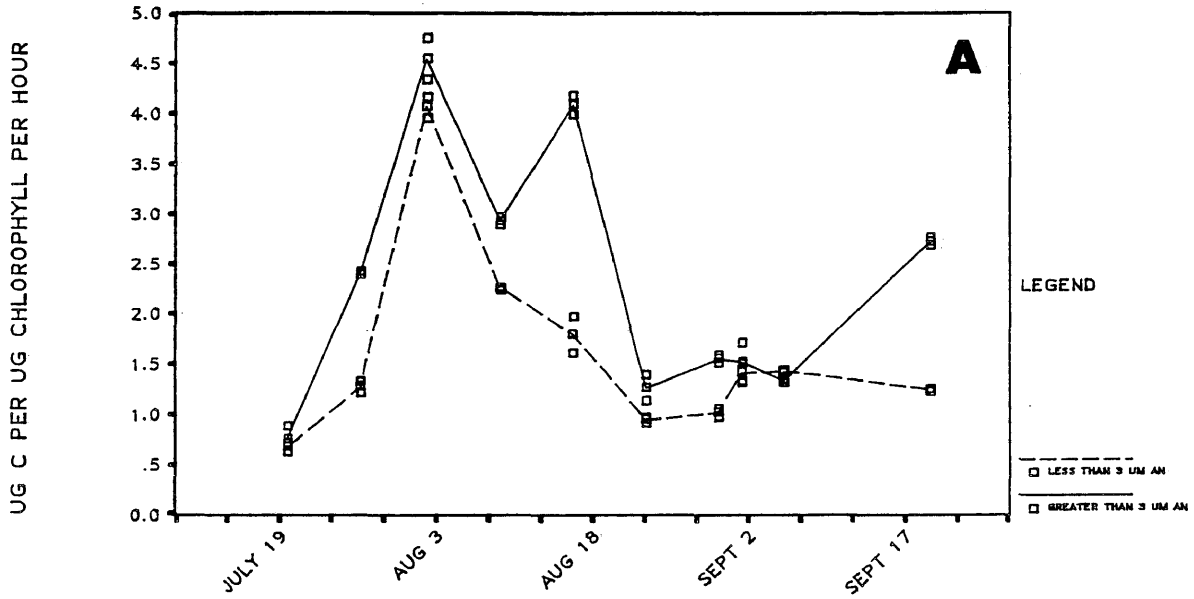


Figure 21: Chlorophyll specific carbon fixation of greater than 3 μm and less than 3 μm autotrophs.

- A. 43 $\mu\text{E m}^{-2} \text{sec}^{-1}$ irradiance
- B. 28 $\mu\text{E m}^{-2} \text{sec}^{-1}$ irradiance.

PHYTOPLANKTON CARBON ASSIMILATION VALUE GREATER AND LESS THAN 3 UM FRACTIONS

43 UE LIGHT INTENSITY



PHYTOPLANKTON CARBON ASSIMILATION VALUE GREATER AND LESS THAN 3 UM FRACTIONS

28 UE LIGHT INTENSITY

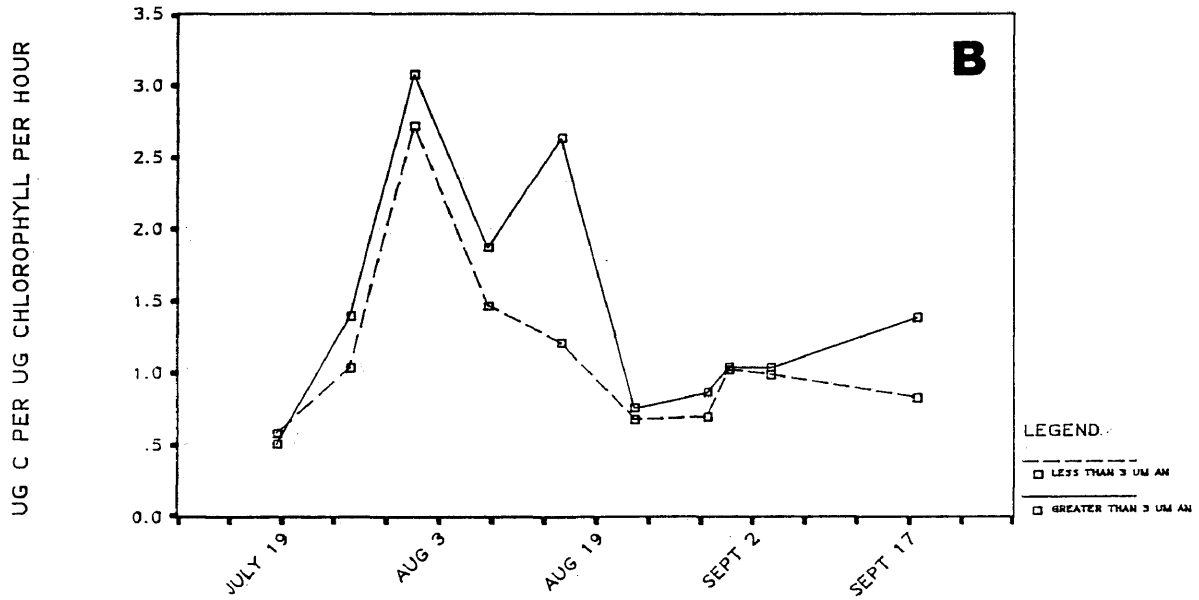


TABLE 5

Carbon specific growth rates (day^{-1})
of greater than 3 μm and less than 3 μm autotrophs
based on ^{14}C uptake values and a 12 hour photo-period

DATE	293		54		43		28	
	>3 μm	<3 μm	>3 μm	<3 μm	>3 μm	<3 μm	>3 μm	<3 μm
19 July	0.259	0.404	0.091	0.331	0.118	0.261	0.078	0.227
26 July	0.888	0.605	0.459	0.480	0.427	0.372	0.262	0.301
02 Aug	2.694	2.962	1.308	2.160	1.051	1.746	0.717	1.168
09 Aug	1.206	1.737	0.760	1.312	0.615	1.158	0.404	0.751
16 Aug	1.593	1.526	0.873	1.006	0.767	0.917	0.555	0.615
23 Aug	0.648	1.014	0.348	0.687	0.332	0.616	0.202	0.442
30 Aug	0.483	0.703	0.254	0.461	0.276	0.444	0.160	0.305
02 Sept	0.502	0.881	0.249	0.756	0.194	0.634	0.133	0.455
06 Sept	0.385	0.727	0.167	0.540	0.208	0.500	0.163	0.346
20 Sept	1.506	0.986	0.603	0.850	0.547	0.903	0.318	0.600
\bar{x}	1.016	1.154	0.511	0.858	0.454	0.755	0.299	0.521
C.V.	74.1%	65.4%	74.2%	63.2%	64.7%	58.9%	67.9%	53.9%

respectively. There was a significant difference ($P < .05$) in decrease in growth rates from 293 to 28 $\mu\text{E m}^{-2} \text{sec}^{-1}$ between the greater than 3 μm and less than 3 μm autotrophs (Rank Sum non-parametric test) (i.e. the decrease in growth rates between the highest and lowest light intensities was greater for the larger organisms). The same test revealed no significant difference in the decreases in growth rates from 54 to 28 $\mu\text{E m}^{-2} \text{sec}^{-1}$ light intensities between the greater than 3 μm and the less than 3 μm autotrophs.

Alpha values (initial slope of chlorophyll specific uptake versus irradiance) are listed in table 6. The mean seasonal alpha value of the greater than 3 μm fraction was 0.06 and ranged from 0.03 to 0.11 (using those alpha values with correlation coefficients greater than 0.8). Mean seasonal alpha of the less than 3 μm fraction was 0.05 and values ranged from 0.03 to 0.09 (discounting alpha values with correlation coefficients below 0.8). The initial slope was calculated both by forcing the the line through the origin and without forcing. The means of the alpha values for both fractions were not significantly different when calculated with or without forcing the line through the origin. The decision to use alpha values from lines forced through the origin was made based on the fact that photosynthesis should not occur in the absence of light.

