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Using frequency of dividing cells in estimating autotrophic picoplankton growth and productivity in the Chesapeake Bay

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Abstract

In situ incubations of natural autotrophic picoplankton populations during a 15 month study were used to test the frequency of dividing cells procedure in estimating phototrophic picoplankton growth rates. These rates were estimated using dilution experiments and compared to the average frequency of dividing cells over the same time interval. The regression equation of $\mu = 2.85 \times 10^{-3}$ (FDC) + 0.022 was calculated to relate autotrophic picoplankton growth rate and the frequency of dividing cells in this study. The resulting relationship was compared to ^{14}C-bicarbonate derived growth rates. Productivity estimates using frequency of dividing cells correlated closely to sodium ^{14}C-bicarbonate results and indicated a range of productivity by autotrophic picoplankton of 55.6% the total phytoplankton primary productivity in July to a January rate of 2.3%. Annual autotrophic picoplankton abundance varied seasonally in the lower Chesapeake Bay ranging from 7.26×10^6 cells l^{-1} in winter to 9.28×10^8 cells l^{-1} during late summer.

Introduction

Picoplankton is defined as plankton between 0.2 and 2.0 μm in size (Sieburth *et al.*, 1978). There are a variety of heterotrophic and autotrophic organisms included in the picoplankton classification of aquatic systems (Johnson & Sieburth, 1982). Originally, the term 'picoplankton' was used as a collective term to identify both heterotrophic and autotrophic organisms that could pass through a 2.0 μm filter, however, many microbiologists prefer to identify the heterotrophic component of picoplankton as 'bacterioplankton' (Fuhram *et al.*, 1980; Hagström *et al.*, 1984). Using this distinction for heterotrophic forms, picoplankton is more commonly used to denote the photoautotrophic organisms in the picoplankton size range that fix carbon by photosynthesis using chlorophyll and accessory pigments (Johnson & Sieburth, 1982). Autotrophic picoplankton is composed of a variety of both eukaryotes and prokaryotes including the common coccoid cyanobacterium *Synechococcus* (Thomsen, 1986; Waterbury *et al.*, 1986). For this study, the term 'picoplankton' will refer to those autotrophic cells that are within the size range defined by Sieburth *et al.* (1978).

There are numerous studies indicating picoplankton as widespread and a major contributor to phytoplankton abundance and total cell volume in marine environments (Johnson & Sieburth, 1979; Li *et al.*, 1983; Takahashi &

Bienfang, 1983; Takahashi & Hori, 1984; Waterbury *et al.*, 1979). A popular method to measure productivity of marine autotrophic picoplankton is with timed incubations using sodium ^{14}C-bicarbonate (Gieskes *et al.*, 1979; Li *et al.*, 1983; Platt *et al.*, 1983; Takahashi & Bienfang, 1983). An alternative to the ^{14}C method is the use of frequency of dividing cells (FDC) as a more direct measure of productivity. This method is based on both theoretical and experimental evidence that the frequency of dividing cells of a population is dependent on the population growth rate (Hagström *et al.*, 1979; Newell & Christian, 1981). The study of cell division as part of the phytoplankton growth process includes work by Gough (1905) and Apstein (1911). The frequency of dividing cells technique was first developed to measure growth rates of bacterioplankton in aquatic systems by Hagström *et al.* (1979). FDC has also been used to measure bacterioplankton productivity (Newell & Christian, 1981; Davis & Sieburth, 1984; Hanson *et al.*, 1983). However, past studies using the FDC method to estimate productivity have concentrated on the heterotrophic population, or a combination of the heterotrophic and autotrophic populations in marine systems. In these studies, the established relationship between FDC and μ (used to estimate productivity) was often based on *in vivo* culturing experiments (Christian *et al.*, 1982; Hagström *et al.*, 1979; Newell & Christian, 1981). Hanson *et al.*, (1983) have indicated these laboratory incubations alter the growth characteristics of natural assemblages, not giving a true indication of growth rates in natural environments.

A common method to calculate picoplankton growth rates *in situ* involves the mathematical formula describing phytoplankton growth as derived from McDuff & Chrisholm (1982). This equation requires estimates of the fraction for dividing cells and duration of division in order to calculate picoplankton specific growth rates. Campbell & Carpenter (1986) found natural *Synechococcus* populations in the Sargasso Sea have a diel pattern in their frequency of dividing cells. Calculated growth rates using the equation derived from McDuff & Chrisholm (1982) revealed a strong correlation with instantaneous daily growth rates calculated from on-deck incubation experiments. Fahnenstiel *et al.* (1991), used the same growth equation to estimate picoplankton growth rates in freshwater lakes of Michigan. Diel patterns of FDC were observed in field populations of *Synechococcus* during thermal stratification with FDC maxima occurring in afternoon and early evening hours. Pick & Bérubé (1992) employed the equation from McDuff & Chrisholm (1982) to compare diurnal patterns of cell division in freshwater picocyanobacteria to their seasonal population and growth patterns in a small mesotrophic lake in Canada.

