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A comparison of the utermohl and epifluorescent microscopic techniques for quantifying natural picophytoplankton : final report

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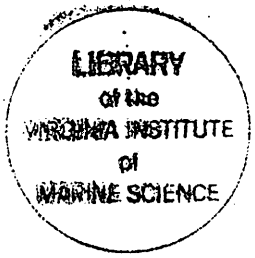

Final Report on Project:

A Comparison of the Ütermohl and Epifluorescent Microscopic Techniques for Quantifying Natural Picophytoplankton.

Submitted to:

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INTRODUCTION

The phytoplankton monitoring program in the Virginia portion of the Chesapeake Bay was initiated in July 1985. A total of 13 stations (7 in the lower Chesapeake Bay and 6 in the major tributaries) are sampled on a regular basis to quantify phytoplankton (Birdsong et al., 1987;1988). A primary objective of the monitoring program is to document long-term trends in phytoplankton abundance and species composition and use this information as one means of evaluating the effectiveness of pollution abatement strategies, particularly related to the reduction of nutrients to the Chesapeake Bay.

The philosophy of monitoring programs is to consistently apply widely accepted quantification techniques over long periods of time as the best means of determining long-term trends in ecosystem states. At the inception of Virginia phytoplankton monitoring program, a modified Ütermohl inverted microscope technique (Marshall, 1984) was the method chosen for phytoplankton identification and enumeration. However, questions have been raised about the effectiveness of this technique to adequately quantify the smaller-sized phytoplankton cells, and it has been suggested that epifluorescence microscopy is more effective in this regard. As new and possibly improved techniques are developed for quantifying ecosystem state variables, it is a difficult decision if and how to accommodate these techniques into a well-established monitoring program, either by substituting new techniques for old or adding the new techniques to the program. The advantages of gathering more and/or better information must be weighed against the added cost and established consistency of the historical data base. The present study compares two microscope techniques, the Ütermohl technique and epifluorescence microscopy, for their effectiveness in quantifying picophytoplankton in the Chesapeake Bay.

BACKGROUND

The food chain of the Chesapeake Bay is a phytoplankton based system. Directly or indirectly the living resources of the Bay are primarily dependent on the primary production of phytoplankton. Although a sufficient level of phytoplankton production is necessary to sustain a productive Chesapeake Bay ecosystem, an overabundance of phytoplankton resulting from nutrient enrichment, a process known as eutrophication, may contribute to decreased water quality. In tidal freshwater portions of estuaries, eutrophication may be manifested in surface scums or mats of algae which may alter the taste of the water, clog water intakes and generally make the water unsuitable for many human uses (Paerl and Ustach, 1982; Expert Panel, 1985). An example in the Chesapeake Bay region is the tidal freshwater portion of the upper Potomac River where noxious algal blooms during the summer were a common occurrence (Expert Panel, 1985). In the saline portions of estuaries the manifestations of eutrophication are neither as dramatic nor as well documented (Neilson and Cronin, 1981 and references therein). In the Chesapeake Bay it is generally accepted that excess algal growth, stemming from nutrient enrichment, contributes significantly to hypoxia, or lack of oxygen, especially in waters below the pycnocline. It is proposed that the excess algae sink through the water column and their subsequent decomposition is instrumental in initiating the depletion of oxygen (Malone et al., 1986, 1988). Eutrophication may also change the species composition of phytoplankton and in so doing alter the abundance of species higher up the food chain, some of commercial importance. For example it has been proposed that nutrient enrichment leads to the dominance of small phytoplankton such as cyanobacteria and phytoflagellates compared to larger phytoplankton such as diatoms under unenriched conditions. The former are thought to support a food chain terminating in jelly plankters (jellyfish and ctenophores) while the latter support a food chain more likely to support finfish as the top trophic level.

Phytoplankton may be classified on the basis of size as follows: picoplankton, 0.2-2.0 μm effective diameter; nanoplankton, 2.0- 20 μm; microplankton, 20-200 μm (Sieburth, 1978). Since the 1930's the Ütermohl technique (Ütermohl, 1931) has been the method of choice for counting and identifying phytoplankton. Over the past three decades there have been numerous modifications to this methodology to improve its effectiveness (Ütermohl, 1958; Lund et al., 1958; Willen, 1976; Hasle, 1978; Hewes et al., 1984; Reid, 1983; Marshall, 1984). Since this technique relies on phytoplankton cells in a water sample to settle into a counting chamber and uses a relatively low magnification for observation, its use has tended to emphasize the importance of the larger phytoplankton (microplankton and larger nanoplankton). However in the last decade, additional techniques for phytoplankton enumeration and identification have been developed such as electron microscopy, epifluorescence microscopy and flow cytometry. A significant conclusion of the application of these techniques is the growing appreciation of the important role of smaller phytoplankton (pico and smaller nanoplankton) in the world's oceans (Platt and Li, 1986 and references therein; Stockner and Antia, 1986) It is now commonly accepted that in most of the world's oceans, the phytoplankton primary production and standing stock is dominated by phytoplankton less than 3-5 μm in effective diameter.

Despite advances in our understanding of phytoplankton size distribution and species composition in oceanic and coastal waters, comparatively little research of this type has been performed in temperate estuaries. Studies in Narragansett Bay indicate that picoplankton (in this case cells less than 5 μm diameter) accounted for 29% of the total summer chlorophyll (Furnas, 1983). In both Long Island Sound and the lower York River, observations during the summer period of high production indicate that picoplankton account for about 10% of the total primary production (Carpenter and Campbell, 1988; Ray et al., 1989). However, observations in the main stem of the Chesapeake Bay during the summer of 1989 indicate that phytoplankton less than 3 μm accounted for >80% of the phytoplankton standing stock based on chlorophyll (Haas et al., 1989). Clearly, considerable research remains to adequately define the role of small phytoplankton in the ecosystem dynamics of estuaries.

