

Dynamics of an estuarine ecosystem: the influence of flow patterns on phytoplankton trends in the Chesapeake Bay

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ABSTRACT

Long term trends in the phytoplankton of the lower Chesapeake Bay were identified using flow corrected and uncorrected data sets, with 22 of 23 significant trends similar under both conditions. The major trends between 1985-1992 were for: 1) reduced phytoplankton concentrations; 2) decreasing numbers of phytoplankton taxa during spring, summer and fall months; and 3) seasonally mixed trends for diatom abundance in waters below the pycnocline, with spring months having decreasing densities, and increasing abundance trends in November and December. The flow patterns had different effects on the trends. Flow diminished the magnitude of the trends for total phytoplankton concentrations, so these trends were greater in the flow corrected data. There were mixed patterns of influence when considering diatom abundance, with the presence of flow conditions showing greater trends for the number of taxa per sample.

RÉSUMÉ

Dynamique d'un écosystème estuaire : effets des courants sur les tendances du phytoplancton dans la baie de Chesapeake.

Les tendances à long terme du phytoplancton dans le bas de la baie de Chesapeake sont identifiées grâce à un ensemble de données brutes et de données corrigées de l'effet des courants : 22 des 23 tendances significatives sont similaires dans les deux cas. Les tendances majeures observées entre 1985 et 1992 sont les suivantes :

- 1) diminution des concentrations de phytoplancton ;
- 2) diminution du nombre des taxons de phytoplancton pendant le printemps, l'été et l'automne ;
- 3) tendances variables avec les saisons dans l'abondance des diatomées au-dessous de la pycnocline, avec des densités décroissantes au printemps et des tendances à une abondance accrue en novembre et décembre.

Les variations du courant ont des effets différents sur les tendances. Le courant atténue leur ampleur dans les concentrations totales de phytoplancton, de sorte que ces tendances sont plus marquées dans les données corrigées. Plusieurs effets sont observés dans l'abondance des diatomées, avec des conditions de courant présentant des tendances plus grandes dans le nombre de taxons de chaque échantillon.

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INTRODUCTION

Previous studies on the composition and distribution of phytoplankton in Chesapeake Bay have been reviewed by Marshall (1994). Trends within the phytoplankton populations of Chesapeake Bay were described for the period 1985 through 1990 by Marshall and Alden (1991). They noted modest, but significant seasonal trends of increased abundance above the pycnocline and a decrease below the pycnocline. When these data sets were combined, these trends negated each other and were not established for the entire water column. The abundance increase above the pycnocline was most developed in July and associated with higher concentrations of phytoflagellates and small centric diatoms. The reduced abundance below the pycnocline was greatest during mid-winter (January), due to a decrease in the diatom concentrations.

Since this monitoring began in 1985, it was apparent that a common fluctuating event was associated with phytoplankton development in the Bay. This was the changing pattern and amount of water flow into this system. Due to differences in the width and depth of various segments of this estuary, fluctuations in the amount and rate of flow were common. In addition, the average amount of monthly flow was influenced by the timing of the seasonal rains and the passage of the spring freshet, both of which varied in their occurrence year to year. Pilson (1985) studied these differences of flow rate and residence time within regions of Narragansett Bay and concluded flushing time was an important ecological variable to any biota present. Smetacek (1986) has also presented several case studies where the seasonality of river discharge effects phytoplankton composition and productivity. Interannual variations of phytoplankton abundance in Chesapeake Bay have also been associated with river flow (Malone *et al.*, 1988; Harding, 1994). River discharge into San Francisco Bay was recognized by Cloern *et al.* (1985) as a significant variable influencing phytoplankton composition. They found relationships between the residency time of water to phytoplankton growth and composition, productivity, and turbidity. Schaub and Gieskes (1991) have associated greater phytoplankton biomass to increased river discharge in the region of the North Sea that is effected by Rhine River outflow. Schmidt (1994) emphasized variations in hydrological features as most important in determining phytoplankton biomass in the Danube River, and noted an inverse relationship between phytoplankton development to the amount of river discharge and suspended matter. Kiss *et al.* (1994) have also identified low water flow conditions as favoring high phytoplankton density. The rate of flow through an aquatic system will also influence the availability of nutrients to the phytoplankton (De Groodt and de Jonge, 1990; Kiss *et al.*, 1994; Schmidt, 1994).

The various interactions that exist between the water flow within an estuarine system, to the nutrients, turbidity, and other factors mentioned above, may have both direct and indirect effects on the phytoplankton. The phytoplankton community will also be responding to other ecological forces that will produce short term and seasonal changes in their composition and abundance, or even result in long

term trends. The objectives of this study are to first identify any long term trends within the phytoplankton populations in Chesapeake Bay, and then to examine the influence of water flow on these trends. Results taken from a flow corrected data set are compared to those where the raw data were not corrected for the effects of flow. Specific areas of analysis included total phytoplankton abundance and biovolume, species diversity, and the abundance of diatoms, dinoflagellates, and autotrophic picoplankton.