In support of using dividing cells as an indicator of picoplankton growth, an alternative approach that relates FDC and picoplankton growth rate is presented in this study. A relationship between FDC and picoplankton growth rate was developed from a series of *in situ* incubation studies. Autotrophic picoplankton productivity was estimated using this established relationship for the lower Chesapeake Bay. Seasonal patterns of picoplankton abundance and productivity were also identified for the lower Chesapeake Bay.

Methods

The sampling site was the end of a fishing pier on South Island, located in the Chesapeake Bay Bridge Tunnel complex (36°58″N. Lat., 76°07″ W. Long.). The fishing pier is 4.8 km north of the southern end of the Chesapeake Bay Bridge Tunnel and is adjacent to Thimble Shoals Channel. At mean high water, the depth of the water column is 7.0 m, with an average tidal range of 0.46 m (NOAA, 1989). Mean total phosphorous and total nitrogen concentrations for this area of the Chesapeake Bay, over a five year period (July 1985–June 1990), were 0.040 and 0.37 mg l^{-1} respectively (Marshall, 1991).

To establish the relationship of FDC and μ for the autotrophic picoplankton, a total of 15 *in situ* incubations were made from the pier station between June 1988 and October 1989. Seasonal changes influenced temperature, light intensity

and nutrient variability of the *in situ* incubations. Experiments were carried out in a variety of weather conditions and sea states (Table 1). A composite water sample was collected from hydrocasts over one meter increments throughout the water column (7 m) and placed in a 20 liter plastic carboy. One liter of filtered sterilized seawater (seawater passed through a 0.2 μm Nuclepore filter) was mixed with one liter of unfiltered composite sample to reduce grazing pressure and possible nutrient limitations. Two 125 ml subsamples of the diluted composite were placed in Nalgene plastic sampling bottles containing glutaraldehyde (1% final concentration) and returned to the laboratory for epifluorescence analysis. An average cell count for the two samples represented the abundance of picoplankton at the start of incubation (t_o).

Subsamples from diluted composite samples were placed in six 300 ml incubation bottles and allowed to incubate one meter below the water's surface for approximately 12 hours (actual incubation time varied with the light period, ranging from 9.6 to 14.7 hours; NOAA, 1988, 1989). Start times for all incubations were coordinated with the beginning of the light period of each sampling day. Approximately every two hours of the incubation, two 125 ml subsamples were taken from one of the six incubation bottles and fixed in glutaraldehyde (1% final concentration) for enumeration and FDC counts. Care was taken not to introduce excessive turbulence in the sample collection process as this might influence FDC counts.

Table 1. Weather conditions, sea state and average water temperature for 15 *in situ* incubations.

Month	Average water temperature (°C)	Weather condition	Sea state
June 1988	22.42	sunny	calm
July 1988	25.50	partly cloudy	calm
September 1988	22.58	overcast	0.5 m
October 1988	15.88	rain	calm
November 1988	13.50	partly cloudy	0.5 m
December 1988	6.00	sunny	calm
February 1989	4.62	sunny	1.0 m
March 1989	9.92	sunny	calm
April 1989	14.29	partly cloudy	calm
May 1989	18.42	sunny	calm
June 1989	25.17	partly cloudy	calm
July 1989	26.00	sunny	calm
August 1989	25.92	partly sunny	calm
September 1989	26.25	sunny	0.5 m
October 1989	20.50	sunny	calm