In most instances in which the species composition of the picophytoplankton has been characterized, two basic types of cells tend to predominate - eukaryotes and coccoid cyanobacteria (prokaryotes) (Thomsen, 1986; Stockner and Antia, 1986). The former is composed mostly of single celled, flagellated and non-flagellated cells generally in the size range of 1-10 μm, and typically belonging to the algal classes Cryptophyceae, Chrysophyceae, Prymnesiophyceae and Chlorophyceae (Thomsen, 1986). The latter are single celled, roughly spherical non-flagellated cells ranging in size from 0.5-1.0 μm in diameter belonging to the genus *Synechococcus* (Stockner and Antia, 1986). These latter cells were first observed in the marine plankton in the late 1970's (Johnson and Sieburth, 1979; Waterbury et al., 1979) and considerable research on their physiology and ecology has ensued (Platt and Li, 1986). In many oceanic and coastal systems coccoid cyanobacteria of the genus *Synechococcus* dominate both the picophytoplankton and total phytoplankton biomass and production (Fogg, 1987). Here again comparatively little work has been done on the role of coccoid cyanobacteria in estuarine plankton dynamics. However, recent observations in the mainstem Chesapeake Bay indicate extremely high concentrations of cyanobacteria during the summer months (ca.3-5 x 10^6 ml^{-1}) (Haas et al., 1989) compared to typical values of 10^5 ml^{-1} for coastal waters and 10^4 ml^{-1} for open ocean systems (Fogg, 1987, Stockner and Antia, 1986). Coccoid cyanobacteria are also commonly observed in the tidal freshwater regions of coastal plain estuaries where, under certain conditions of water flow, temperature and nutrient loadings, they are the principal contributors to nuisance blooms in the summer months (Paerl, 1982; 1983; Expert Panel, 1985).

METHODS AND MATERIALS

Sampling

For the purposes of this study, four stations were selected from the water quality stations to provide as large a range of salinity as possible (Fig 1). Station CB 7.4 was on the bayside of the Chesapeake Bay Bridge Tunnel, Station TF 5.5 was just downriver of the Hampton Roads Bridge Tunnel, Station RET 5.2 was in the vicinity of Jamestown Island and Station LE 5.5 was downriver from Hopewell, Virginia. Samples from each station were collected once a month during the regular water quality cruises from October 1987 through December 1988. At each station a 15 liter composite water sample was obtained from 5 depths above the pycnocline (3 liters per depth) using a submersible pump.

Ütermohl Procedure

Two sets of subsamples of 125 and 500 ml were taken and preserved with Lugols solution. The 500 ml sample was left to settle for 48 hours and a two step siphoning process started on the second day to bring the sample to 250 ml. After another 48 hour settling period the second siphoning resulted in a concentrate of less than 40 ml. This concentrate was thoroughly mixed and depending on the concentration of cells, a known volume of a sub-sample was transferred to a settling chamber used with the inverted plankton microscope. It was allowed to settle for another 24 hours before being placed on the microscope stage to be analyzed for cell counts. The sample was examined at 500X magnification for all cells in the size range between 1.5 and 3.0 µm. A counting procedure was followed using a minimum combination of random fields (10) and cell counts (200).

The 125 ml sample was returned to the laboratory, thoroughly mixed and 2 ml were transferred from this sample to a settling chamber, and allowed to settle for 48 hours. The cells were counted in a similar fashion as mentioned above with the inverted plankton microscope. Replicate samples for both the 125 and 500 ml samples were analyzed. Mean values were then used in plotting the cell concentrations. References to these two methodologies in the figures will be to the Ütermohl for the 500 ml samples and modified Ütermohl for the 125 ml set.

Emphasis was placed on discernible cyanobacteria to be counted, with identification based on characteristics (cell size, cell shape, colony formation, etc.) as presented in Geitler (1932), Desikachary (1959) and Prescott (1951). However, cells from other taxonomic categories could have been included in these counts, especially small spherical shaped cells. The size ranges of cells counted was mainly between 1.5 and 2.0 µm. Isolated cells less than 1.5 µm could not be counted with confidence as cyanobacteria. However, there were cells less than 1.5 µm in size associated in characteristic colonial formations, that were identified as cyanobacteria (e.g. *Merismopedia*).

Epifluorescence Procedure

A 40 ml aliquot was taken from the composite sample and either used immediately for slide preparation or preserved with 2 ml of 6% glutaraldehyde (0.03% final concentration) for slide preparation within two days. This difference in procedure has no effect on cyanobacterial cell counts. The procedure for preparing slides for cell counts followed that of Haas (1982). A 2 ml sample is placed in a filter apparatus containing a prestained (irgalan black 0.2% in 2% acetic acid) Nuclepore filter (0.2 µm pore size, 25 mm diameter). Proflavin (0.033% in distilled water) is added to the sample (20 µl/ml) followed by glutaraldehyde (6%, 50 µl/ml) if the sample has not been previously preserved. DAPI (4',6-diamidino-2-phenyl-indole, 1 µg/ml in distilled water) is then added to the sample (100 µl/ml) which is allowed to sit for several minutes. A vacuum is then applied to the sample (<15cm mercury) and after the meniscus disappears, the filter is placed sample side up on a standard microscope slide spread with a thin layer of low fluorescence immersion oil (Resolve, Stephens Scientific). A small drop of oil is then placed on the filter and a cover slip gently pressed onto the filter.