DILUTION CHAMBERS

Numbers of heterotrophic and autotrophic bacteria in replicate chambers after the 24 hour incubation were often not well duplicated. (Table 7). The mean C.V. (Coefficient of Variation) for replicate

TABLE 6

Alpha values (initial slope of P vs I curve) for
greater than 3 μm and less than 3 μm phytoplankton
based on chlorophyll specific carbon uptake at
54, 43 and 28 $\mu\text{E m}^{-2} \text{sec}^{-1}$ (line forced through origin)

DATE	>3 alpha	SE	r^2	<3 alpha	SE	r^2	>3/<3
19 July	.014	.002	-.906	.017	.001	.520	0.8
26 July	.052	.003	.894	.031	.002	.827	1.7
02 Aug	.106	.001	.993	.094	.001	.996	1.1
09 Aug	.068	.000	.998	.050	.002	.942	1.4
16 Aug	.092	.005	.826	.040	.002	.851	2.3
23 Aug	.027	.002	.844	.021	.001	.758	1.3
30 Aug	.031	.003	.546	.021	.002	.589	1.5
02 Sept	.036	.000	.995	.033	.001	.922	1.1
06 Sept	.029	.003	-.424	.031	.002	.765	0.9
20 Sept	.058	.003	.934	.025	.003	.252	2.3
* \bar{x}	.063			.050			

SE = standard error of alpha

* mean of alpha values with r^2 greater than 0.80

TABLE 7

Bacterial numbers and coefficients of variation in replicate chambers
after 24 hour in situ incubation

CHAMBER TREATMENT

	100% 3um filtered	C.V.	50% 3um/50% unfiltered	C.V.	100% unfiltered	C.V.
Heterotrophic bacteria						
DAY						
07 Sept	7,383,917	9,192,604	15.4%	9,435,005	14,021,984	27.7%
10 Sept	7,421,210	7,520,656	0.9%	6,358,373	6,787,237	4.6%
23 Sept	2,498,598	2,386,721	4.0%	3,853,559	3,555,219	5.7%
					10,255,440	27.7%
					6,470,251	32.0%
					9,845,223	2.2%
					5,780,339	13.0%
Red-fluorescing cyanobacteria						
DAY						
07 Sept	200,758	256,386	17.2%	48,480	1,631,547	35.2%
10 Sept	277,829	127,417	52.5%	383,180	760,767	46.7%
23 Sept	31,233	27,503	9.0%	48,481	47,859	0.9%
					1,566,285	63.5%
					1,271,674	3.3%
					257,313	36.8%
Orange-fluorescing cyanobacteria						
DAY						
07 Sept	6,215	24,240	83.7%	52,210	85,773	34.4%
10 Sept	8,080	6,215	18.4%	19,579	54,074	66.2%
23 Sept	6,526	6,526	0.0%	18,614	26,726	25.2%
					82,044	62.6%
					31,699	45.3%
					61,533	19.2%
					50,345	19.2%

chamber heterotrophic bacterial numbers was 11.7% and ranged from 0.9 to 32.0%. Orange fluorescing cyanobacteria had a mean C.V. for replicate chamber numbers of 39.4% and ranged from 0.0% to 83.7%. Red fluorescing cyanobacteria had a mean C.V. for replicate chamber numbers of 29.5% and ranged from 0.9% to 63.5%. None of the organisms counted exhibited obvious patterns in replicate chamber variation due to treatment or day of experiment. Estimated autotrophic carbon at t=24 hours in replicate chambers were not consistently duplicated (mean C.V. was 22%). Chlorophyll *a* values in replicate chambers were not consistently duplicated either, but carbon : chlorophyll values for replicate chambers did show better consistency (mean C.V. was 14%, Table 8). Carbon : chlorophyll values had significant difference ($P < .025$) between the less than 3 μm chambers and the unfiltered chambers.

Calculated doublings day^{-1} using chlorophyll and carbon values (Table 9) had similar means and ranges, but pair-wise comparisons between the methods showed no significant correlations. Growth rates for the greater than 3 μm autotrophs ranged between 0.23 and 1.64 for carbon and -0.34 to 1.67 for chlorophyll. The less than 3 μm autotrophs had ranges of -0.39 to 1.52 doublings day^{-1} for carbon and -0.17 to 1.22 for chlorophyll. Ranges of doublings day^{-1} were greater for the carbon estimates than the chlorophyll estimates.

Undiluted chamber *r* values for heterotrophic bacteria, orange fluorescing cyanobacteria and red fluorescing cyanobacteria are shown (Table 10). Mean *r* values for the series of chamber experiments for heterotrophic bacteria were 0.80, for orange fluorescing

TABLE 8

Carbon : Chlorophyll values
 from growth chamber experiments
 after 24 hour incubation
 Summer, 1985

	100% 3um filtered C.V.			50% 3um/50% unfiltered C.V.			100% unfiltered C.V.		
DAY									
28 Aug	59	*		59	*		56	*	
07 Sept	26	21	15%	60	67	8%	62	71	10%
10 Sept	18	20	7%	49	69	24%	49	54	7%
23 Sept	10	17	37%	35	31	9%	36	32	8%

* No replicate chambers on August 28.

TABLE 9

Autotrophic growth rates derived from chlorophyll and carbon changes over a 24 hour incubation period in diffusion chambers.

Date	Chlorophyll				Carbon			
	>3 um fraction		<3 um fraction		>3 um fraction		<3 um fraction	
28 Aug	1.666	*	0.638	*	0.907	*	1.524	*
7 Sept	1.134	0.320	-0.345	0.046	0.853	0.227	-0.280	-0.105
10 Sept	1.139	1.224	0.562	-0.168	0.600	0.793	0.225	-0.394
23 Sept	0.995	0.922	0.396	0.383	1.636	1.578	0.126	-0.037
\bar{x}	1.009		0.269		0.938		0.300	
1 S.D.	0.213		0.332		0.471		0.807	
Range	0.32 - 1.2		-0.34 - 0.64		0.23 - 1.64		-0.39 - 1.52	

* No replicate chambers on 28 August

TABLE 10
 Bacterial intrinsic rate of increase (r)
 mean growth (k) and grazing (g) coefficients and coefficient of variation
 from growth chamber experiments

	r	k	C.V.	g	C.V.
Heterotrophic bacteria					
DAY					
28 Aug	0.75	1.11	23%	0.40	85%
07 Sept	0.55	1.06	59%	0.28	336%
10 Sept	0.72	0.35	54%	- 0.45	64%
23 Sept	1.17	0.37	119%	- 1.08	90%
Red-fluorescing cyanobacteria					
DAY					
28 Aug	0.52	1.45	20%	0.88	40%
07 Sept	0.88	- 0.11	1,609%	- 1.63	161%
10 Sept	1.25	- 0.27	741%	- 0.96	285%
23 Sept	1.38	- 0.62	244%	- 1.43	173%
Orange-fluorescing cyanobacteria					
DAY					
28 Aug	0.69	- 0.30	777%	- 0.98	342%
07 Sept	0.74	1.04	122%	- 0.15	1,260%
10 Sept	0.92	0.99	242%	1.24	330%
23 Sept	0.90	- 0.65	332%	- 0.70	494%

$$\text{Coefficient of Variation (C.V.)} = \frac{\text{Standard Deviation} * 100}{\bar{X}}$$

cyanobacteria, 0.81, and for red fluorescing cyanobacteria 1.01. The red fluorescing cyanobacteria had the lowest and highest r values for the undiluted chambers: 0.52 on August 28 and 1.38 on September 24.

Mean growth and grazing coefficients of the heterotrophic and autotrophic bacteria are presented in table 10. Heterotrophic bacteria had a mean growth coefficient of 0.72 and a mean coefficient of grazing of -0.21 for the 4 experiments. Orange fluorescing cyanobacteria had a mean growth coefficient of 0.27 and a mean coefficient of grazing of -0.15. Red fluorescing cyanobacteria had mean growth and grazing coefficients of 0.11 and -0.78, respectively. The Rank Sum test indicated significant difference ($P < .05$) between the k and g coefficients of the heterotrophic bacteria over the season, but indicated no significant difference between k and g coefficients of either types of cyanobacteria. The Rank Sum test found no significant difference between growth or grazing coefficients of the two types of cyanobacteria observed. Growth and grazing coefficients varied widely, depending on what two experimental treatments were used simultaneously to solve for k and g. The mean C.V. of k for all organisms and dates was 305%, and ranged 40 - 1260%. For g, the mean C.V. for all days and organisms was 362% and ranged from 20 to 1609% (Table 10).