To aid in distinguishing eukaryotic and prokaryotic picoplankton, the fluorochrome DAPI (4′, 6-diamidino-2 phenylindole) was used (Porter & Feig, 1980). A subsample (2 to 10 ml, depending upon cell density) was taken from the sample and incubated in the dark with 100 μl ml^{-1} of DAPI for seven minutes. After incubation, this subsample was filtered on a 0.2 μm Nuclepore filter stained with Irgalan Black, at a vacuum pressure of 10 cm of Hg. The filter was placed on a slide and a drop of immersion oil was placed above the filter and covered with a cover glass. A Zeiss epifluorescence inverted microscope, equipped with a 100 watt mercury bulb and three filter sets (Zeiss 365 excitation filter, 395 dichromatic mirror, 420 barrier filter; Zeiss 450–490 excitation filter, 510 dichromatic mirror, 520 barrier filter, and a Zeiss 546 excitation filter, FT 580 dichromatic mirror and 590 barrier filter) were used. Picoplankton cells that autofluoresced a yellow to red color while using either the 450–490, or 546 excitation filter were counted as autotrophic cells. Two cells with a complete cross wall between them were counted as a dividing cell. The total number of dividing cells viewed in 30 randomly chosen microscope fields were counted to determine frequency of dividing cells for autotrophic picoplankton. FDC was determined by dividing the number of dividing cells by the number of total cells per field. Dividing cells were counted as two separate cells in calculating total abundance.

Changes in autotrophic picoplankton abundance and FDC were plotted over the incubation period for each sampling date. A best fit line was calculated for data points that were determined within the growth phase of the incubation period. The calculation of the growth phase for all incubations was based on the maximum frequency of dividing cells. The growth phase was defined in

this study as all abundance values of the incubation that occurred from t_o through t_{max+1}. The value of t_{max+1} corresponded to one data point beyond the time when the maximum dividing cells was observed. For those months where the maximum FDC was during the final collection of the incubation period, all data points of the incubation were used to calculate the best fit line. The calculation of the best fit line for data points within the growth phase was based on a linear fit model relating $\log_{10}$ (abundance) to time by minimizing the sum of squares of the residuals for the fitted line. The origin of the best fit line for all *in situ* incubations was the picoplankton abundance value at t_o.

Using the best fit line, specific growth rates (μ) of the autotrophic picoplankton component for all incubations were determined from a change in cell numbers over time:

$$\mu = (\log_{10}Z - \log_{10}Z_0)2.303/(t - t_0)\,,$$

where Z and Z_o represent the abundance of picoplankton at the incubation times of t_{max+1} and t_o respectively (Stanier *et al.*, 1979).

The FDC procedure in this study was calibrated in a similar fashion to Newell & Christian (1981) and Hanson *et al.* (1983), where an independent variable representing FDC behavior was compared to growth rate. To account for the diurnal effect of *Synechococcus* (Waterbury *et al.*, 1986) and the resulting influence this phenomenon has on FDC counts, average FDC values were calculated over the incubation period and used as independent variables.

Linear regression was performed using average FDC values (independent variable) and μ values (dependent variable) observed for all 15 incubations. The regression equation calculated from this procedure was used to express the relationship between FDC at any given time and the growth rate of the autotrophic picoplankton. To check the validity of using the regression equation to estimate autotrophic picoplankton growth rates and productivity, five blind tests were run comparing the FDC technique to sodium ^{14}C-bicarbonate analysis for measuring picoplankton productivity (Strickland & Parsons, 1972). These occurred in July, August, September, October (1989) and January (1990). Using the same water sample for each procedure, an estimation of picoplankton productivity was calculated using both sodium ^{14}C-bicarbonate and the frequency of dividing cells technique. For ^{14}C-bicarbonate analysis, picoplankton was separated from total phytoplankton by passing the water sample through a 2.0 μm Nuclepore filter at vacuum pressures not exceeding 10 cm of Hg. The filtering process involved small aliquots of water (25 ml) to avoid clogging the filter; which would inhibit the passage of picoplankton cells. After a 30 minute acclimation period, a one hour incubation period with ^{14}C-bicarbonate followed, with water temperatures and light intensities representing *in situ* conditions. For each blind test, growth rates calculated from FDC values were compared to growth estimates determined by ^{14}C-bicarbonate uptake. A conversion to carbon biomass was made by multiplying cell growth per hour by a value (115 fgC per cell) representing the cellular carbon content of one picoplankton cell (Ray *et al.*, 1989).

Results

In situ incubations, representative examples of picoplankton growth, associated FDC, and calculated best fit lines are presented in Fig. 1. Generally, growth was varied over the light period. Patterns of cell division fluctuated seasonally with dividing cells reaching maximum values in the afternoon hours for the majority of incubation studies. Over 95% of the autotrophic picoplankton observed in this study were *Synechococcus* sp. (both phycocyanin and phycoerythrin enriched cells).