Cell counts were made using a Zeiss standard microscope equipped with a 50 W high pressure mercury lamp, 12.5X calibrated ocular and either a 63X Plan Neofluar or a 100X Neofluar objective. Three epifluorescence filter sets are used for identification and enumeration of the plankton. Blue excitation (450-490 nm; Zeiss #487709) is used to observe the green fluorescence of cytoplasm and flagella resulting from the proflavin fluorochrome and the crimson red autofluorescence of chlorophyll. Ultra-violet excitation (365 nm; Zeiss #487702) is used to observe the blue-white fluorescence of cell nuclei resulting from the DAPI fluorochrome. Neither of these excitation filters is the most effective for enumerating cyanobacteria in estuarine waters. Cyanobacteria typically contain two auxiliary photosynthetic pigments, phycoerythrin (PE) and phycocyanin (PC). Under blue light excitation PE-dominant cyanobacteria typically autofluoresce gold-orange while PC-dominant cyanobacteria are not readily visible. Under green light excitation (510-560 nm; Zeiss #487714) PE cyanobacteria autofluoresce a bright orange red, and PC cyanobacteria autofluoresce a bright crimson red (Ray et al., 1989; Wood et al. 1985; Waterbury et al. 1986). Typically high salinity oceanic and coastal systems contain only PE cyanobacteria (Murphy and Haugen, 1985; Waterbury et al. 1986; Campbell and Carpenter, 1987; Booth, 1988) and previous observations in the James River indicated that the PE type are not observed below 10 °/oo salinity (Haas and Paerl, 1988). PC/PE ratios in the lower Chesapeake Bay and lower York River have been reported to be ca. 3 and 8 respectively (Falkenhayn, 1990; Ray et al. 1989). Cyanobacterial counts reported for the epifluorescence technique in this report are total cyanobacteria counted under green light excitation.

RESULTS

Cyanobacterial cell counts for each of the three counting methods and the four stations are shown in Table 1. The data for each station are graphed in Figures 2a-d and for each counting method in Figures 3a-c. In general all three counting methods show a summer (June-August) peak in cyanobacterial abundance at each of the four stations. However the magnitude of the summer peak and temporal characteristics of the annual cycle differ, depending on the counting method used. The modified Ütermohl method indicates constant and relatively similar cyanobacterial concentrations from February - May at all four stations and relatively moderate increases into the summer (Fig. 3a), resulting in a dampened annual cycle, especially at the two estuarine stations. At LE 5.5 eleven of the fifteen values are between 15K and 30K (i.e. one doubling) and at station CB 7.4 fourteen of the fifteen values are between 15K and 60K (two doublings).

The Ütermohl method indicates a greater annual range in picoplankton abundance than the modified Ütermohl method at all four stations; that is higher maxima and lower minima (Fig. 3b). At three stations the annual maxima occurs in July and the magnitude of each maxima substantially exceeds the respective abundances indicated by the modified Ütermohl method. At the fourth station (LE 5.5), the annual maxima occurs in March and again the magnitude greatly exceeds that indicated by the modified technique. Three of the four stations (RET 5.2, CB 7.4 and LE 5.5) indicate an annual minimum during November, 1987 and a winter-spring (February-April) peak in picoplankton abundance, a phenomenon that is not evident in the modified Ütermohl data. At station RET 5.2 the winter-spring maxima exceeds the summer abundances.

In general the epifluorescent counting technique shows the most pronounced annual range in cyanobacterial abundance of the three counting methods, and similar to the modified Ütermohl method, indicates only one annual maxima (Fig. 3c). At all four stations cyanobacterial abundance decreased from the fall of 1987 to an annual minima during February-April. Concentrations increased rapidly during April-June and generally peaked during June-August. The most consistent difference between both Ütermohl methods and the epifluorescent method occurred during the winter-spring. The distinct winter minimum in the epifluorescence counts is not reflected in the modified Ütermohl counts which indicates relatively constant picoplankton abundances at all four stations during the first five months of the year. In addition, during the period December 1987-January 1988 the epifluorescence counts indicated decreasing cyanobacterial numbers at all four stations progressing to a February-April minima, while the Ütermohl method indicated increasing cyanobacterial abundance progressing to a February-April maxima at three stations.

In order to more directly compare counting methods over the course of the study, counts for each of the three counting techniques were averaged for each station over the period March-November, 1988, a time for which data were available for each method (Fig. 4). All three methods indicate the greatest number of picoplankton at the most upriver station (TF 5.5) and the lowest abundances at the Bay mouth station (CB 7.4). It is also apparent that the epifluorescent method indicates approximately 2-3 times more

cyanobacteria at each station than either of the Ütermohl methods, averaged over this time period.

The dominant cyanobacterial morphology observed in this study was a coccoid cell approximately one µm in diameter. At stations LE 5.5 and CB 7.4, this was virtually the only cyanobacterial cell type observed, occurring predominantly as a single isolated cell, occasionally as a doublet (i.e. recently divided) and less often as aggregations (clumps and chains) of 3-5 cells. Coccoid cyanobacteria were also the dominant morphological type observed at the two freshwater stations. However, in addition to the single and doublet cells, both stations exhibited colonial cyanobacterial strains consisting of large numbers of coccoid cells. The two most common colony forms were cells arranged in rows and columns much like a page of stamps (*Merismopedia* spp) or as dense aggregations containing hundreds of individual cells (*Microcystis* spp.). Because of the three-dimensional nature of the latter form, an accurate count of the total number of cells in a colony is problematical. Thus, during those times of year when this species is common it may be expected that confidence intervals for counts will be increased.

DISCUSSION

The basic premise of the Ftermohl cell counting method is that in processing the algal flora a representative subset will be concentrated on the bottom plate of a settling chamber. This chamber is placed on the stage of an inverted light microscope to allow the bottom plate to be examined. The major criticism of this technique is that the smaller plankton (picoplankters and smaller nanoplankton) due to their size, their low specific gravity and large surface area to volume ratio, will not completely settle to the bottom plate and therefore will not be adequately enumerated. Those smaller forms that do settle out may be obscured by the settled detrital material present in the chamber. It is also possible that the fixatives typically employed in the Ftermohl method may destroy the more fragile, smaller algae (van der Veer, 1982).