METHODS

The data set comes from monthly water samples taken at seven stations in the Chesapeake Bay between July 1985 and December 1992 (Fig. 1). At each station, a series of 3-liter samples were taken at five equidistant depths between the surface and pycnocline, and were mixed in a carboy. Another set of samples was taken at five depths between the pycnocline and bottom to produce another composite sample. From these composite samples, subsamples were taken for phytoplankton (500 ml) and autotrophic picoplankton (125 ml) analysis. The samples analyzed for phytoplankton (cells > 2 μm) counts were fixed in Lugol's solution and passed through a series of settling and siphoning steps to produce a 20 ml concentrate. This concentrate was placed in a settling chamber and examined with an inverted plankton microscope using a modified Utermöhl procedure (Marshall and Alden, 1990). The entire concentrate was first scanned at 125 \times for net phytoplankton; then using a minimum count basis of 200 cells and 10 random fields, cells were counted at 315 \times

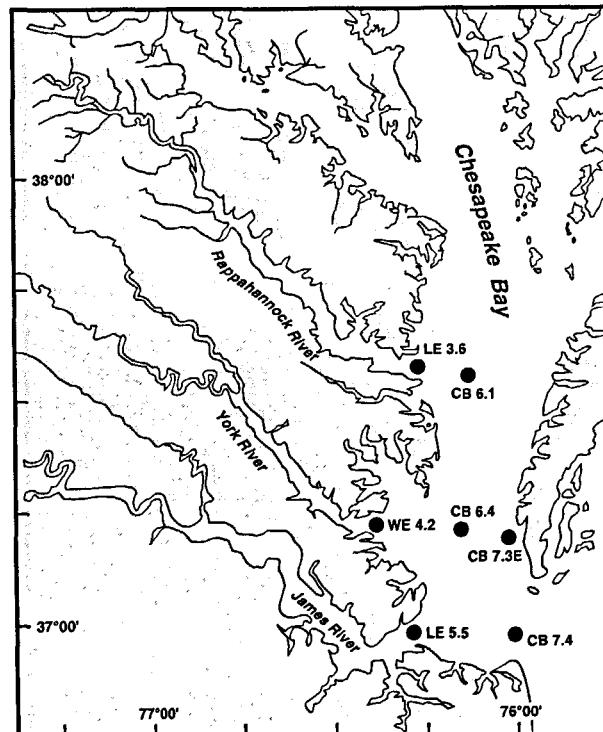


Figure 1

Station locations in Chesapeake Bay.

and 500 \times to produce an 85 % accuracy estimate for cell concentrations. Qualitative and quantitative analysis followed using an inverted plankton microscope. The autotrophic picoplankton (cells < 2.0 μm) samples were fixed with glutaraldehyde, filtered on a 0.2 μm Nuclepore filter, and examined for autotrophic components using a green filter set with an epifluorescence microscope (Marshall, 1995), and cells counted at 1000 \times .

The initial trend analysis of the data was based on the seasonal intra-block sign test from the Kendall Tau statistic (Seasonal Kendall) described by Hirsch *et al.* (1982) and the aligned rank test (Sen's Tau) by Sen (1968). A Chi square protocol by Van Belle and Hughes (1984) was used to analyze trends unique to certain seasons, stations, and the interaction of seasons and stations. The median slopes of significant trends ($p < 0.01$) were determined by the seasonal Kendall slope estimator (Gilbert, 1987). This analysis gave results uncorrected for flow. These data were then reanalyzed following the flow correction process.

The evaluation of flow effects involved the estimation of flushing times for regions of the mainstem Bay, followed by a series of nonlinear regression models to determine the relationship between each water quality variable and the flushing rates. Flushing time calculations were based on the "fraction of fresh water" method described by various investigators (*e.g.* Ketchum, 1950, 1951; Dyer, 1973; and Pilson, 1985). The equation for the calculation of flushing times was:

$$T = Q/R \quad (1)$$

where Q is the total amount of freshwater in the segment of the estuary and R is the estimated average flow into the segment. Flow estimates were provided monthly for various segments of the Chesapeake Bay by the U.S. Geological Survey (USGS). These were based on daily fall-line discharge data from the major tributaries to the Bay and the estimation methods of Bue (1968) to calculate 2-month rolling average flow rates (R). The mean values for flow data were determined from the month of collection and the preceding month. The Q values were calculated by:

$$Q = fv \quad (2)$$

where f is the fraction of freshwater in the segment and v is the total volume of the segment. Segment volumes were obtained from estimates provided by Cronin (1971). The f values were calculated using:

$$f = (S_1 - S_2)/S_1 \quad (3)$$

where S_2 is the average salinity in the segment and S_1 is the bottom salinity of the next seaward segment. The flushing times were calculated for each of the seven phytoplankton stations using the USGS river flow values and salinity measurements by Alden (1997).