A summary of the average FDC, maximum FDC, and growth rates is given in Table 2. The average FDC varied from 4.07% to 16.94% with higher FDC values occurring in the summer months (Table 2). The maximum number of dividing cells varied from 5.05% to 19.35%. Specific growth rates calculated from each incubation varied from 0.23 d^{-1} to 1.10 d^{-1}, with higher growth rates common for summer.

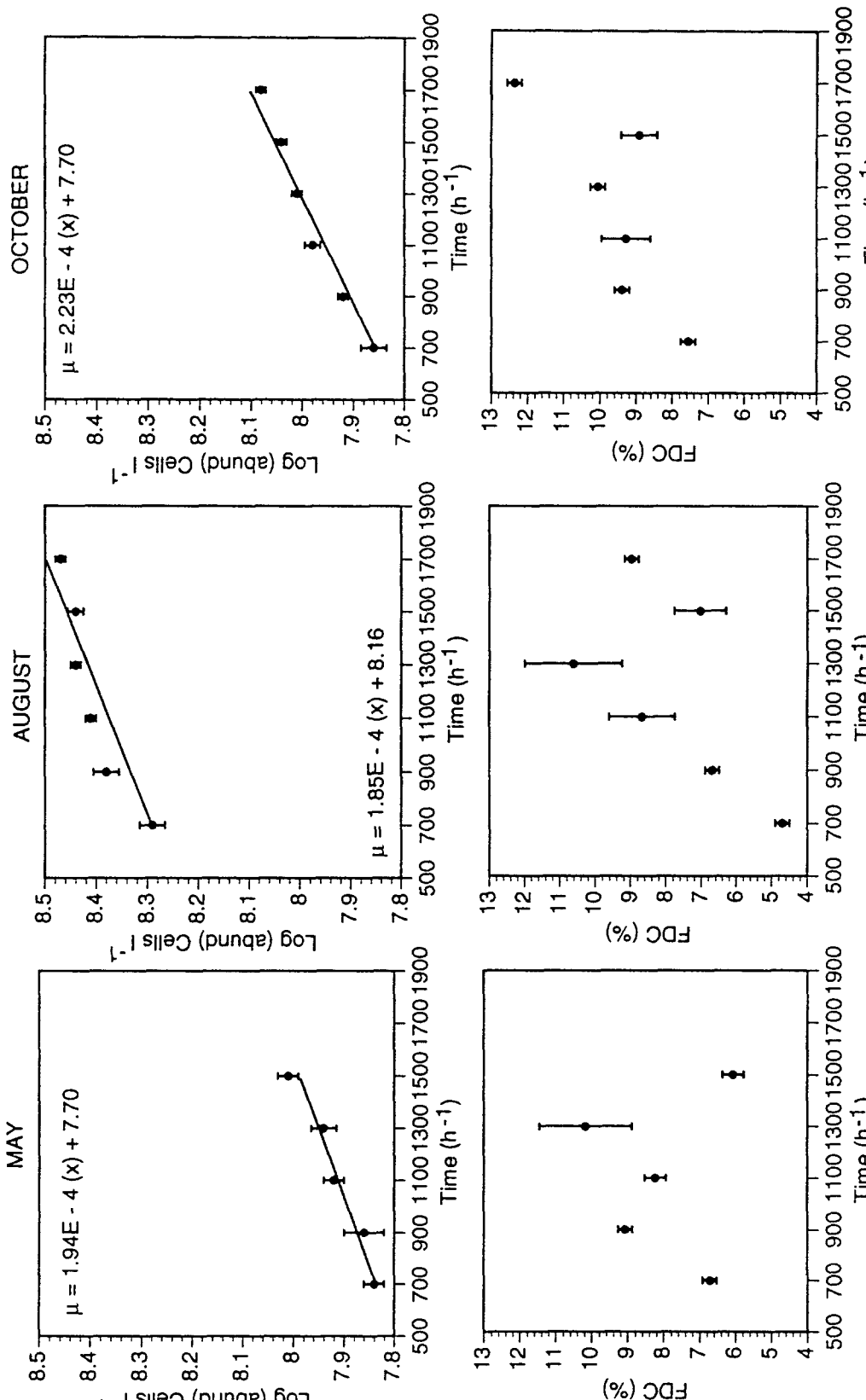

Fig. 1. Representative examples of picoplankton growth, best fit lines; frequency of dividing cells patterns for May, August and October 1989. Error bars indicate standard error of two replicate samples.

Table 2. Growth rates, average and maximum FDC values for 15 *in situ* incubations.