DISCUSSION

I. The Role of Picoplankton in Primary Production

This study refers to picoplankton as those organisms passing through a 3 μm pore size Nuclepore filter and retained on a 0.2 μm Nuclepore filter. The reason for not using a 2.0 μm Nuclepore filter and remaining consistent with Sieburth et al. (1978) nomenclature was the concern of retaining less than 2 μm organisms on the 2.0 μm filter and underestimating their role in the community. Use of 3 μm pore size filters alleviated the problem somewhat, but retention of less than 2.0 μm organisms was still observed (e.g. Fig.5). Some organisms larger than 3 μm in diameter were observed, but this was relatively rare (autotrophic dinoflagellates in the less than 3 μm fraction had a modal diameter of 5 μm). Most of the organisms in the less than 3 μm fraction were smaller than 2 μm in diameter.

STANDING STOCK

Chroococcoid cyanobacteria were the most numerically important picophytoplankton, with densities in the range of 10^5 ml^{-1} . Densities of *Synechococcus* species on the order of $10^5 \text{ cells ml}^{-1}$ have been observed in other estuarine studies - Woods Hole Harbor (Waterbury et

al. 1979), Carmans River Estuary, Great South Bay, New York (Campbell et al. 1983) and Chesapeake Bay (Perkins et al. 1981).

Most studies have equated picophytoplankton with cyanobacteria, but this is not necessarily the case. On July 3, 19, 26, 29, and August 19-23, the percentage of cyanobacterial carbon making up the picophytoplankton biomass fell below 50%. No other category of phytoplankton was singularly responsible for a large increase in contribution to phototrophic picoplankton biomass on those days. Autotrophic dinoflagellates in the less than 3 μm fraction were responsible for 25% and 40% of the autotrophic carbon on July 2 and July 26, respectively. Diatoms contributed 27% and 28% of the autotrophic carbon in the picoplankton fraction on July 3 and August 21, respectively. Autotrophic flagellates were responsible for 28% of the biomass on July 29 and 25% on August 21 in the picophytoplankton. Cyanobacterial carbon on those days of low contribution showed no particular trends (e.g. consistently low values). The contribution to phototrophic picoplankton carbon by the "other autotrophs" category was relatively minor at all times. Other studies have documented organisms making up the phototrophic picoplankton community. Glover et al. (1985a) reported that cyanobacteria made up 50-99% of the picophytoplankton (<3.0 >0.2) biomass in the Gulf of Maine in 1982. On a 1983 cruise from the Gulf of Maine to the Sargasso Sea, Glover et al. (1985b) found eucaryotic ultraphytoplankters (<3.0 >0.2 μm) made up 12 - 78% of the ultraplankton community abundance. Others have found prasinophytes, prymnesiophytes, chrysophytes and chlorophytes in

the ultra and picoplankton fractions (Glover et al. 1985b, Johnson and Sieburth 1982).

Picophytoplankton biomass in this study ranged from 9 to 148 $\mu\text{g C L}^{-1}$, and less than 15 μm autotrophic biomass ranged between 14 and 1365 $\mu\text{g C L}^{-1}$. Furnas, 1983, studied phytoplankton dynamics in Narragansett Bay, Rhode Island in 1979. He found that relative and absolute contribution of the less than 5 μm phytoplankton to total standing stock was greatest during the early and mid-summer period. Between June and September, estimated phytoplankton biomass ranged between 200 - 2,700 $\mu\text{g C L}^{-1}$ for the total fraction, and less than 400 $\mu\text{g C L}^{-1}$ for the less than 5 μm fraction (except for a peak of approximately 1350 $\mu\text{g C L}^{-1}$ in the beginning of June). Total values are similar to those I estimated. The less than 5 μm fraction is higher than what I reported, but the difference seems reasonable considering the the different pore size filters used.

In the larger than 3 μm fraction, 2 distinct peaks were observed in autotrophic carbon - the first week in July and late August - early September. The first peak was mainly due to autotrophic flagellates (including dinoflagellates), while the peak in late August was made up primarily by diatoms. Patten et al., 1963, observed the same phenomena at the mouth of the York River in 1960.

Carbon : chlorophyll *a* (C:Chl) values were significantly lower in the picophytoplankton than the larger phytoplankton (seasonal means of 22 and 55, respectively). Chlorophyll *a* per volume has been found to be inversely related to cell size in other studies (see Malone 1980). Putt and Prezelin (1985) calculated C:Chl values for 0.2 - 5.0 μm

phytoplankton in the Santa Barbara Channel during the 1983 "el Nino" event when chroococcoid cyanobacteria were twice as abundant as usual. Using $45.6 \text{ fg C cell}^{-1}$ for converting cyanobacterial numbers to carbon biomass, they obtained C:Chl values of 2 - 9. They also used a value of $294 \text{ fg C cell}^{-1}$ (based on Cuhel and Waterbury 1984), and obtained a ratio of 49.8, which was still lower than their mean ratio of 80 which was measured for 4 whole water samples taken at the chlorophyll maximum. Values for carbon biomass per cell for bacteria are controversial at this time. the value of 0.22 g C cm^{-3} (Bratbak and Dundas 1984) is high compared to previously reported values (c.f. Bratbak 1985), but conforms to the measurements of Cuhel and Waterbury (1984) and Kana and Glibert (submitted 1986) (means of 294 and 250 fg C cell^{-1} , respectively, for Synechococcus cultures. The carbon value I used for the cyanobacteria was intermediate (152 fg cell^{-1}), but still resulted in lower relative C:Chl values. These lower values may be due, in part, to underestimation of the picophytoplankton carbon biomass. Lower values for the smaller forms indicate that the autotrophic picoplankton might be more photosynthetically active at low light intensities than the larger autotrophs, an observation that has been suggested elsewhere (Glover et al. 1985a, Putt and Prezelin 1985, Platt et al. 1983).

CARBON FIXATION RATES

Although picophytoplankton accounted for approximately 7% of the seasonal autotrophic standing stock, they were responsible for 9% of the total carbon uptake at $293 \text{ uE m}^{-1} \text{ sec}^{-1}$ (ca. 15% of noon surface

light irradiance) and increased their contribution to 13% of the total carbon uptake at $28 - 54 \text{ uE m}^{-2} \text{ sec}^{-1}$ (1-2% of noon surface light irradiance). Again, this indicates that phototrophic picoplankton are more active than larger phytoplankton at carbon fixation at low light intensities.

Based on literature values, $293 \text{ uE m}^{-2} \text{ sec}^{-1}$ was presumed to represent saturating light intensity for both fractions and that chlorophyll specific uptake rates at this intensity represented P_{max} . Harris, (1980), in an examination of the literature, found that a large number of observations of I_k fell in the range of $50 - 120 \text{ uE m}^{-2} \text{ sec}^{-1}$. Putt and Prezelin (1985) performed ^{14}C productivity studies with $0.2 - 5.0$ and $5.0 - 30 \text{ um}$ post filtered Santa Barbara Channel water. They found that the I_k values for the small and large fractions ranged between $26 - 32$ and $38 - 55 \text{ uE m}^{-2} \text{ sec}^{-1}$, respectively (incubated at 16°C). In this study it was apparent that light saturation values were always above $54 \text{ uE m}^{-2} \text{ sec}^{-1}$ for both fractions, as uptake rates at $293 \text{ uE m}^{-2} \text{ sec}^{-1}$ were always greater than at $54 \text{ uE m}^{-2} \text{ sec}^{-1}$.

P_{max} values reported in this study (Table 4, 1.66 - 11.68 for the greater than 3 um autotrophs and 1.05 - 6.90 for the less than 3 um autotrophs) are well within values reported in the literature for natural populations of phytoplankton (Harris 1980). P_{max} values of Chesapeake Bay natural phytoplankton populations were determined in 1982 by Harding et al. (1985). Values under white light incubations ranged from 1.5 to $11 \text{ ug C (ug Chl}_a\text{)}^{-1} \text{ hr}^{-1}$, and I_k values were between 47.5 and $195 \text{ uE m}^{-2} \text{ sec}^{-1}$ (with one outlying value of 322).

P_{\max} values for total plankton samples in the York River in August 1978 were reported by Haas et al. (1981b) to range between 8 and 18 $\mu\text{g Chl}_a^{-1} \text{hr}^{-1}$.