The phytoplankton indices from the two depths at each station were regressed against the reciprocal of the flushing times (*i.e.* the flushing rates, hereafter designated as "flow rates" or "flow") using regression models developed by Smith *et al.* (1982) for the USGS NASQAN Program. The statistically significant ($p < 0.01$) regression model with

the highest R^2 value was employed to correct for flow effects. Trend analyses were conducted on the grand mean-centered residuals from the selected regression models. Trend results were produced for two sets of phytoplankton data, identified as either "uncorrected", or "corrected" for flow effects.

Two individual sets of phytoplankton data were examined. These were the composite water samples taken from above and below the pycnocline at each station. Six phytoplankton indices were analyzed in this study. These were: 1) total phytoplankton abundance (excluding picoplankton concentrations), 2) autotrophic picoplankton abundance, 3) abundance of dinoflagellates, 4) abundance of diatoms, 5) total phytoplankton and picoplankton biovolume, and 6) the number of phytoplankton taxa per sample. The abundance data were recorded as numbers of cells per liter, with biovolume based on the mean geometric cell measurements as determined for each species. The indices were selected as representing those components and variables in the phytoplankton community that would likely be influenced by changing trophic and water quality conditions over time.

RESULTS

Trends uncorrected for flow

There were 23 significant ($p < 0.01$) long-term trends identified for the period of study (Table 1). These trends were observed across all regions of the lower Bay (*i.e.* no regional or station-specific trends were observed). The trends were: decreasing total phytoplankton abundance (2), both above and below the pycnocline; seasonally mixed trends (5) in diatom abundance below the pycnocline (decreasing the three spring months of March, April and May, and increasing in November and December); and declining monthly diversity (numbers of taxa per sample) above (7) and below (8) the pycnocline, with one month having increased species diversity (1).

Table 1

Significant trends ($p < 0.01$) in flow corrected (C) and flow uncorrected (U) waters above and below the pycnocline in Chesapeake Bay from July 1985 to December 1992.

Above the pycnocline

1. Decreasing total phytoplankton abundance (C and U).
2. Decreasing species diversity (C and U).
(March, April, May, June, Aug., Sept. and Oct. U only)

Below the pycnocline

1. Decreasing total phytoplankton abundance (C and U).
2. Increasing diatom abundance (C and U).
(Nov., Dec.)
3. Decreasing diatom abundance (C and U).
(March, April, June)
4. Increasing species diversity (C and U).
(Feb.)
5. Decreasing species diversity (C and U)
(March, April, May, June, July, Aug., Sept., Oct.)

Trends corrected for flow

Of the 84 combinations of phytoplankton indices, depths and sites, most (nearly 60 %) displayed significant relationships with flow. The R^2 values for the significant regressions with flow ranged from 0.03 to 0.44 (50 % ranged from 0.03 to 0.10; 29 % ranged from 0.10 to 0.20; and 21 % were above 0.20). The stations near the mouths of the three major rivers (LE5.5, WE4.2 and LE3.6) displayed the strongest relationships, and had the largest R^2 values. The phytoplankton indices from the station in the mouth of the Bay (CB7.4) displayed the fewest relationships to flow. Diversity (number of taxa per sample) was the index that consistently displayed the strongest relationship to flow (R^2 values ranging from 0.24 to 0.44; for negative hyperbolic relationships). Thus, a quarter to nearly half of the variance in the number of phytoplankton taxa per sample is related to river flow (i.e. the greater the flow, the fewer the number of taxa per sample).

There were 22 significant trends for the flow corrected data for the lower Chesapeake Bay (Table 1). These were: decreasing total phytoplankton abundance (2), above and below the pycnocline; decreased diatom abundance (3) below the pycnocline in March, April and May, and increasing abundance (2) in November and December; and declining monthly diversity (numbers of taxa per sample) above (6) and below (8) the pycnocline, with one month having increased (1) species diversity. As with the uncorrected data, these trends were found across all stations. While there were differences in the magnitude of some of the trend rates changed in comparison to the uncorrected data set (see below), the direction of all trends for the corrected data were the same as observed for the uncorrected data.

Total phytoplankton trends

In the flow corrected and uncorrected data sets, there were two similar trends of decreasing phytoplankton abundance, both above and below the pycnocline (Figs. 2, 3). The long-term annual rate of decrease (trend slope) above the pycnocline intensified slightly from -2.0×10^5 cells $l^{-1} yr^{-1}$ to $-2.3 \times 10^5 l^{-1} yr^{-1}$ in data corrected for flow. Likewise, the rate of decrease for phytoplankton below the pycnocline intensified from -1.9×10^5 cells $l^{-1} yr^{-1}$ for uncorrected data to $-2.17 \times 10^5 l^{-1} yr^{-1}$ for flow corrected data. Thus, flow patterns in the Bay appeared to have slightly influenced the magnitude of the decreasing trends for phytoplankton. Both the uncorrected and corrected data sets displayed long-term changes that were within the same order of magnitude, but these differences within the water column may be negligible ecologically. In contrast, there were no trends associated with the total phytoplankton biovolume above or below the pycnocline in either the corrected or uncorrected flow data sets.