Month	Growth rate		AVG FDC	Max FCD
	(hr^{-1})	(day^{-1})	(%)	(%)
June 1988	7.46×10^{-2}	1.10	14.29	15.81
July 1988	4.81×10^{-2}	0.68	7.14	9.80
September 1988	4.65×10^{-2}	0.58	4.97	5.99
October 1988	3.94×10^{-2}	0.47	4.07	5.05
November 1988	4.47×10^{-2}	0.45	6.27	7.22
December 1988	2.39×10^{-2}	0.23	6.00	7.05
February 1989	2.58×10^{-2}	0.28	5.84	7.21
March 1989	4.35×10^{-2}	0.54	6.37	9.42
April 1989	5.94×10^{-2}	0.80	12.01	13.11
May 1989	4.47×10^{-2}	0.64	8.05	10.17
June 1989	5.85×10^{-2}	0.85	14.48	16.28
July 1989	6.63×10^{-2}	0.98	16.94	19.35
August 1989	4.26×10^{-2}	0.56	7.54	10.62
September 1989	4.35×10^{-2}	0.54	9.50	11.80
October 1989	5.14×10^{-2}	0.57	9.59	12.36

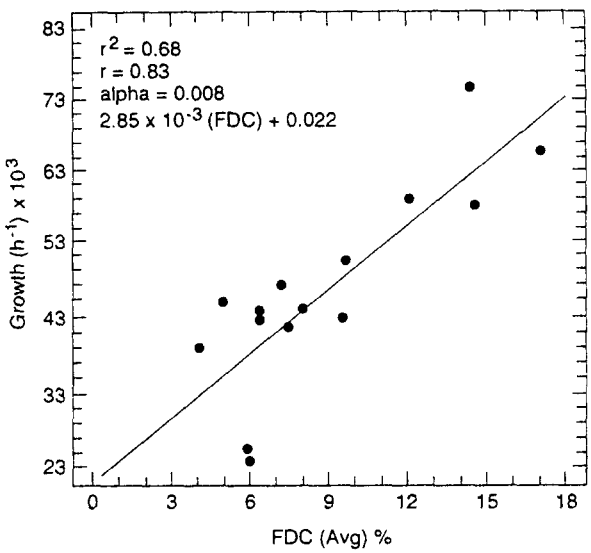

Fig. 2. Relationship of frequency of dividing cells and growth rates over the 15 month study. The independent variable (FDC) represents the average frequency of dividing cell values calculated over the incubation period.

Relationship between FDC and μ – Regression analysis relating average FDC values over the 15 month study and μ indicated 68.0% of the variation in μ can be explained by the regression equation of:

$$\mu = 2.85 \times 10^{-3}(\text{FDC}) + 0.022\,,$$

where μ equals the growth rate (h^{-1}); FDC is the average FDC value observed in natural picoplankton populations (Fig. 2). In order to make the equation useful for field application, FDC values obtained in the field were used as independent variables.

Comparison of sodium ^{14}C-bicarbonate and FDC techniques, measured by the FDC and sodium ^{14}C-bicarbonate methodology, is given in Table 3. Using ^{14}C, peak productivity by the picoplankton occurred in July (6.58 μgC l^{-1} h^{-1}), with the lowest value in January (0.109 μgC l^{-1} h^{-1}). Similar picoplankton productivity rates were obtained using the FDC technique, ranging from a summer high in July (6.85 μgC l^{-1} h^{-1}), to a winter low in January (0.108 μgC l^{-1} h^{-1}). A positive correlation coefficient of 0.980 (slope 0.91) was calculated when comparing the two methods. The amount of picoplankton produc-

Table 3. The comparison of the total phytoplankton productivity in the lower Chesapeake Bay to that portion attributed to the autotrophic picoplankton measured by the sodium ^{14}C-bicarbonate and FDC techniques. Standard error of two replicate samples is shown in parentheses.

Month	^{14}C technique (μgC l^{-1} h^{-1})	Standing stock corrected prod. (^{14}C-μ)	% of total	FDC technique (μgC l^{-1} h^{-1})	Standing stock corrected prod. (FDC-μ)	% of total	Total ^{14}C prod (μgC l^{-1} h^{-1})
Jul 1989	6.58 (0.602)	0.062	53.4	6.85 (0.315)	0.064	55.6	12.32 (1.02)
Aug 1989	1.52 (0.075)	0.034	29.7	1.57 (0.003)	0.035	30.7	5.11 (2.28)
Sept 1989	1.95 (0.142)	0.032	14.2	2.72 (0.004)	0.045	19.9	13.67 (0.913)
Oct 1989	1.48 (0.012)	0.089	7.5	0.718 (0.012)	0.044	3.7	19.59 (2.48)
Jan 1990	0.109 (0.019)	0.076	2.2	0.108 (0.008)	0.075	2.3	4.76 (0.119)