This study found significantly lower P_{\max} values for the picoplankton fraction than the larger plankton. At lower light intensities, however, chlorophyll specific uptake rates were not significantly different. Takahashi and Bienfang, 1983, reported P_{\max} values of 3.24 - 14.51 for less than 3 μm phytoplankton in subtropical waters off Oahu, Hawaii, while 3 - 20 μm phytoplankton showed a trend towards slightly higher values (one-third higher for 2 out of 3 experiments). During the 1983 "el Nino" event, P_{\max} values of the 0.2 - 5.0 μm and 5.0 - 30 μm fractions were 1.85 - 4.65 and 2.08 to 5.34, respectively, in the Santa Barbara Channel (Putt and Prezelin 1985). Other studies (see Malone 1980) have indicated higher P_{\max} values for nanoplankton than net plankton.

Growth rates based on P_{\max} values were higher for the picophytoplankton than the larger phytoplankton in 8 out of 10 experiments (Table 5). At the 3 lower light intensities, only on one day in one treatment was the growth rate estimate greater for the larger than 3 μm autotrophs than the less than 3 μm autotrophs. P_{\max} growth rates, 0.259 - 2.694 for larger autotrophs and 0.404 - 2.962 for picoautotrophs, resemble growth rates reported in other studies. Furnas, (1982) estimated less than 10 μm phytoplankton doubling rates in Narragansett Bay, Rhode Island, using ^{14}C and cell numbers to carbon conversions. Doubling rates per day ranged from 0.5 to 1.9 in 1978 and 0.9 to 2.0 in 1979. Doublings per day for marine

cyanobacteria have been studied by others (see Putt and Prezelin 1985) and values range from 0.22 for the oligotrophic North Atlantic to 12.8 near Nova Scotia, with most values between 0.7 and 2.5.

It is interesting to compare the chlorophyll specific uptake rates and growth rates based on carbon uptake and biomass estimates, for the picoplankton and larger plankton. At $293 \text{ uE m}^{-2} \text{ sec}^{-1}$ intensity, the greater than 3 μm phytoplankton chlorophyll specific uptake rates for each experiment were always higher than the picoplankton fraction, and yet the picoplankton fraction showed greater growth rates. This again indicates that the carbon conversion values for the autotrophic picoplankton were too low. The alternative is that chlorophyll *a* values for the greater than 3 μm fraction were underestimates. Assuming total chlorophyll was measured correctly, if chlorophyll in the less than 3 μm fraction was overestimated then carbon to chlorophyll ratios for the less than 3 μm fraction would be higher than those presented. This could be possible if larger cells were broken up in filtering samples through the 3 μm Nuclepore filter, releasing chlorophyll into the filtrate. Both alternatives are plausible and not mutually exclusive.

Alpha values are considered to be a measure of low light photosynthetic efficiency, with higher values indicating higher efficiency (Malone and Neale 1981). The present study found that alpha values for the greater than 3 μm fraction were consistently higher than the less than 3 μm fraction (Table 6) but the difference between seasonal means was not statistically significant (0.06 and 0.05 respectively). It has been suggested (c.f. Malone 1980) that

algal photosynthetic capacity increases with decreasing cell size. Malone and Neale (1981) in the lower Hudson Estuary found the mean alpha values for phytoplankton less than 22 μm at 17 to 26 $^{\circ}\text{C}$ to be 0.07 and for larger phytoplankton at the same temperatures, 0.02. Platt et al. (1983) found the mean alpha value of the less than 1 μm phytoplankton to be double of that of the greater than 1 μm organisms (0.074 and 0.038 respectively) on the mid-Atlantic ridge around 36 $^{\circ}$ N Latitude. Other workers, however, have found the alpha values of the smaller cells to be less than those of larger organisms. Putt and Prezelin (1985) found alpha values of 0.06 to 0.1 for phytoplankton 0.2 to 5.0 μm in size, in the Santa Barbara Channel. Phytoplankton 5.0 to 30 μm in size from the same location in the sub-surface chlorophyll maxima were found to have alpha values of 0.09 to 0.16. Prezelin et al. (1986) found Synechococcus dominated size fractions had lower assimilation rates than larger phytoplankton fractions in the North Atlantic. The reason for the difference in these findings is not clear, but it may be due to estimated chlorophyll values in the smaller size fractions. Criticisms have been made concerning the large amount of chlorophyll found per cell by Prezelin et al. (1986) (T. Kana, personal communication). Lower chlorophyll values in the smaller size fractions would lead to increased alpha values (and higher carbon : chlorophyll values). In the present study it is also possible that $54 \mu\text{E m}^{-2} \text{sec}^{-1}$ was close to the inflection point of the P v I curve. If so, including this value in the calculation would cause alpha to be lower than the actual value.

DILUTION CHAMBER ESTIMATES

Large variations in organism densities and chlorophyll values between replicate chambers indicated experimental design problems. Larger volume chambers and more replicates could perhaps give more definitive answers to questions the chamber experiments attempted to address. Use of chambers that are more light transparent and/or allow light attenuation to be measured within the chamber would be desirable.

Growth coefficients, (k), indicate gross growth rate. Negative k values resulted from simultaneous equation solving where autotroph abundances in chambers with lesser numbers of grazers was much lower than those with greater numbers of grazers or where a decrease over the incubation period occurred in the number of autotrophs in chambers having lower grazer densities. It is possible that autotrophic picoplankton growth rates are increased in the presence of grazers, as suggested by the predominance of negative grazing coefficients. The negative g values indicate higher growth rates in chambers having a greater density of grazing heterotrophs. Goldman et al. (1979) suggested that heterotrophs excrete compounds that can be limiting to phytoplankton growth. Webb and Haas (1976) found urea, a regenerated nitrogen compound, to be a significant source of nitrogen for phytoplankton in the York River, especially for the less than 15 μm fraction organisms. Haas, 1975, suggested that a close link could exist between zooplankton and/or microzooplankton excretion and nutrient availability for phytoplankton. Under low nutrient conditions, these grazers could be an important nutrient source. It

was assumed but never tested, that the Nuclepore membrane filters would allow for ambient nutrient conditions inside the chambers. If nutrient conditions inside the chambers were low, the presence of grazers could have had an impact on picoplankton growth. It is also possible that fractionation prior to incubation had a deleterious effect upon the picoplankton. This problem certainly cannot be dismissed, although microscopic examination of the picoplankton fraction revealed no obvious cell damage. One way around this problem would be to prefilter sample water through a 0.2 μm Nuclepore filter and use the dilution technique described by Landry et al., 1984.

II. Plankton Dynamics in a Variable Vertical Salinity Structure Estuary

PHYSICAL PARAMETERS

Mean surface water temperatures for July and August were within 0.5 $^{\circ}\text{C}$ of 5 year means taken from the Virginia Institute of Marine Science Ferry Pier, approximately 1.5 km across the York River from the Coast Guard Pier (Computer Center records from 1979-1984). The mean monthly temperature for September was approximately 1.0 $^{\circ}\text{C}$ higher in 1985 than 1979-1983 (no 1984 data was available). Mean monthly surface salinities were 2 to 4 ppt higher in 1985 than the 25 year monthly mean (Wojcik 1981), with July 1985 surface salinities having the greatest difference from the 25 year mean (23.0 and 19.2 respectively). This reflects the lower than average summer

precipitation values for 1985 (U.S.G.S. Estimated Stream Flow entering the Chesapeake Bay 1986).

Haas, 1975, first described the effect of the neap-spring tidal cycle on the vertical salinity structure of the York River. The York River oscillates between a stratified and a well-mixed water column on a fortnightly tidal cycle. This stratified-destratified cycle is well correlated with tidal height. This study spanned 3 monthly lunar tidal cycles - in the beginning of July, the water column was just beginning to restratify, and this was the case again at the end of September. Top to bottom salinity differences, an indication of water column stratification, were inversely correlated with predicted highest daily tide height ($r^2 = .315$, $P < .001$). Predicted monthly highest tidal height occurred about 3 days after lowest monthly delta salinity (Fig.3). Temperature and extinction coefficient were not significantly correlated to either the delta salinity or the daily highest tide height. This pattern conforms to previous observations of Haas (1977) and Hayward et al. (1982).