Figures 2 and 3 also show seasonal variations in abundance, and annual differences in the occurrence and magnitude of phytoplankton populations. Various seasonal combinations for abundance maxima appear.

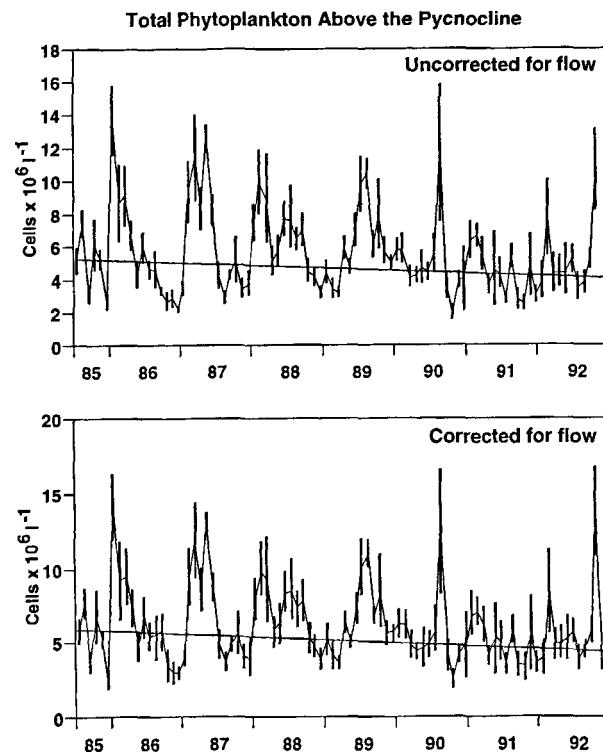


Figure 2

Total phytoplankton abundance above the pycnocline, showing the trend lines for data sets uncorrected and corrected for flow, 1985-1992.

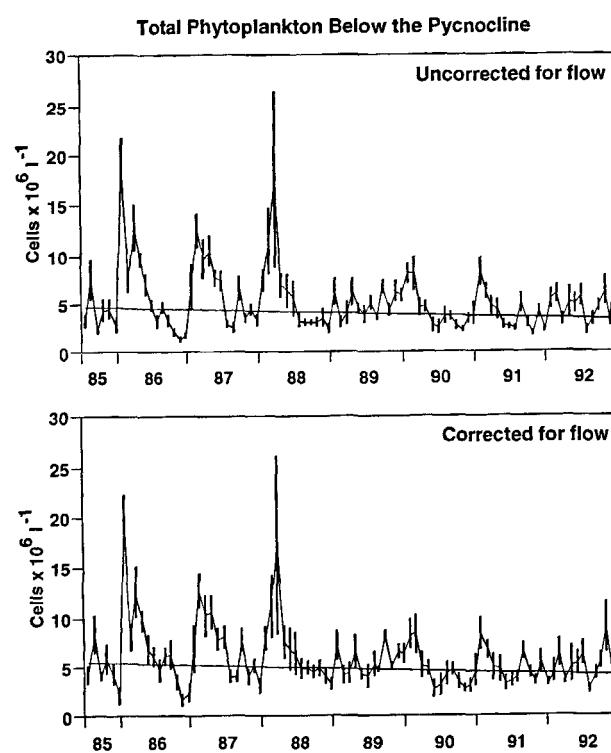


Figure 3

Total phytoplankton abundance below the pycnocline, showing trend lines of data sets uncorrected and corrected for flow, 1985-1992.

These include the occurrence of spring-summer, spring-fall, spring-summer-fall, summer-fall, summer and spring maxima during different years of the study. Corresponding phytoplankton assemblages often differed in composition, yet, specific species remained numerically dominant during many of these periods, e.g. *Skeletonema costatum*, *Katodinium rotundatum*, *Cyclotella* spp., *Cryptomonas* spp. In the uncorrected set above the pycnocline, the phytoplankton abundance ranged from winter lows to spring peaks of 2.0 to 15.8×10^6 cells l^{-1} respectively. In the flow corrected data, these concentrations ranged from 1.9 to 16.6×10^6 cells l^{-1} .

Below the pycnocline there were large spring blooms from 1986 to 1988 that exceeded concentrations above the pycnocline. The spring bloom was much less developed the following four years (1989-1992), with greater counts occurring above the pycnocline, and usually higher concentrations associated with the flow corrected data. In general, there was close similarity in the seasonal patterns and abundance levels determined for total phytoplankton abundance in both the flow corrected and uncorrected data sets. Abundance levels below the pycnocline ranged from winter lows to spring peaks of 1.0 to 26.2×10^6 cells l^{-1} in the uncorrected data, and 0.9 to 25.9×10^6 cells l^{-1} for data that was flow corrected.