Another problem in the use of the Ütermohl method is the inability to distinguish between pico- and nanoplankton heterotrophs and autotrophs. Small flagellates may be autotrophic (contain photosynthetic pigments) or heterotrophic (contain no photosynthetic pigments), and in some instances the latter may equal or exceed the former in abundance. Small cyanobacteria (autotrophs) are roughly the same size as bacteria (heterotrophs), and in this case a specific colonial formation, shape and size is necessary for identification and enumeration of cyanobacteria as autotrophs. In order to recognize the presence of cells that cannot be categorized with assurance as either heterotrophs or autotrophs with the Ütermohl method, many investigators have chosen to place such cells in general size categories. Marshall (1984) has used general categories for cells where identification of species or trophic mode was not possible and therefore were not included in other counts. He placed these cells in size groups as: less than 3 µm, 3-5 µm and 5 to 10 µm. The lower size range for cells counted was 1.5 µm (Marshall, 1988; Marshall and Ranasinghe, 1989) so unless cells of smaller dimensions were recognized by their colonial traits, they were not counted. Thus there was a considerable under estimation of cell numbers for the picoplankton component of the phytoplankton (Birdsong et al., 1988). Despite the potential for underestimating these cells, in most instances the unidentified, 1.5-10 µm size component constituted 50-70% of the total phytoplankton counts (Birdsong et al., 1987; 1988; Marshall and Alden, 1990).

A variety of studies over the past decade and more have demonstrated the diminished efficacy of the Ütermohl method to enumerate small phytoplankton in natural samples (Ballantine, 1953; Bernhard et al., 1967; Booth et al., 1982; Davis and Sieburth, 1982; Hewes et al., 1984; Paerl, 1977;1978; Reid, 1983). Davis and Sieburth (1982) determined that the Ütermohl technique will severely underestimate counts of heterotrophic organisms at the lower end of the nanoplankter size range. Paerl (1978) using fresh water samples compared various counting methods and found the Ütermohl procedure and membrane filter methods as underestimating counts of cells less than 5 microns in size. He recommends an autoradiographic method to improve objectivity and be more accurate. Reid (1983) used the Ütermohl method for estimating pico and nanoplankton biomass and also found this method underestimated the picoplankton and noted the problem was associated with the non-settling cells. She suggested the Ütermohl method should be supplemented with either a membrane filter protocol, or epifluorescence procedure for analyzing the

picoplankton to more fully cover the phytoplankton. In contrast, Davis and Sieburth (1982) indicate that epifluorescence microscopy has insufficient resolution to be of value in the species identification of even the dominant nanoplanktors, a task they suggest requires the use of electron microscopy.

Epifluorescence microscopy overcomes many of the deficiencies of the Ftermohl method for counting picophytoplankton. Since all of the particles in a water sample, except perhaps for viruses, are retained on the membrane filters, the entire picoplankton assemblage is available for enumeration. At the magnifications typically used, cells as small as heterotrophic bacteria (ca. 0.5 µm effective diameter) may be accurately counted. With the use of appropriate fluorochrome stains, small flagellates are easily partitioned between heterotrophic and autotrophic forms (Haas, 1982). For both groups the capability for species identification using epifluoresence microscopy is as good or better than with the Ftermohl method. Electron microscopy remains as the necessary procedure for many species identifications at this level. Coccoid cyanobacteria are readily distinguished from heterotrophic bacteria and other algae on the bases of a distinct autofluorescence and size respectively.

During the period of peak summer cyanobacterial abundance, the epifluorescence and Ütermohl methods appeared to compare best at the most upriver station and least well at the lower Bay station. In general the Ütermohl method indicated lower numbers of picoplankton (which included cyanobacteria) and a shorter duration of the summer maxima than did the epifluorescence method at the two estuarine stations. This is most likely a reflection of the presence or absence of colonial cyanobacterial taxa at these stations. At the two estuarine stations the predominant cyanobacterial cell type is the single, isolated cell. Measurements of cyanobacterial cell size during the summer of 1989 from the mainstem of the lower Bay using image analysis indicated mean cell diameters in the range of 0.7-0.9 µm (Falkenhayn, 1990). Since the lower size limit for the Ütermohl technique is ca. 1.5 µm for non-colonial forms the effectiveness of this method is likely compromised for this type of cell. On the other hand, the presence of colonial cyanobacteria at the two upriver stations and especially at the most upriver station, is likely to enhance the capability of the Ütermohl method to adequately assess cyanobacterial numbers. Since the colonies constitute a larger mass, they are more likely to settle out into the counting chambers than are individual cells or doublets. Also, because of their larger size the colonies are more likely to be noticed among the detrital material and the individual cells enumerated. Thus, one might expect a better agreement between the epifluorescent and Ftermohl methods at these stations.

A distinct characteristic of the cell count data is the large annual range in the number of cyanobacteria, spanning four orders of magnitude (i.e. 10^2 - 10^6 ml^{-1}). Counts of eukaryotic algae at these stations typically range between 10^2 - 10^3 and only rarely exceed 10^4 ml^{-1} (Birdsong et al., 1987; 1988; Marshall and Alden, 1990). Thus, during the summer period of peak cyanobacterial abundance (10^5-10^6 ml^{-1}), the inclusion of eukaryotic picophytoplankton (ca. 10^3 ml^{-1}) in the Ftermohl cell counts would increase the cyanobacterial numbers only minimally, and their influence would be greater during other seasons. Because of the small size of the cyanobacterial cells, their contribution to total algal

biomass or production is likely to be small at cell concentrations similar to those for eukaryotic algae. However at peak abundances, cyanobacteria may constitute an important if not dominant component of the total phytoplankton assemblage. In the mainstem of the lower Chesapeake Bay during summer 1989, cyanobacterial abundances on the order of $3\text{-}5x10^6$ ml^{-1} were estimated to constitute 40-90% of the total phytoplankton biomass (Falkenhayn and Haas, 1990).