STANDING STOCK

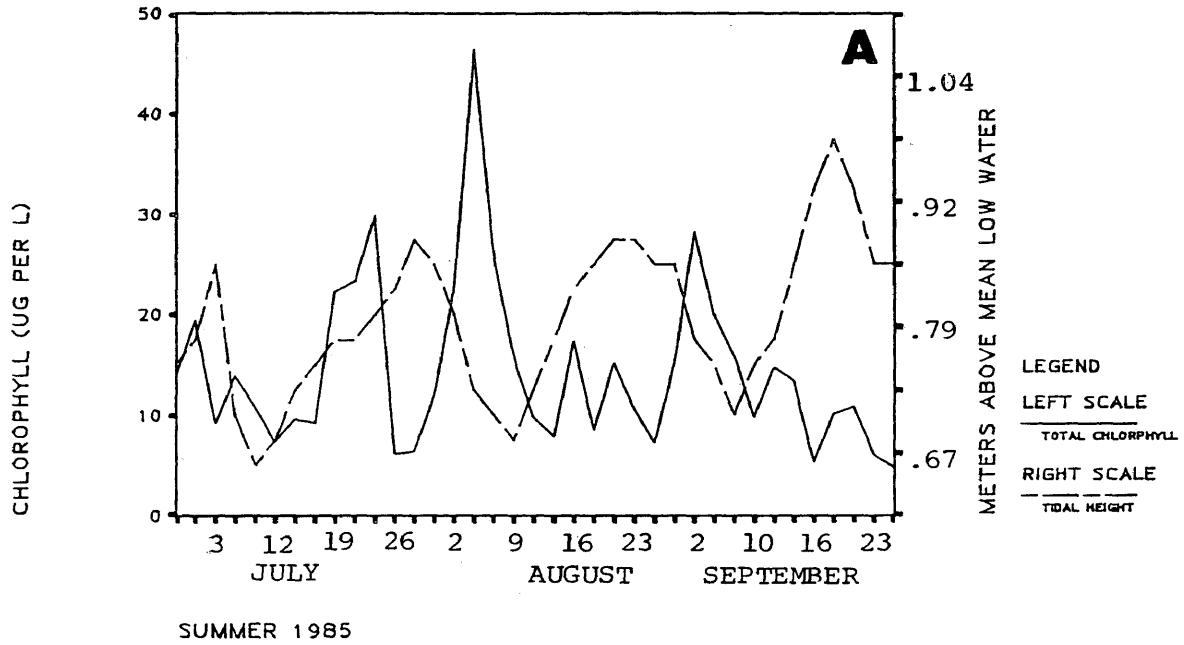
Surface chlorophyll values in all three fractions increased during monthly restratification (Figs.4,22). Total fraction values increased 10-660%, with increases lasting 3 to 6 days before declining. The less than 3 μ m fraction increased 3-154% over the same periods. The lowest percentage increase in chlorophyll a values after monthly high spring tide for both fractions occurred at the end of September, after surface water temperatures had dropped from 28 to 23

Figure 22: Surface chlorophyll a and highest tide by day.

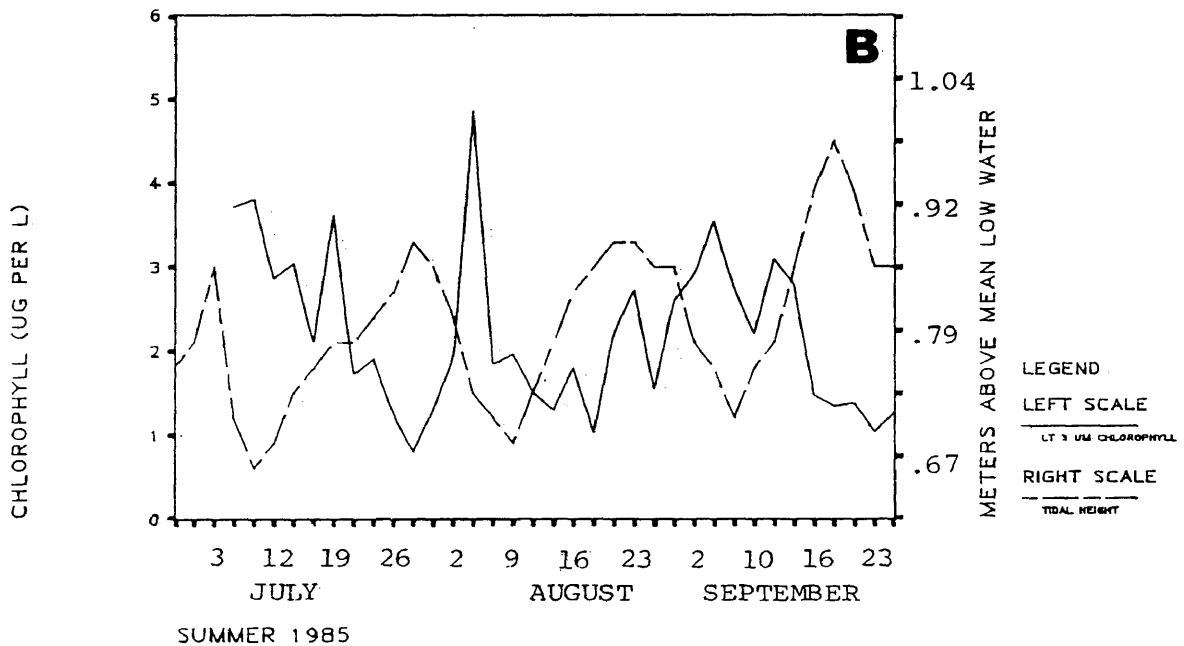
A. Total chlorophyll a

B. Less than 3 um fraction chlorophyll a.

SURFACE CHLOROPHYLL AND TIDAL HEIGHT TOTAL FRACTION



SURFACE CHLOROPHYLL AND TIDAL HEIGHT LESS THAN 3 UM FRACTION



°C. Two other notable increases in chlorophyll occurred which were not subsequent to monthly highest spring tides, June 26 and July 16-26 (in June, samples were take only once per week). Both periods were associated with a destratification event. Delta salinity decreased from 3.01 to -0.01, June 20-26. Delta salinity decreased from 3.14 to 0.87 between July 14-16. This last destratification period could have been due to a spring tide, coming 14 days prior to the monthly highest spring tide.

Both autotrophic and heterotrophic plankton decreased in numbers during destratification events, reaching low densities at highest monthly spring tide. Total plankton biomass was correlated to autotrophic biomass over the sampling season. The total fraction had an r^2 coefficient of .385 ($P < .001$) and the less than 3 um fraction had a correlation coefficient of .890 ($P < .001$). Organism categories with large biovolumes had very large biovolume Standard Deviations (Table 2). This, coupled with erratic distribution on the filters, makes interpretation of the total sample biomass responses over time difficult. Increased counts of the rarer categories could help alleviate the problem, but would represent a considerable increase in slide counting time. In spite of this problem, the higher correlation coefficient between the heterotrophs and autotrophs for the less than 3 um fraction suggests that the response to physical processes by the picoheterotrophs was more similar or closely coupled to the picoautotrophs than the larger heterotrophs and autotrophs. Heterotrophic bacteria abundances were the most highly correlated

abundances to the daily tidal height with an r^2 value of 0.512 and $P < 0.001$.

Densities of cyanobacteria increased 2 to 30 fold after the spring tides, except for the September spring tide (sampling ceased prior to restratification at the end of September, Figs.3,23). Cyanobacterial numbers reached their lowest density of the season on August 19. This day marked the lowest delta salinity of the monthly tidal cycle. Subsequently, the cyanobacteria increased to their highest seasonal density over the span of 3 weeks. The precipitous drop in cyanobacterial numbers on September 13 coincided with a destratification of the water column- delta salinity remained above 1 ppt from August 23 to September 10 and then decreased to 0.58 ppt.