Autotrophic picoplankton trends

The data for autotrophic picoplankton are based on a smaller number of collections than the other phytoplankton data, since this component of the program was not added until September 1989. Abundance trends did not appear in the raw, uncorrected data or in the Bay sets corrected for flow. Throughout the study, larger concentrations of picoplankton were associated with waters above the pycnocline. These consisted of mainly cyanobacteria, e.g. *Synechococcus* spp. Cell abundance throughout the water column in the Bay was generally from 10^6 cells l^{-1} in winter to 10^9 cells l^{-1} during summer. Annual concentrations were characterized by a major summer maximum found throughout the water column.

Diatom abundance trends

The concentrations of diatoms showed five significant monthly trends below the pycnocline in both the flow corrected and uncorrected data sets. No trends appeared above the pycnocline. There were decreasing long-term trends of diatom abundance during March, April, and May. Examples of these patterns for March and April are given in Figs. 4 and 5. The degree of trend expression was similar for both data sets, with the mean abundance greater in the uncorrected data sets. The second diatom trend is increasing abundance in November and December. There are similar patterns for the flow corrected and uncorrected data, with a higher mean level in the corrected data for November and in the uncorrected data for December (Figs. 6, 7). There were no trends indicated for a changing abundance pattern of the dinoflagellates either above or below the pycnocline.

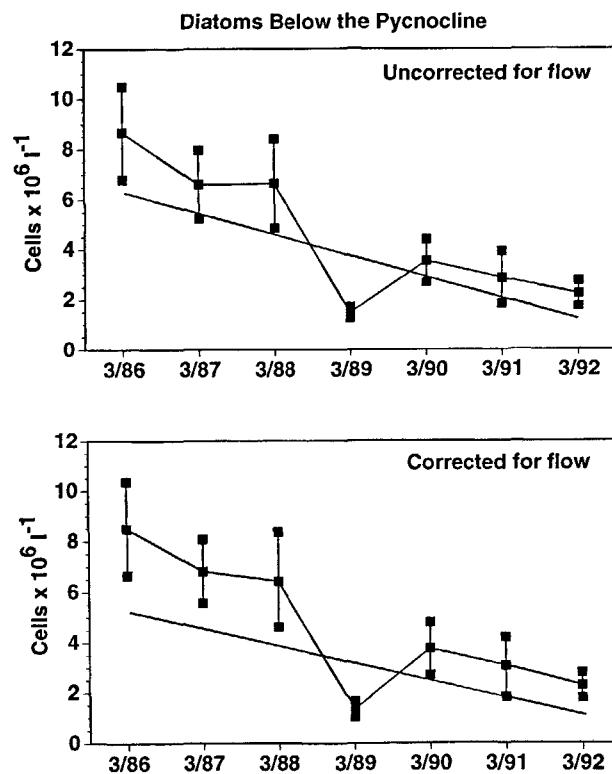


Figure 4

Total diatom abundance below the pycnocline in March, showing trend lines of data sets uncorrected and corrected for flow, 1986-1992.

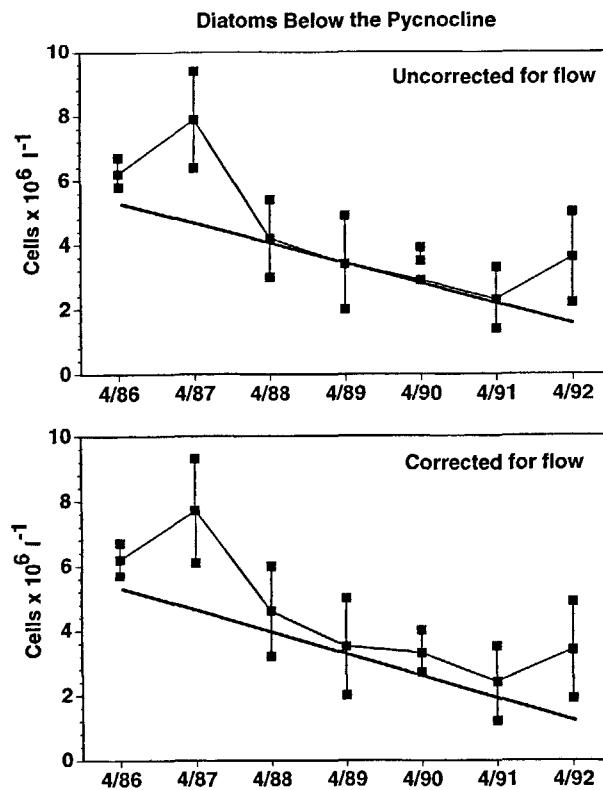


Figure 5

Total diatom abundance below the pycnocline in April, showing trend lines of data sets uncorrected and corrected for flow, 1985-1992.