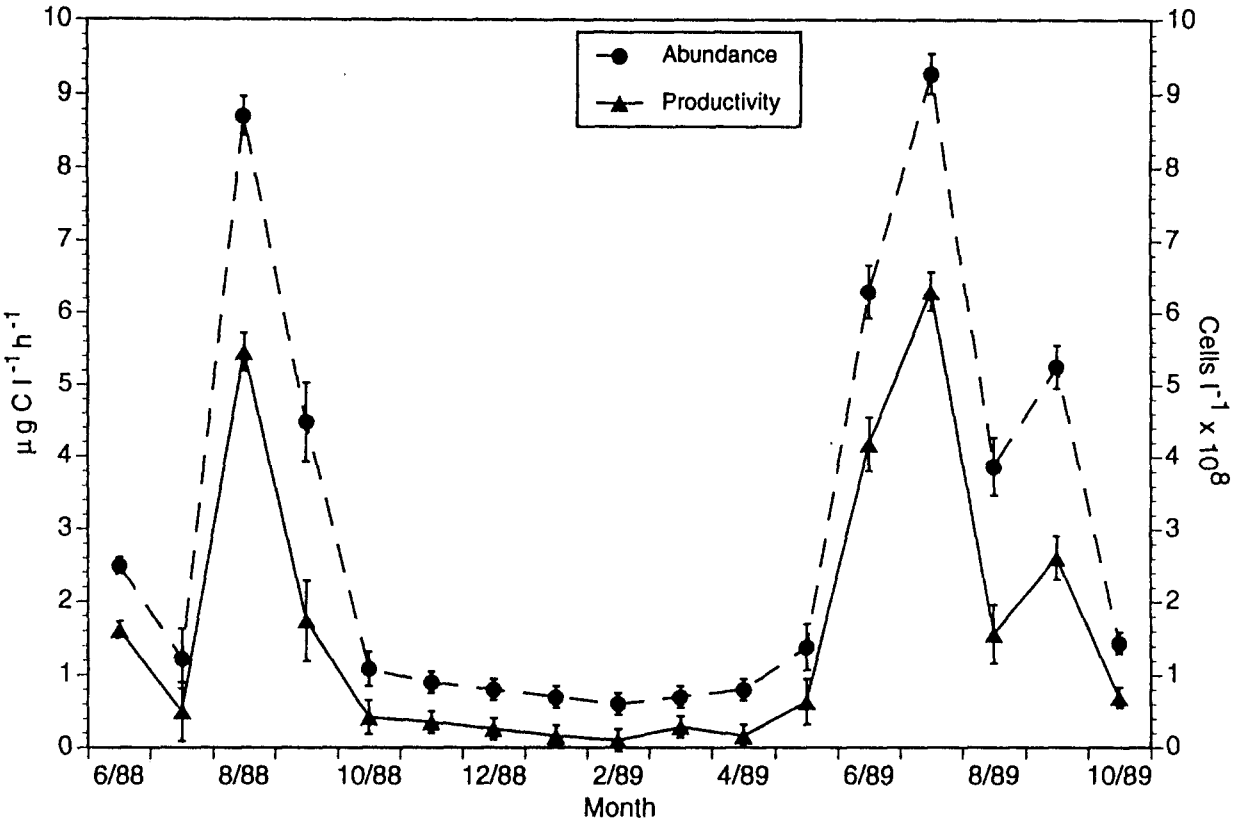

Fig. 3. Seasonal patterns of abundance (dotted line) and productivity (solid line) for autotrophic picoplankton in the lower Chesapeake Bay between June 1988 and October 1989. Error bars indicate standard error from two replicate samples.

tivity measured by the FDC and ^{14}C methods, compared to the total phytoplankton productivity, is given in Table 3. These results indicate that in July, the picoplankton were responsible for over half (53.4 to 55.6%) of the total productivity at the collection site. However, picoplankton productivity decreased into winter, when it represented between 2.2 and 2.3% of total productivity in January, as measured by these two methods. In comparison, the greatest differences between the methods, occurred during periods when concentrations of cells were decreasing, as in October, when the ^{14}C technique produced values that were lower than the FDC method. The results were most similar in summer (July and August) and winter (January) when cell concentrations were at their highest and lowest concentrations.