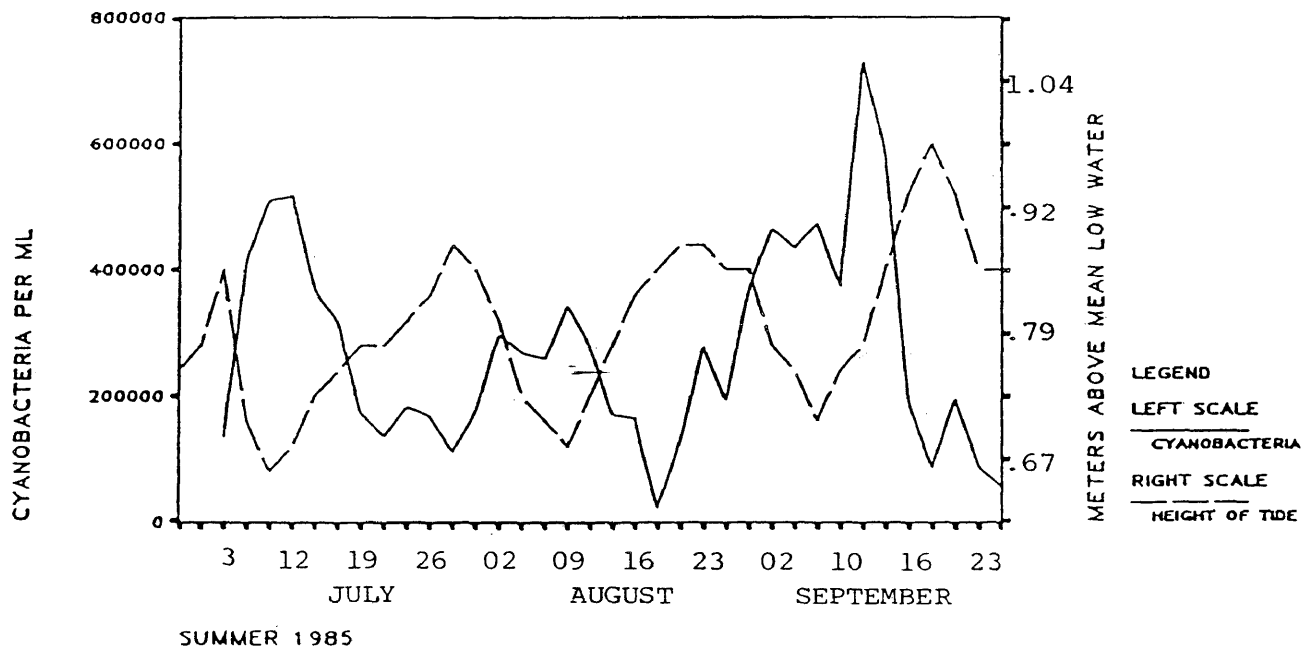
Both red and orange fluorescing cyanobacteria were negatively correlated with daily highest tide ($r^2 = .269$ and $.230$, respectively). Red fluorescing cyanobacteria were negatively correlated with the mean surface salinity ($r^2 = .317$, $P < .001$) and positively correlated with delta salinity ($r^2 = .417$, $P < .001$). The negative correlation between red fluorescing cyanobacteria and mean surface salinity indicates that these organisms are advected into the bay by fresher upriver water and suggests a fresh water source. The orange fluorescing cyanobacteria exhibited weak correlations with both delta salinity and mean surface salinity ($r^2 < .05$). Because of the lower than normal freshwater inflow in 1985, red fluorescing cyanobacteria may have been present in lower numbers than normally found.

The inverse relationship between organism numbers and daily tidal height (Fig.23) is probably due to physical dilution of organisms.

Figure 23: Cyanobacteria abundance and highest tide by day.

—

CYANOBACTERIAL DENSITIES AND TIDAL HEIGHT



The low densities of orange fluorescing cyanobacteria on July 29 and August 19 were also reflected in the red fluorescing cyanobacterial densities. On July 29 delta salinity was 0.38 ppt, and on August 19, delta salinity was -0.01 ppt, indicating a vertically homogeneous water column. These days were also days of predicted monthly highest spring tides. Once the water column restratified, as indicated by higher delta salinities or lower predicted daily tidal heights, cyanobacterial densities increased. The reason for the decrease in percentage of orange-fluorescing cyanobacteria in the cyanobacterial population over the course of the summer is not apparent. It is possible that the orange fluorescing cyanobacteria do not do well at higher temperatures. At the time of highest densities of red fluorescing cyanobacteria (September 4- September 12, Fig.5), temperatures were ca. 28°C, while orange fluorescing cyanobacteria reached their maximum densities early in the season when temperatures were ca. 26°C. This conforms to the hypothesis that the orange fluorescing forms are more adapted to oceanic environments and lower temperatures. If higher temperatures are limiting to phycoerythrin-containing cyanobacteria, years with more average temperatures in late August and September would be predicted to show higher numbers of orange fluorescing cyanobacteria at the Yorktown Coast Guard Pier.

The increase in the total fraction diatom density, beginning August 23, occurred 3 days later than the cyanobacterial increase. The decline of the diatom population began sooner than that of the cyanobacteria, shortening the time span of the diatom increase with respect to that of the cyanobacteria.

PRIMARY PRODUCTION

The lack of information on in situ nutrient conditions hampers the interpretation of the effects of stratification-destratification upon carbon uptake and assimilation values as affected by nutrients. It has been reported that the magnitude of assimilation numbers are affected by nutrient availability (see Harris 1980), assimilation being lower under low nutrient conditions than high nutrient conditions. In a 1978 study of a York River stratification-destratification event, Haas et al. (1981b) reported prior and post destratification surface water P_{\max} values of 18 and 16 $\mu\text{g C } (\mu\text{g Chl}_a)^{-1} \text{ hr}^{-1}$. P_{\max} values of surface waters during destratification dropped to 10 $\mu\text{g C } (\mu\text{g Chl}_a)^{-1} \text{ hr}^{-1}$, but during the destratification event a surface water dinoflagellate bloom was sampled and P_{\max} for this water was 20 $\mu\text{g C } (\mu\text{g Chl}_a)^{-1} \text{ hr}^{-1}$. He concluded that the decrease in P_{\max} during destratification was due to shade adaptation by the phytoplankton. However, Haas et al. (1981b) hypothesized that observed increases in diatom numbers subsequent to destratification, may have resulted from nutrient enrichment of surface waters from nutrient rich bottom waters. Webb and D'Elia (1980) concluded in a study of a stratification-destratification event in the York River that mixing of the water column allows benthic-regenerated nutrients to be pulsed into euphotic surface waters. It is interesting that lowest values of ammonia and phosphate occurred during the observed destratification period. Ducklow (1982) observed maximum heterotrophic bacterial productivity during maximum water column homogeneity in the York River, 3 to 4 days after maximum tide

in 1980. Also observed was a redistribution in the water column of ammonium and oxygen.

Carbon uptake rates and chlorophyll specific carbon uptake rates were greatest for both greater and less than 3 μm phytoplankton on August 2, 4 days after the highest monthly spring tide and the day of lowest monthly tidal delta salinity (when samples were taken for the productivity study delta salinity was -0.03 , the lowest value of the study). It is interesting that biomass estimates on this day were relatively low, perhaps due to a dilution effect with the mixing of the water column. The water column had delta salinity values below 1.0 ppt for 8 days prior to this date and was restratified (delta salinity > 1.0 ppt) prior to the subsequent sampling date. Other destratification events did not produce carbon fixation values of this magnitude.

There is a trend in the data for a biweekly increase/decrease in chlorophyll specific uptake rates for the greater and less than 3 μm phytoplankton, but there are discrepancies which need to be explained. Assimilation values are low on July 19, where one might expect higher values due to the low delta salinity. Delta salinity on that day was 0.87, but had been above 2.0 since July 8. It is possible that surface waters were still low in critical nutrients, or that the water column had not yet fully destratified. On August 16, the greater than 3 μm organisms showed an increase in chlorophyll specific carbon uptake, but the less than 3 μm fraction did not. On September 20, the same type of discrepancy was again noted. In both cases low chlorophyll values in the total fraction could account for the

increased chlorophyll specific uptake in the greater than 3 um fraction. The reason for the low chlorophyll specific carbon fixation rates by the less than 3 um autotrophs is not apparent but can perhaps be explained as an overestimate of chlorophyll a concentration in the less than 3 um fraction.

Alpha values for both greater than 3 um and less than 3 um fractions were at their highest at low delta salinity values (Fig.3 and Table 6). The highest alpha values for both fractions were noted on August 2, when delta salinity was 0.03 ppt. Correlation coefficients were low ($r^2 < .10$ and $P > .10$) when regressions were run on both fractions against delta salinity and daily highest tide, indicating the importance of other factors on photosynthetic efficiency. The data does suggest, however, that under natural conditions both size fractions do adapt to lower average light levels (due to the greater surface mixed layer) by increasing their photosynthetic efficiency, a response that has been observed in the laboratory.