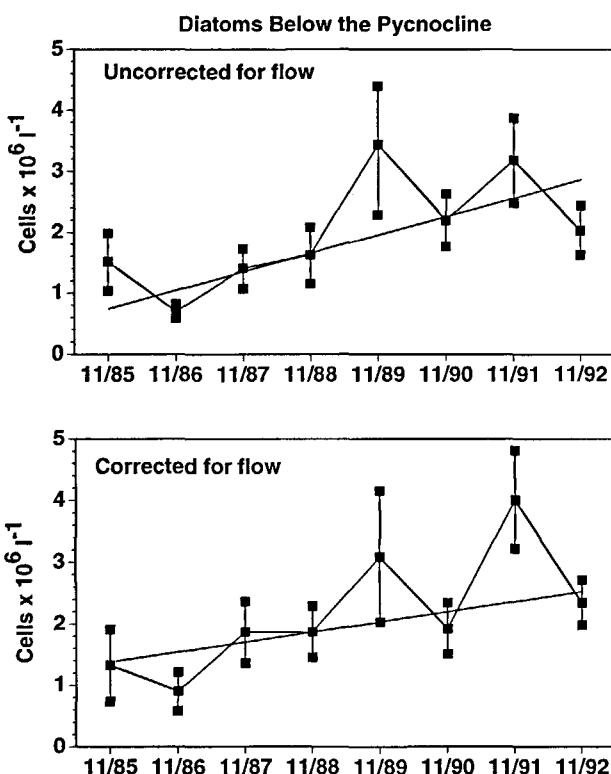


Figure 6

Total diatom abundance below the pycnocline in November, showing trend lines of data sets uncorrected and corrected for flow, 1985-1992.

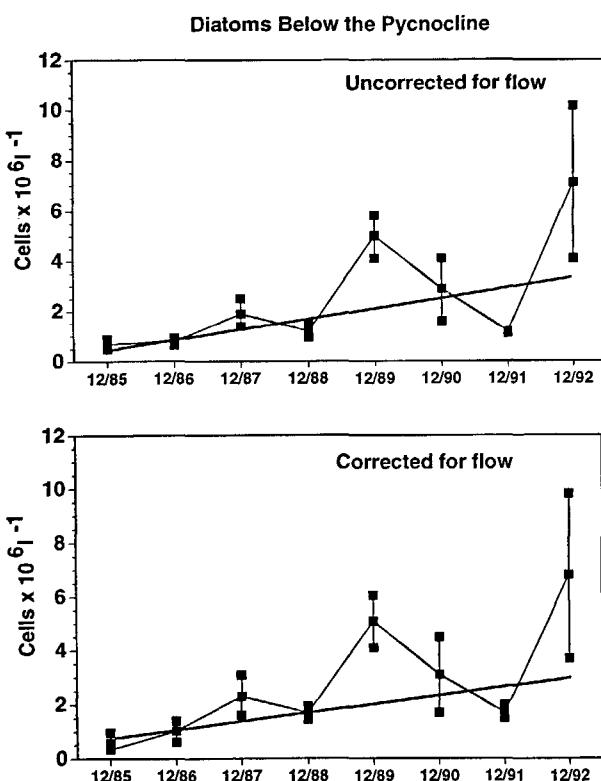


Figure 7

Total diatom abundance below the pycnocline in December, showing trend lines of data sets uncorrected and corrected for flow, 1985-1992.

Trends for number of taxa

A characteristic pattern of decreasing diversity (numbers of taxa per sample) was evidenced by negative slopes for 15 of 16 significant trends for phytoplankton taxa from above and below the pycnocline (Table 1). Both the uncorrected and corrected flow data from above the pycnocline had this negative trend from March through June, plus August and September, with an October downward trend also occurring in the uncorrected set. Both data sets had similar trends below the pycnocline. These included a positive trend for February and negative trends from March through October. Examples of these trends are given in Figures 8 and 9 for above and below the pycnocline for April. In all these trends, the flow corrected data indicated a lower loss in taxa over time.

DISCUSSION

Water flow may affect phytoplankton communities in several different ways. The hydraulic flushing effect of high flow periods can reduce the residence time of phytoplankton to the point where the reproductive potential of some species is never attained and densities cannot be maintained in certain regions of the estuary. Increased river flow also elevates suspended solid loads (Alden, 1997), possibly leading to light limitation for phytoplankton development and production (Gosselain *et al.*, 1994; Harding, 1994; Schmidt, 1994). On the other hand, flow can influence the input of nutrients into the estuary, possibly leading to either the stimulation or reduction of primary production (Cloern *et al.*, 1985; de Jonge, 1995; Harding, 1994). For instance, Chauvet and Fabre (1990), in the Ariège and Garonne Rivers, found a negative correlation between particulate phosphorus content and discharge, with high discharge associated with lower phosphorus and lower algal pigment concentrations. Lapierre and Planas (1987) also related decreased water flow to increased algal biomass and productivity downstream in two rivers. Madariaga *et al.* (1992), in studying short term successional changes in the Gernika estuary, found river discharge and residence time the main factors that influenced the composition and growth dynamics of the plankton. Alden (1997) indicates that the concentration of nitrogen and phosphorus based nutrients in tributaries of the lower Chesapeake Bay are positively correlated to flow in relationships that are best described by various hyperbolic models. Thus, periods of increased river flow could affect the phytoplankton community in either a positive or negative manner.