Discerning annual cycles in abundance in dynamic aquatic environments from monthly samples is problematical at best. A variety of shorter term physical and biological processes can influence cell abundance and obscure any seasonal cycle. For example advective processes may transport an adjacent water mass with a different algal composition and abundance into the sampling site for a short period of time just when a sample is collected. Growth and mortality may also obscure seasonal trends. During the summer months cyanobacterial growth rates range between 1 and 2 doublings per day (Falkenhayn, 1990). Thus four doublings (i.e. about two days), in the absence of mortality or other losses such as sinking or advection, will increase cell numbers from $1x\ 10^5$ ml^{-1} to 1.5×10^6 ml^{-1}. Grazing mortality is also likely to be enhanced since the probable grazers of the cyanobacteria are protozoans with higher growth rates, and hence shorter response times, than larger zooplankton.

Despite these concerns, the most consistent feature of all three counting techniques is the presence of a summer picoplankton peak in cyanobacterial abundance at all four stations. The summer dominance of cyanobacteria in tidal freshwater systems is well established (Paerl, 1982; Paerl and Ustach, 1982) A distinct summer maximum and winter minimum in cyanobacterial abundance also appears to be a consistent feature of temperate estuarine and coastal waters, occurring in most all instances in which such counts have been made (Ray et al., 1989). What is unusual about the Chesapeake Bay is the high numbers of cyanobacteria observed. In most temperate estuarine and coastal systems seasonal maxima are on the order of 10^4-10^5 ml^{-1} and cyanobacterial densities exceeding 10^6 ml^{-1} are rarely reported in the literature. In addition to the present study, observations in the mainstem of the mesohaline portion of the Chesapeake Bay during the summer of 1989 indicated cyanobacterial numbers in excess of 4×10^6 ml^{-1}. It appears that the Chesapeake Bay contains extraordinarily high numbers of cyanobacteria during the summer months.

Although each of the counting methods indicate a summer peak of cyanobacterial abundance, there are differences, often substantial, with regard to other aspects of the annual cycle among the various techniques. The modification of the Ütermohl technique in the present study was for the purpose of enhancing the capability for this technique to count picoplankton. In most instances (40 of 60 samples) the modified Ütermohl method indicated greater numbers of these cells than did the Ütermohl method. However on at least one occasion at each station when the Ütermohl technique indicated a distinct peak in abundance, the modified method substantially ($x = 17\%$) under-counted these cells. These large differences between the two methods coincided with the high seasonal concentrations of diatoms from the spring and summer populations, and were associated with periods of growth maxima for other phytoplankters and the picoplankton. However, it is

not immediately apparent why the modified Ütermohl method indicates substantially fewer cells than the Ütermohl technique for these samples.

The greatest discrepancy among the three counting techniques occurred in the late winter-early spring period. At all four stations the epifluorescent method indicated the lowest yearly counts during this period, while the Ütermohl technique indicated a seasonal peak in abundance at three out of four stations, the most upriver station being the exception. The modified Ütermohl method indicated largely constant numbers throughout this period and at station RET 5.2 did not reflect the seasonal peak in picoplankton indicated by the Ütermohl method. In all cases, the Ütermohl methods indicated much greater concentrations of cells during this time period than did epifluorescence microscopy. Although the emphasis in these counts was directed to the cyanobacteria, there may have been cells included in this series from other taxonomic groups. For instance, small, non-flagellated chlorophyceans, less than 3 mm in size are present in these waters. Chlorophyceans in general will have major spring development (Birdsong et al., 1987) that begins in late winter and their cells may have been included in these samples. Since the Ütermohl procedure itself does not distinguish cyanobacteria from other cells, other cells may be a part of the total counts. The lack of a similar pulse at this time in the modified Ütermohl method may be a procedural phenomenon and needs further investigation. As discussed above, a variety of observations in temperate coastal and estuarine systems indicate a seasonal minimum for cyanobacteria during this time of year. However, Shapiro and Haùgen (l988) in Boothbay Harbor, Maine report a winter peak in cyanobacterial abundance in that estuary. Marshall (1988), Marshall and Alden (1990) and Birdsong et al. (1987; 1988) report a variety of fall, winter and spring cyanobacterial maxima (including filamentous and colonial forms) in Back Bay, Virginia the lower Chesapeake Bay and tributaries with peak abundances on the order of 1-5 x 10^4 ml^{-1}. If such a winter-spring cyanobacterial bloom is occurring in the Chesapeake Bay and its tributaries, then the pigment or fluorescence characteristics of the cyanobacteria are such that they are apparently not being enumerated by the epifluorescent technique.

At present, it is not clear to what extent the high concentrations of coccoid cyanobacteria in the saline portions of the lower Chesapeake Bay represent a normal condition for temperate estuaries or a response to nutrient enrichment. Cyanobacteria, both filamentous and coccoid, are well known as indicators of nutrient overenrichment in tidal freshwater portions of estuaries and in lakes. It is also likely that the general decline in cyanobacterial abundance from Hopewell to the Chesapeake Bay mouth reflects the decreased nutrient availability which typically occur along such estuarine salinity gradients (Fisher et al., 1988).