CONCLUSION

Over the course of the study, the autotrophic picoplankton represented a small but measurable and possibly significant standing stock of carbon. Alpha values indicated that the picoplankton were not as photosynthetically efficient (on a per chlorophyll basis) as larger phytoplankton, but their higher contribution to total carbon fixation at lower light intensities suggests that at times of increased turbidity or deeper surface mixed layer their role in primary production is greater than their actual carbon or chlorophyll biomass suggests. Both the autotrophic and heterotrophic picoplankton in surface waters showed a much closer correlation with the neap-spring tidal cycle than the larger plankton, but the reason for this is unclear. Is it because they were more, or less affected by ambient nutrient conditions? Perhaps metabolic rates of the picoplankton allowed faster adaptation to changing light conditions during warmer temperatures, or was the closer correlation due simply to the higher error associated with the lower number of large organisms on the filters?

Diffusion chamber experiments were not definitive enough to answer the question of the role of autotrophic picoplankton in the estuarine food web. Slide counts did reveal that there were heterotrophic eucaryotes as small as 1 - 2 μm in diameter. Iturriaga

and Mitchell (1986), Gast (1985), Landry et al. (1984), Fenchel (1982) and Haas and Webb (1979) all observed grazing of picoplankton by heterotrophic eucaryotes. This suggests that picoplankton do play a role in the estuarine food web. An interesting question brought out by this study is whether or not picoplankton do in fact have higher growth rates in the presence of grazers, a question that is closely tied to the question of nutrient effects and limitations on the picoplankton.

The decline of both smaller fraction phytoplankton abundance and contribution to total autotrophic carbon in temperate estuaries in the fall has been observed by others (Bruno et al. 1983, Campbell et al. 1983, Furnas 1983, Van Valkenberg and Flemmer 1974). It appears that temperature has some role in this decline, but a hypothesis concerning the reason for this occurrence has yet to be documented. The determination of the underlying cause of the fall decline in picophytoplankton would help resolve larger questions concerning the role of physical factors on phytoplankton processes.

LITERATURE CITED

- Ambrose, H.W. and K.P. Ambrose. 1978. A Handbook for Biological Investigation, 2nd Edition. 170 p. Winston-Salem, North Carolina. Hunter Publishing Company.
- Boynton, W.R., W.M. Kemp and C.W. Keefe. 1982. A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production. pp69-90. In: V. Kennedy, ed. Estuarine Comparisons. Academic Press.
- Bratbak, G. 1985. Bacterial biovolume and biomass estimations. Applied and Environmental Microbiology 49(6):1488-1493.
- Bratbak, G. and I. Dundas. 1984. Bacterial dry matter content and biomass estimations. Applied and Environmental Microbiology 48(4):755-757.
- Bruno, S.F., R.D. Staker, G.M. Sharma and J.T. Turner. 1983. Primary productivity and phytoplankton size fraction dominance in a temperate North Atlantic estuary. Estuaries 6(3):200-211.
- Campbell, L., E.J. Carpenter and V.J. Iacono. 1983. Identification and enumeration of marine chroococcoid cyanobacteria by immunofluorescence. Applied and Environmental Microbiology 46(3):553-559.
- Cowles, R.P. 1930. A biological study of the offshore waters of Chesapeake Bay. Bulletin of the Bureau of Fisheries 46:277-381.
- Cuhel, R.L. and J.B. Waterbury. 1984. Biochemical composition and short term nutrient incorporation patterns in a unicellular marine cyanobacterium, Synechococcus (WH7803). Limnology and Oceanography 29(2):370-374.
- Ducklow, H.W. 1982. Chesapeake Bay nutrient and plankton dynamics. 1. Bacterial biomass and production during spring tidal destratification in the York River, Virginia, estuary. Limnology and Oceanography 27(4):651-659.
- Eppley, R.W., F.M. Reid and J.D. Strickland. 1970. Estimates of phytoplankton crop size, growth rate, and primary production. Bulletin of the Scripps Institute of Oceanography 17:33-42.
- Fenchel, T. 1974. Intrinsic Rate of natural increase: the relationship with body size. Oecologia 14:317-326.

- Fenchel, T. 1982. Ecology of Heterotrophic Microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. *Marine Ecology - Progress Series* 9:35-42.
- Fritz-Thompson, A. 1986. Trophodynamics of nanoplankton in salt marsh ecosystems. Doctoral dissertation, School of Marine Science, College of William and Mary, Gloucester Point, Virginia.
- Furnas, M.J. 1982. Growth rates of summer nanoplankton (<10 μm) populations in lower Narragansett Bay, Rhode Island, USA. *Marine Biology* 70:105-115.
- Furnas, M.J. 1983. Community structure, biomass and productivity of size-fractionated summer phytoplankton populations in lower Narragansett Bay, Rhode Island. *Journal of Plankton Research* 5(5):637-655.
- Gast, V. 1985. Bacteria as a food source for microzooplankton in the Schlei Fjord and Baltic Sea with special reference to ciliates. *Marine Ecology - Progress Series* 22:107-120.
- Glover, H.E., D.A. Phinney and C.S. Yentsch. 1985a. Photosynthetic characteristics of picoplankton compared with those of larger phytoplankton populations, in various water masses in the Gulf of Maine. *Biological Oceanography* 3(3):223-248.
- Glover, H.E., A.E. Smith and L. Shapiro. 1985b. Diurnal variations in photosynthetic rates: comparisons of ultraphytoplankton with a larger phytoplankton size fraction. *Journal of Plankton Research* 7(4):519-535.
- Goldman, J.C., J.J. McCarthy and D.G. Peavey. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* 279:210-215.
- Haas, L.W. 1975. Plankton dynamics in a temperate estuary with observations on a variable hydrographic condition. Doctoral dissertation, School of Marine Science, College of William and Mary, Gloucester Point, Virginia.
- Haas, L.W. 1977. The effect of the spring-neap tidal cycle on the vertical salinity structure of the James, York, and Rappahannock Rivers, Virginia, U.S.A. *Estuarine and Coastal Marine Science* 5:485-496.
- Haas, L.W. 1982. Improved epifluorescence microscopy for observing planktonic micro-organisms. *Annales de L'Institut Oceanographique* 58(S):940-946.
- Haas, L.W., F.J. Holden and C.S. Welch. 1981a. Short term changes in the vertical salinity distribution of the York River estuary associated with the neap-spring tidal cycle. pp 585-599. In: B.J.

- Neilson and L.E. Cronin, editors. Clifton, New Jersey. Humana Press.
- Haas, L.W., S.J. Hastings and K.L. Webb. 1981b. Phytoplankton response to a stratification-mixing cycle in the York River estuary during late summer. In: Estuaries and Nutrients. pp 619-636. In: B.J. Neilson and L.E. Cronin, editors. Clifton, New Jersey. Humana Press.
- Haas, L.W., and K.L. Webb. 1979. Nutritional mode of several non-pigmented microflagellates from the York River estuary, Virginia. *Journal of Experimental Marine Biology and Ecology* 39:125-134.
- Haines, E.B. 1979. Interactions between Georgia salt marshes and coastal waters: a changing paradigm. pp.35-46. In: R. J. Livingston, ed. *Ecological Processes in Coastal and Marine Systems*. New York, New York. Plenum Press.
- Harding, L.W., B.W. Meeson and T.R. Fisher. 1985. Photosynthesis patterns in Chesapeake Bay phytoplankton: short- and long-term responses of P-I curve parameters to light. *Marine Ecology - Progress Series* 26: 99-111.
- Hayward, D. and K.L. Webb. (Draft). Chlorophyll extraction of estuarine and marine phytoplankton with Dimethyl Sulfoxide (DMSO). *Canadian Journal of Fisheries and Aquatic Sciences*.
- Hayward, D., C.S. Welch and L.W. Haas. 1982. York River destratification: an estuary-subestuary interaction. *Science* 216: 1413-1414.
- Harris, G.P. 1980. The Measurement of Photosynthesis in Natural Populations of Phytoplankton. pp. 129-187. In: I.M. Morris, ed. *The Physiological Ecology of Phytoplankton*. 625p.. Studies in Ecology, vol. 7. Berkeley, California. University of California Press.
- Hendee, W.R. 1973. Chapter 15 - Liquid Scintillation Counting, Quench Corrections. *Radioactive Isotopes in Biological Research*. New York, New York. John Wiley and Sons. pp. 208-212.
- Ikeda, T. 1970. Relationship between respiration rate and body size in marine plankton animals as a function of the temperature of habitat. *Bulletin of the Facilities of Fisheries, Hokkaido University* 21(2):91-112.
- Iturriaga, R. and B.G. Mitchell. 1986. Chroococcoid cyanobacteria: a significant component in the food web dynamics of the open ocean. *Marine Ecology - Progress Series* 28:291-297.