The autotrophic picoplankton concentrations ranged from 7.36×10^6 cells l^{-1} (in winter) to 9.28×10^8 cells l^{-1} (in summer). These abundance values represented average autotrophic picoplankton concentrations for the water column. Productivity patterns using the FDC method were similar to picoplankton abundance patterns and ranged from 0.019 μgC l^{-1} h^{-1} in winter, to a summer rate of 6.85 μgC l^{-1} h^{-1} (Fig. 3).

Discussion

A criticism of the FDC technique using *in vitro* cultures is the inability to mimic *in situ* growth conditions (Hanson *et al.*, 1983; Newell & Christian, 1981). In this study, the relationship between FDC and μ was determined with *in situ* incubations of picoplankton assemblages common to the estuary. By using results over a 15 month period, a more comprehensive response of

picoplankton seasonal behavior was assessed. To reduce the influence of grazing pressure, the *in situ* incubations were performed using diluted picoplankton populations. Based on *in situ* picoplankton incubation data, only two of the 15 incubations contained evidence of a lag phase in growth (Affronti, 1990), and this may be due to the sampling frequency. By sampling at higher frequencies (*i.e.*,every hour), a lag phase would be more evident. To be consistent in determining picoplankton growth rates, the beginning data point of the growth phase was set at t_o. With this approach, more conservative estimates of picoplankton growth rate would be expected where a lag phase was present.

Changing light, temperature, and nutrient variability can in part explain the varied patterns of growth observed in the incubation experiments. Changes in weather conditions have been shown to influence picoplankton growth (Waterbury *et al.*, 1986). One goal of diluting picoplankton with filtered water was to increase the availability of nutrients thus encouraging growth. Estimating growth based on the best fit line was an attempt to 'average' the variation in growth over time. With this procedure being performed consistently for each incubation, estimates of growth were obtained.

A determination was made to use the average FDC calculated over the incubation time interval as the independent variable for relating FDC and picoplankton growth. Other studies have used FDC at a variety of time intervals throughout the growth phase as independent variables for relating FDC and growth (Newell & Christian, 1981; Hanson *et al.*, 1983). The method to follow is determined by the specific goals of each study.

Average FDC values of picoplankton from late spring to early summer (May and June) were similar to those reported by Waterbury *et al.* (1986) for *Synechococcus* in Buzzards Bay, with higher FDC values for summer. These findings are consistent with data reported by Campbell & Carpenter (1986), where higher FDC rates were observed in pure cultures of *Synechococcus* grown at higher temperatures and light intensities. Regression analysis relating FDC and μ noted reasonable scattering of μ on FDC. A $r^2 = 0.68$ provides reasonable confidence in the predictive capacity of the regression line $\mu = 2.85 \times 10^{-3}$ (FDC) + 0.022 in estimating picoplankton growth rates from FDC values in the Chesapeake Bay. The picoplankton productivity can easily be determined using direct counting procedures and obtaining: (1) the percent of dividing cells in the population, and (2) the picoplankton abundance. Advantages of this procedure include a rapid method to determine picoplankton productivity, without the need for continuous incubating procedures, or the use of radioisotopic techniques. Li & Dickie (1991) concluded a positive correlation between FDC and population size in their picoplankton study in the North Atlantic. The equation between FDC and growth in the lower Chesapeake Bay was developed from an initial incubation series using *in situ* picoplankton. However, this relationship may vary in other ecosystems.

Duration of division (t_d) plays a major role in the accuracy of the FDC technique. Physical factors such as nutrient status, turbulence and temperature all influence t_d. The goal of the design of this study was to sample picoplankton growth and FDC over a wide range of physical factors to include those particular conditions that would effect t_d. Duration of division influencing FDC values was evident in incubations for December and February. Because of cold water temperatures, relatively high FDC values were observed for corresponding low growth rates. Similar results were reported by Campbell & Carpenter (1986) using picoplankton common to oceanic environments where t_d was observed to increase in water temperatures less than 15 °C. By eliminating FDC and growth rate data for December 1988 and February 1989, the predictive capacity of the regression equation increases (Fig. 4). Another indication that t_d was being influenced by one, or a unique combination of physical factors, is seen by the various times FDC_{max} was reported over the different months of incubation. By understanding and correcting for the effects of t_d on FDC, more predictable relationships between FDC and growth rate could be determined.