On the other hand, coccoid cyanobacteria in oceanic areas are typically most abundant in stable, oligotrophic areas where nutrients are below conventional detection limits. It is proposed that under these circumstances, cyanobacteria are sustained primarily by nutrients which are recycled by an active microbial plankton component. In a like fashion, the primary seasonal peak of cyanobacteria in the lower Chesapeake Bay occurs during the period of maximal annual vertical stability of the water column when nutrient input via runoff and standing stock of nutrients are both at their annual minima. Thus, cyanobac-

teria appear to dominate when the estuary is in its most "oligotrophic" state. In this circumstance as well, cyanobacteria would appear to be primarily dependent on recycled nutrients. If this is the case, then the peak summer abundance of cyanobacteria is probably not a direct response to the advection of nutrients into the Chesapeake Bay but is complicated by the role of the estuarine food web in conserving and recycling essential nutrients.

SUMMARY

1. A comparison of three methods for counting picophytoplankton in the lower Chesapeake Bay estuary (Ütermohl, modified Ütermohl and epifluorescence microscopy) indicates significant differences in algal abundances and temporal cycles among the methods.

2. A modified Ütermohl method quantified a greater number of picophytoplankton than did the Ütermohl method in 66% of paired samples, but on four occasions when the latter method indicated a substantial temporal peak in abundance, the modified Ütermohl method undercounted by an average 83%. The Modified Ütermohl method would appear to provide no advantage in counting picoplankton.

3. At three out of the four stations sampled the Ütermohl method indicated summer maxima and a smaller late winter-early spring peak in abundance. During summer periods of maximal abundance the Ütermohl counts most closely matched the epifluorescence counts at those stations (freshwater) where colonial cyanobacteria were most abundant and most under counted compared to the epifluorescence method at those stations (estuarine) where single cyanobacterial cells less than 1.5 mm were the predominant form.

4. At all four stations sampled, the epifluorescence method indicated a winter minima and summer maxima in cyanobacterial abundance. At the estuarine stations the epifluorescence method indicated a longer duration for the peak abundance than did the Ütermohl method. If a late winter-early spring peak in picophytoplankton occurred, then the fluorescent characteristics of the cells were such that they were not being detected by epifluorescence microscopy.

5. The epifluorescent procedure is recommended for the enumeration of autotrophic picoplankton cells, and specifically for the cyanobacteria in this size range.

6. The Chesapeake Bay exhibits extremely high concentrations of cyanobacteria which may be related to water quality considerations, particularly aspects of nutrient enrichment. A successful phytoplankton monitoring program should effectively enumerate picophytoplankton in general and coccoid cyanobacteria in particular.

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Table and Figure Legends

Table I. Cyanobacterial cell counts.

Figure 1. Station locations in the James River and lower Chesapeake Bay.

Figure 2. Cyanobacterial abundance at stations TF 5.5 (A), RET 5.2 (B), LE 5.5 (C) and CB 7.4 (D) using three different counting techniques.

Figure 3. Cyanobacterial abundance at four sampling sites using the modified Ütermohl (A), Ütermohl (B) and epifluorescence (C) microscope methods.

Figure 4. Cyanobacterial abundance averaged over the period March through November, 1988 for each of the three microscope methods at the four sampling sites.

TABLE I

Date	Station TF 5.5			Station RET 5.2			Station LE 5.5			Station CB 7.4		
month\year	UM	MUM	EPI	UM	MUM	EPI	UM	MUM	EPI	UM	MUM	EPI
10/87	6.51	2.55	5.80	0.52	1.49	7.00	0.55	2.29	nd	0.22	4.61	2.10
11/87	6.41	1.65	2.90	0.28	0.88	nd	0.12	2.11	4.05	0.19	1.03	5.60
12/87	7.32	5.27	0.77	6.31	nd	0.15	2.30	nd	nd	0.20	3.13	nd
1/88	9.33	5.18	nd	3.65	5.14	nd	2.76	2.96	nd	0.55	3.68	nd
2/88	3.27	4.67	nd	7.03	4.05	0.22	4.66	2.18	0.10	3.84	2.31	0.17
3/88	1.65	4.41	0.54	17.00	5.00	0.44	2.79	2.69	0.32	0.89	2.28	0.13
4/88	6.90	4.44	0.27	11.10	5.46	0.20	2.10	2.15	0.32	0.57	1.85	1.13
5/88	3.53	2.58	2.90	2.59	3.06	4.90	1.03	2.11	20.20	0.58	1.80	0.70
6/88	18.50	10.20	205.40	5.72	10.10	29.20	4.97	2.65	0.84	1.11	2.99	23.80
7/88	197.00	44.00	64.90	5.80	9.66	62.00	65.40	6.08	50.80	65.30	6.10	63.10
8/88	6.00	23.50	78.90	13.20	25.10	87.20	3.37	7.11	169.80	0.54	2.99	1.40
9/88	11.30	29.10	37.50	5.59	5.11	29.60	3.80	6.94	50.20	1.06	3.91	36.40
10/88	15.80	34.80	43.30	7.00	7.05	22.00	1.63	1.69	1.59	1.40	1.97	3.18
11/88	5.15	13.60	8.90	15.50	24.00	36.30	2.33	2.26	1.37	1.27	2.70	3.46
12/88	1.69	8.55	1.20	3.56	10.50	12.30	1.11	4.36	nd	1.29	2.45	nd