- Johnson, P.W. and J.M. Seiburth. 1979. Chroococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass. *Limnology and Oceanography* 24(5):928-935.
- Johnson, P.W. and J.M. Sieburth. 1982. In-situ morphology and occurrence of eucaryotic phototrophs of bacterial size in the picoplankton of estuarine and oceanic waters. *Journal of Phycology* 18:318-327.
- Kana, T.M. and P.M. Glibert. (submitted 1986). Effects of irradiances up to 2000 $\mu\text{E m}^{-2} \text{s}^{-1}$ on marine *Synechococcus* WH7803: I Growth, pigmentation and cellular composition. Submitted to Deep Sea Research.
- Kemp, W.M. and W.R. Boynton. 1981. External and internal factors regulating metabolic roles of an estuarine benthic community. *Oecologia* 51:19-27.
- Landry, M.R., L.W. Haas and V.L. Fagerness. 1984. Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. *Marine Ecology Progress Series* 16:127-133.
- Levinton, J.S. 1982. *Marine Ecology*. 526 p. Englewood Cliffs, New Jersey. Prentice-Hall, Incorporated.
- Li, W.K., D.V. Subba Rao, W.G. Harrison, J.C. Smith, J.J. Cullen, B. Irwin and T. Platt. 1983. Autotrophic picoplankton in the tropical ocean. *Science* 219:292-295.
- Malone, T.C. 1980. Algal Size. pp 433-463. In: I. Morris, ed. *The Physiological Ecology of Phytoplankton*. Studies in Ecology volume 7. Berkely, California. University of California Press.
- Malone, T.C. and P.J. Neale. 1981. Parameters of light-dependent photosynthesis for phytoplankton size fractions in temperate estuarine and coastal environments. *Marine Biology* 61:289-297.
- McCarthy, J.J., W.R. Taylor and M.E. Loftus. 1974. Significance of nanoplankton in the Chesapeake Bay Estuary and problems associated with the measurement of nanoplankton productivity. *Marine Biology* 24:7-16.
- Murphy, L.S. and E.M. Haugen. 1985. The distribution and abundance of phototrophic ultraplankton in the North Atlantic. *Limnology and Oceanography* 30(1):47-58.
- Parsons, T.R., Y. Maita and C.M. Lalli. 1984. *A Manual of Chemical and Biological Methods For Seawater Analysis*. New York, New York. Pergamon Press.
- Patten, B.C., R.A. Mulford and J.E. Warinner. 1963. An Annual phytoplankton cycle in the lower Chesapeake Bay. *Chesapeake Science* 4(1):1-20.

- Perkins, F.O., L.W. Haas, D.E. Phillips and K.L. Webb. 1981. Ultrastructure of a marine *Synechococcus* possessing spinae. *Canadian Journal of Microbiology* 27(3):318-329.
- Platt, T., D.V. Subba Rao and B. Irwin. 1983. Photosynthesis of picoplankton in the sea. *Nature* 301:702-704.
- Prezelin, B.B., M. Putt and H.E. Glover. 1986. Diurnal patterns in photosynthetic capacity and depth-dependent photosynthesis-irradiance relationships in *Synechococcus* spp. and larger phytoplankton in three water masses in the Northwest Atlantic Ocean. *Marine Biology* 91:205-217.
- Pritchard, D.W. 1965. Lectures On Estuarine Oceanography. Chesapeake Bay Institute. Baltimore, Maryland. November 1965.
- Putt, M. and B.B. Prezelin. 1985. Observations of diel patterns of photosynthesis in cyanobacteria and nanoplankton in the Santa Barbara Channel during 'el Nino'. *Journal of Plankton Research* 7(6):779-790.
- Rhodes, M.W. and H.I. Kator. 1983. In Situ survival of enteric bacteria in estuarine environments. Virginia Water Resources Research Center Bulletin 140. Virginia Polytechnic Institute and State University. January 1983. 48p.
- Sellner, K.G. 1983. Plankton productivity and biomass in a tributary of the upper Chesapeake Bay. I Importance of size-fractionated phytoplankton productivity, biomass, and species composition in carbon export. *Estuarine, Coastal, and Shelf Science* 17:197-206.
- Sheldon, R.W. 1984. Plankton growth rates in the tropical ocean. *Limnology and Oceanography* 29(6):1342-1346.
- Sieburth, J.M., V. Smetacek and J. Lenz. 1978. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnology and Oceanography* 23(6):1256-1263.
- Sokal, R.R. and F.J. Rohlf. 1981. *Biometry*, 2nd Edition. 859 p. San Francisco, California. W.H. Freeman and Company.
- Sournia, A. (editor). 1978. *Phytoplankton Manual*. Monographs on Oceanographic Methodology, Volume 6. United Nations Educational, Scientific and Cultural Organization. pp236-237, 273-279.
- Taguchi, S. 1976. Relationship between photosynthesis and cell size of marine diatoms. *Journal of Phycology* 12:185-189.
- Takahashi, M and P.K. Bienfang. 1983. Size structure of phytoplankton biomass and photosynthesis in subtropical Hawaiian waters. *Marine Biology* 76:203-211.

- Thayer, G.W., P.L. Parker, M.W. LaCroix and B. Fry. 1978. The stable isotope ratio of some components of an eel grass, *Zostera marina*, bed. *Oecologia* 35:1-12.
- Tide Tables, 1985 High and Low Water Predictions. East Coast of North and South America including Greenland. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service. Issued 1984. 285p.
- Van Valkenberg, S.D. and D.A. Flemer. 1974. The distribution and productivity of nannoplankton in a temperate estuarine area. *Estuarine and Coastal Marine Science* 2:311-322.
- Waterbury, J.B., S.W. Watson, R.R. Guillard and L.E. Brand. 1979. Widespread occurrence of a unicellular marine, planktonic, cyanobacterium. *Nature* 277:293-294.
- Watson, S.W., T.J. Novitsky, H.L. Quinby and F.W. Valois. 1977. Determination of bacterial numbers and biomass in the marine environment. *Applied Environmental Microbiology* 33:940-946.
- Webb, K.L. and C.F. D'Elia. 1980. Nutrient and oxygen redistribution during a spring neap tidal cycle in a temperate estuary. *Science* 207:983-985.
- Webb, K. L. and L.W. Haas. 1976. The significance of urea for phytoplankton nutrition in the York River, Virginia. pp. 90-102. In: M. Wiley, ed. *Estuarine Processes*, Volume 1. New York, New York. Academic Press.
- Wojcik, F.J. 1981. Data Report 17: Monthly Salinity Data for the York River Plotted by River Mile by Month. September 1981. Virginia Institute of Marine Science, Gloucester Point, Virginia.
- Wolfe, J.J., B. Cunningham, N.F. Wilkerson and J.T. Barnes. 1926. An Investigation of the microplankton of Chesapeake Bay. *J. Elisha Mitchell Scientific Society* 42:25-54.

VITA

ROBERT TRIAU RAY

Born in Seattle, Washington, March 15, 1956. Graduated from Evanston Township High School, Illinois in 1974. Pursued a liberal arts education for two years at Saint John's College, Santa Fe, New Mexico. Worked two seasons for the National Forest Service in fire suppression. Worked for the Alaska Department of Game and Fish on the International Salmon Stock Program and lake fertilization research. Received a Bachelor of Science in Biology from the University of New Mexico in Albuquerque in December, 1982. Entered the master's program at the College of William and Mary, School of Marine Science in September, 1983. Served as crew on the 45' ketch Rubaiyat on a trans-Atlantic cruise in December, 1985. Began work at Horn Point Environmental Laboratories, University of Maryland in June, 1986.