Considering the various water quality and hydraulic effects that may be linked to river discharge, it is not unreasonable to expect that long-term trends in estuarine phytoplankton populations may be influenced by the trends in river discharge into the estuary. If this situation occurs in an estuary that is managed to reduce excess nutrients (as is the Chesapeake Bay), the long-term influence of the management efforts may be obscured by changes in natural flow patterns. In the case of the southern Chesapeake Bay, the first four years of the monitoring program were a time of severe drought, so the median river flows of the major

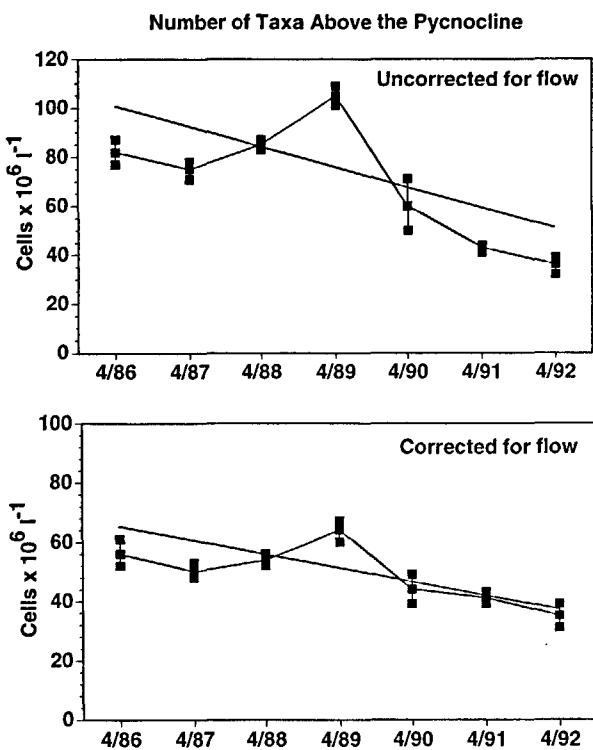


Figure 8

Number of taxa per analyzed sample above the pycnocline in April, showing trend lines of data sets uncorrected and corrected for flow, 1986-1992.

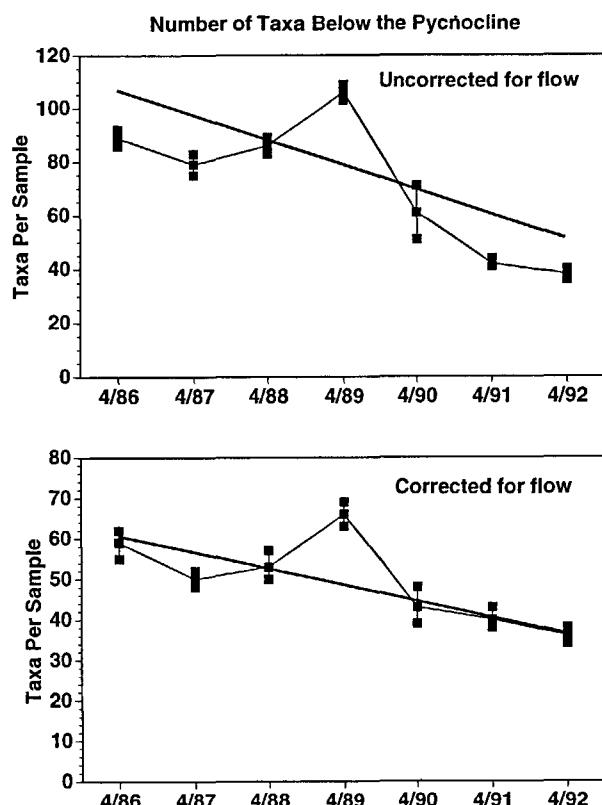


Figure 9

Number of taxa per analyzed sample below the pycnocline in April, showing trend lines of data sets uncorrected and corrected for flow, 1986-1992.

tributaries increased by 55-60 % during the second half of the study period when the rainfall returned to normal levels (Alden, 1997). Therefore, it is quite important that the trends in the phytoplankton which may be related to long-term trends in flow be separated from those that may be related to nutrient management activities in the Chesapeake Bay.