All values are cells x 10^4 ml^{-1}
nd - no data collected

FIGURE 1

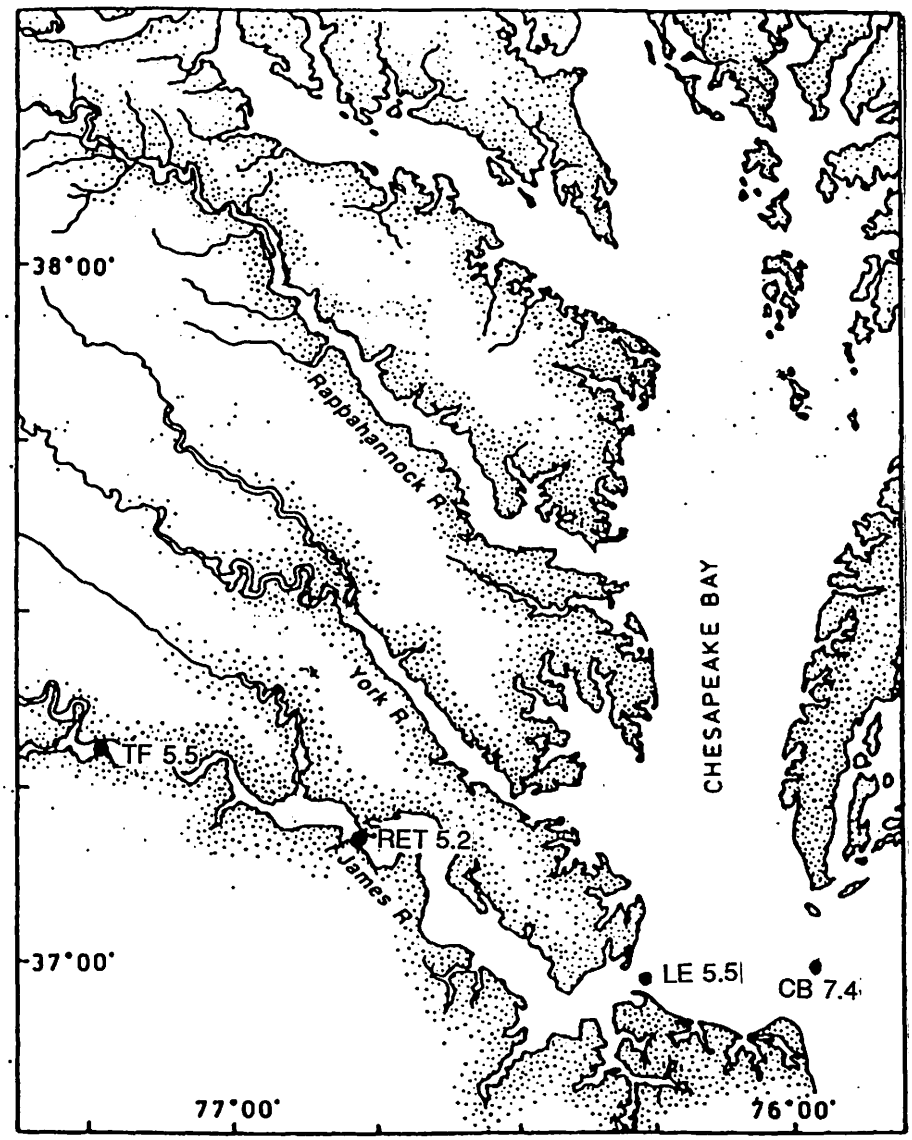

38°00'
Rappahannock R.
York R.
James R.
CHESAPEAKE BAY
TF 5.5
RET 5.2
LE 5.5
CB 7.4
37°00'
77°00'
76°00'