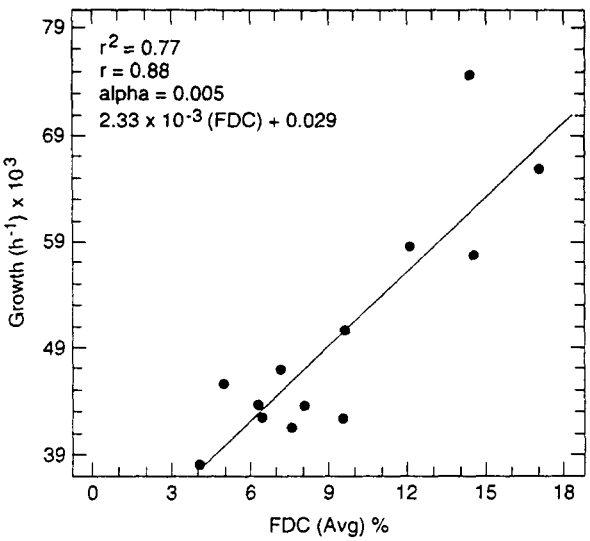

Fig. 4. Relationship of frequency of dividing cells and growth rate over a 13 month period (December 1988 and February 1989 omitted).

Picoplankton productivity was estimated using both the FDC technique and standard fraction methods where picoplankton were incubated using sodium ^{14}C-bicarbonate. The five month study included a wide range of different physical conditions for picoplankton growth (e.g., temperature, light). The greatest similarity between the results of the two methods occurred when picoplankton seasonal abundance was at its peak and at its lowest values, with differences more prevalent during periods of declining abundance. Results obtained in comparing the two methodologies ($r = 0.98$) is encouraging, with productivity values obtained using the FDC techniques similar to those derived using ^{14}C analysis. Further support for the FDC technique being an alternative method to estimate picoplankton productivity was indicated when comparisons were made using standing stock corrected productivity values from the two techniques (Table 3). With the exception of October 1989, there was a correlation coefficient of 0.93 in comparing growth rates of the two methods. The significant deviation of October 1989 data may be explained in part by technique differences. The FDC approach requires estimates of cellular carbon content to calculate productivity values, and population growth is measured by changes in cell abundance. The FDC technique is somewhat limited in measuring growth in terms of an increase in cell mass. To account for this limitation, more specific estimates of picoplankton cellular carbon should be considered. In this study, dilution experiments were used to estimate picoplankton growth. Because grazing influence was reduced, but not eliminated in these experiments, FDC growth rates may have been unfavorably influenced. Campbell & Carpenter (1986) have suggested grazing pressure in *Synechococcus* may be selected toward the dividing cells because they are larger, and Gonzalez *et al.* (1990) also showed a general preference of ciliates to graze larger size bacteria.

A method that may improve the incubation procedure in measuring picoplankton growth rates would be the use of transparent semipermeable membranes as incubation chambers similar to those developed by Landry *et al.* (1984) and Weisse (1988). Membrane pore size would need to retain the picoplankton (> 0.2 μm), yet, allow free flow of nutrients in and out of the membrane. This method would eliminate problems associated with incubating cells in closed containers and more closely mimic *in situ* conditions for picoplankton growth. Image analysis procedures would also ultimately improve the FDC technique. Total time to count picoplankton abundance would decrease, yet, there would be a need to enumerate the cells undergoing division. The specificity of the image analysis technique to distinguish cells undergoing division is low (Sieracki *et al.*, 1985).

The seasonal patterns of picoplankton abundance were similar to other studies in the lower Chesapeake Bay and its river systems, with maximum abundance occurring in summer (Affronti & Marshall, 1990, 1993; Perkins *et al.*, 1980). While maximum picoplankton concentrations were lower than abundance values reported by Affronti & Marshall (1990, 1993), the seasonal counts in this study represented picoplankton abundance from a composite sampling of the water column. Similar seasonal patterns of picoplankton abundance have been reported in Woods Hole harbor,

where maximum peaks occurred in summer (Waterbury *et al.*, 1986).

Conclusions

Phototrophic picoplankton dynamics in the lower Chesapeake Bay were studied from June 1988 to October 1989 using epifluorescence microscopy, frequency of dividing cells and sodium ^{14}C-bicarbonate techniques. The regression equation: $\mu = 2.85 \times 10^{-3}$ (FDC) + 0.022 represented the relationship between frequency of dividing cells and the phototrophic picoplankton growth rate. The frequency of dividing cells method was highly correlated with ^{14}C-bicarbonate fractionation. By calibrating FDC with *in situ* incubations and understanding the limits of this technique and its application to field studies, this method is suggested as an alternative method for measuring phototrophic picoplankton productivity.

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