Of the 23 trends identified for the data sets uncorrected for flow, 22 similar trends were found in the flow corrected data. The exception was an October trend of reduced taxa number above the pycnocline that only appeared in the original uncorrected data set. Overall, both data sets identified long-term trends of decreasing phytoplankton abundance and diversity throughout the water column. This reduction in phytoplankton abundance establishes a more specific trend in the lower Bay than the rather static condition previously described by Marshall and Alden (1991) using a four and one-half year data base. The results reported here support the value of longer term (greater than seven years) data bases for trend analysis evaluations over those of shorter study periods. These results indicate a general curtailment of long-term increases in phytoplankton and picoplankton growth in the system, and are considered responses to practices that have reduced nutrient accumulation in the Bay, or to environmental conditions less favorable for the development of specific flora in the system.

Associated with these reduced phytoplankton concentrations is a similar reduction of total phosphorus (TP), dissolved inorganic phosphorus (DIP), ammonia, and dissolved inorganic nitrogen (DIN) in the southern Bay (Alden, 1997). The seven phytoplankton sampling sites correspond to five segments of the Bay where water quality trend measurements have also been made. In all five segments, there are declining trends for TP, with DIN and DIP decreasing in four of the five segments, and ammonia concentrations declining in two of the segments. Variables showing positive trends at this time were total suspended solids (TSS) and total organic carbon, increasing in four and five of the five segments respectively. The decreasing nutrient levels are associated with the trends for reduced abundance of phytoplankton above and below the pycnocline, but apparently have not yet affected the autotrophic picoplankton abundance.

Both the flow corrected and uncorrected data sets identify diatom abundance decreasing in mid to late spring, and an increasing trend in November and December. The decline of the spring bloom in Chesapeake Bay is substantially responsible for the negative trend exhibited by the total phytoplankton. The decline in the spring bloom within the Chesapeake Bay has previously been associated with TN, TP and silicon limitation (Fisher et al., 1992), so nutrient limitation may explain this pattern. This decline usually occurs after the spring bloom (February to April), when the dominant diatom (*Skeletonema costatum*) is replaced by larger, less numerous species (e.g. *Cerataulina pelagica*). This trend suggests that concentrations of cells normally associated with the spring maximum are declining earlier, and the more typical successional patterns that follow the spring development are being initiated earlier, lengthening

the transition period prior to the establishment of the summer flora. The trend for increased diatom abundance in November and December represents a favorable growth periods where various nutrients (*e.g.* silicon) are reintroduced into the water column and made available for diatom development.

A declining number of taxa per sample characterized the water column assemblages from spring through fall. The magnitude of this decline was greater in the uncorrected data set compared to figures corrected for flow. In either case, the diversity of species decreased over this time period. It may be theorized that during the early stages of this study, water quality conditions favored a more diverse development of species, exposed to a wider range of growth conditions that favored greater diversity. Over the past seven and one-half years, there has been a reduction in certain nutrients, with an increase in TSS. Conditions of declining nutrients and light entering the water due to higher concentrations of the TSS would be expected to reduce the presence, or growth of certain species and productivity (DeGroodt and de Jonge, 1990; de Jonge, 1995; Malone *et al.*, 1988; Harding *et al.*, 1986; Schmidt, 1994). Other species that were not previously present or in low concentrations may also have a greater opportunity for development in a changing environmental setting. For instance, *Pseudo-nitzschia pungens* has long been a common and dominant species in the southern Chesapeake Bay, whereas, *Pseudo-nitzschia pseudodelicatissima* has not been previously recorded till recently noted by Marshall (1994). Electron microscopic examination is necessary to distinguish these two species from each other. Archived samples from Chesapeake Bay collections have been examined with electron microscopy and since the late 1980's, *P. pseudodelicatissima* has become gradually more abundant, with *P. pungens* abundance declining. The importance of increasing concentrations of this species is that it may indicate changes in the trophic structure of this system, and represents a potential toxic bloom producer of domoic acid (Martin *et al.*, 1990).

In Chesapeake Bay, these results indicate a close similarity of the trends identified for phytoplankton abundance,

diatom abundance, and number of taxa per sample using both corrected and uncorrected flow data. However, these trends show the rate of change in total phytoplankton abundance would have been higher if it were not for the influence of the flow rate in the Bay. By removing the influence of flow, the size of the trend would have been greater. Flow patterns tended to mask the decreasing trends of phytoplankton concentrations, and the diatom trends for increasing abundance in December and decreasing numbers in May. In contrast, flow patterns tended to enhance the trends for reduced numbers of taxa, the decrease in diatom abundance in March and April, and for a diatom abundance increase in November.

Without the dual analysis of flow corrected and uncorrected data, it would have been unclear whether the observed phytoplankton trends were attributable to changes in flow. The findings of the present study suggest that the Chesapeake Bay water flow (over this study period) produced little overall effects on the trends observed for the phytoplankton indices, except to partially mask the magnitude of the reductions in overall abundance.

The flow corrected data would be useful in identifying the magnitude of impact that water flow has on different components within an estuarine system. Since flow is a factor beyond the direct control of most management actions, it is important to be able to account for and "filter out" these effects in order to identify any results due to management actions, or other changes in the ecosystem.

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