FIGURE 2

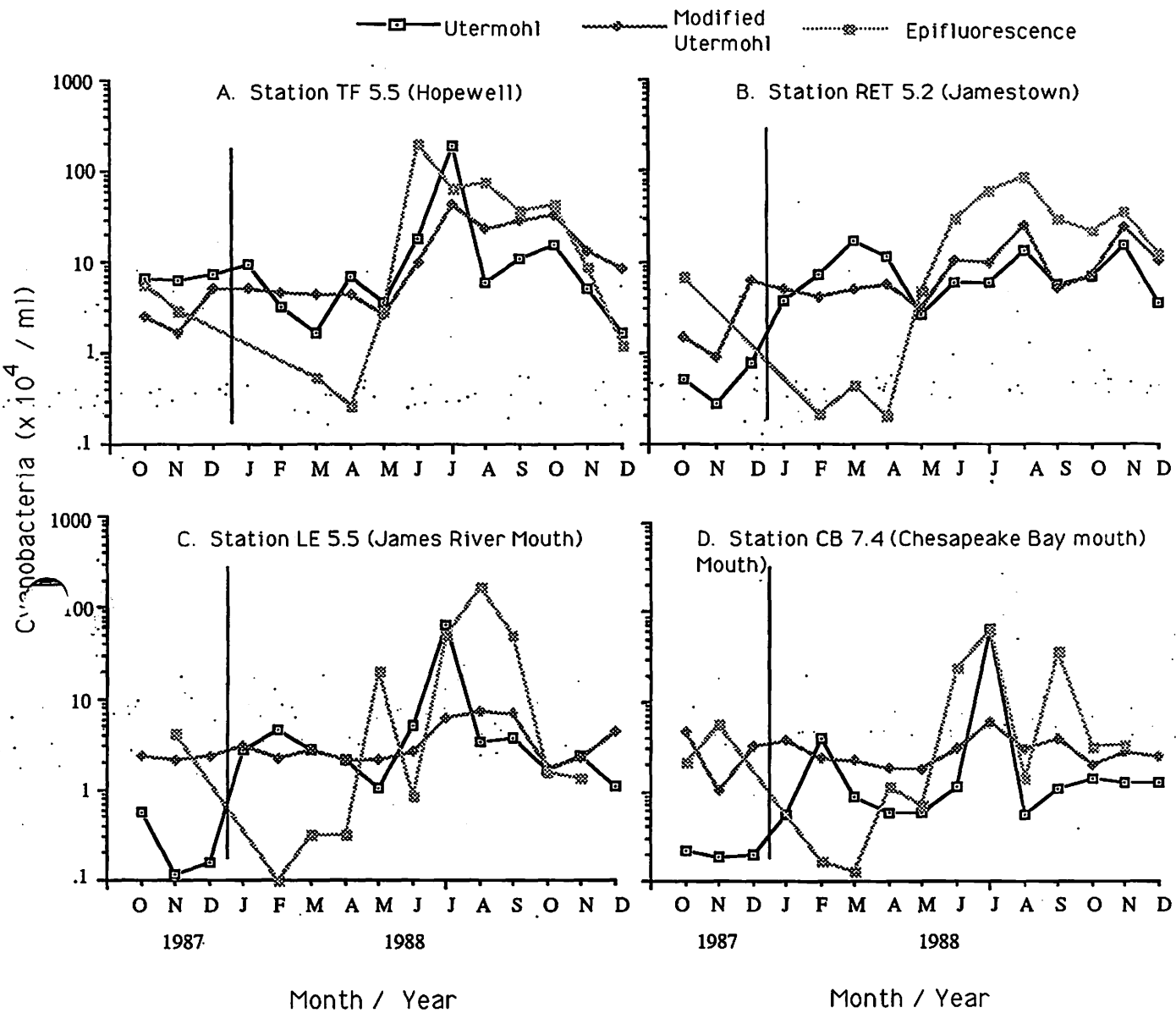

Utermohl
Modified Utermohl
Epifluorescence
A. Station TF 5.5 (Hopewell)
B. Station RET 5.2 (Jamestown)
C. Station LE 5.5 (James River Mouth)
D. Station CB 7.4 (Chesapeake Bay mouth)
Cyanobacteria (x 10^4 / ml)
1000
100
10
1
.1
O N D J F M A M J J A S O N D
1987
1988
Month / Year

FIGURE 3

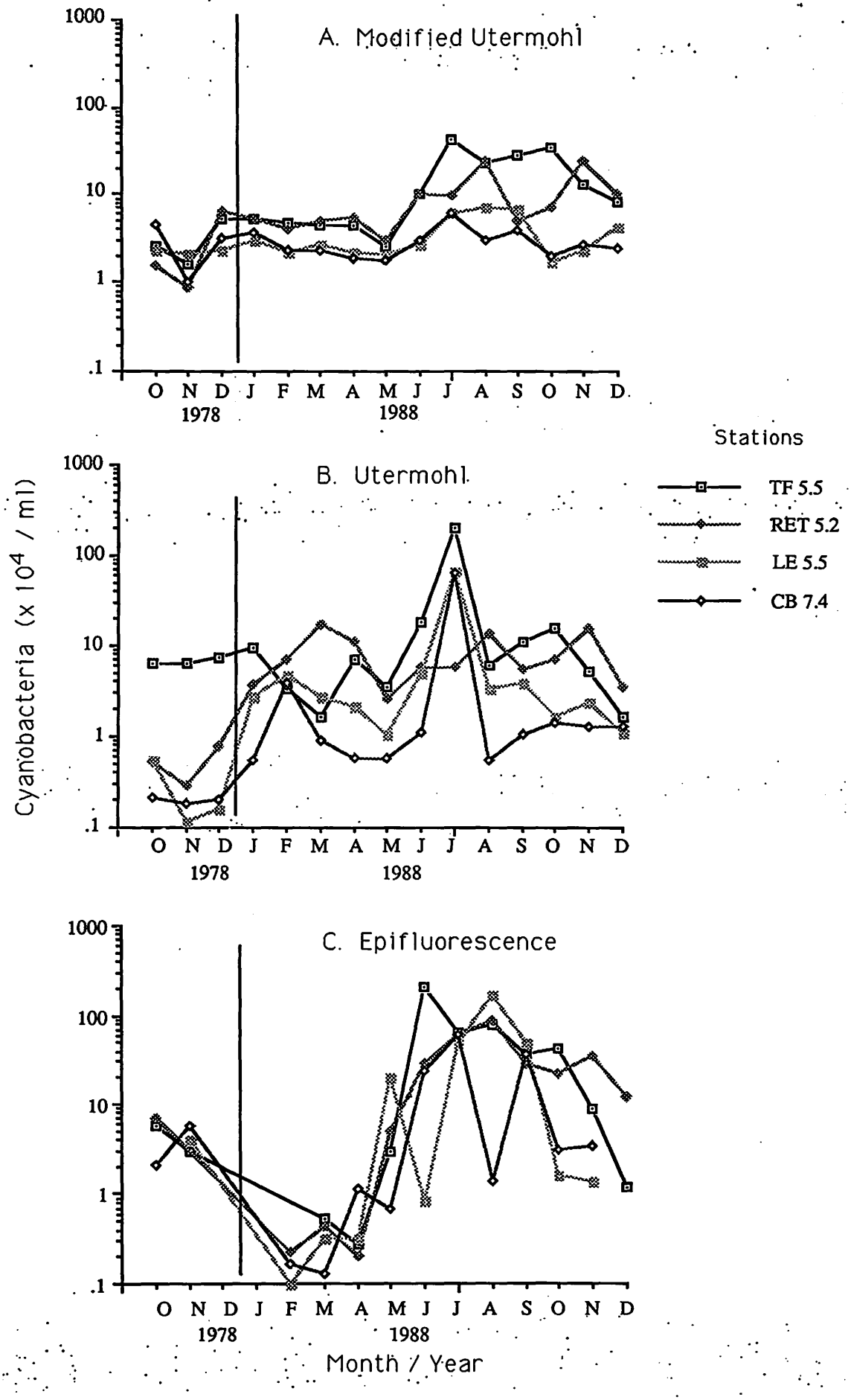
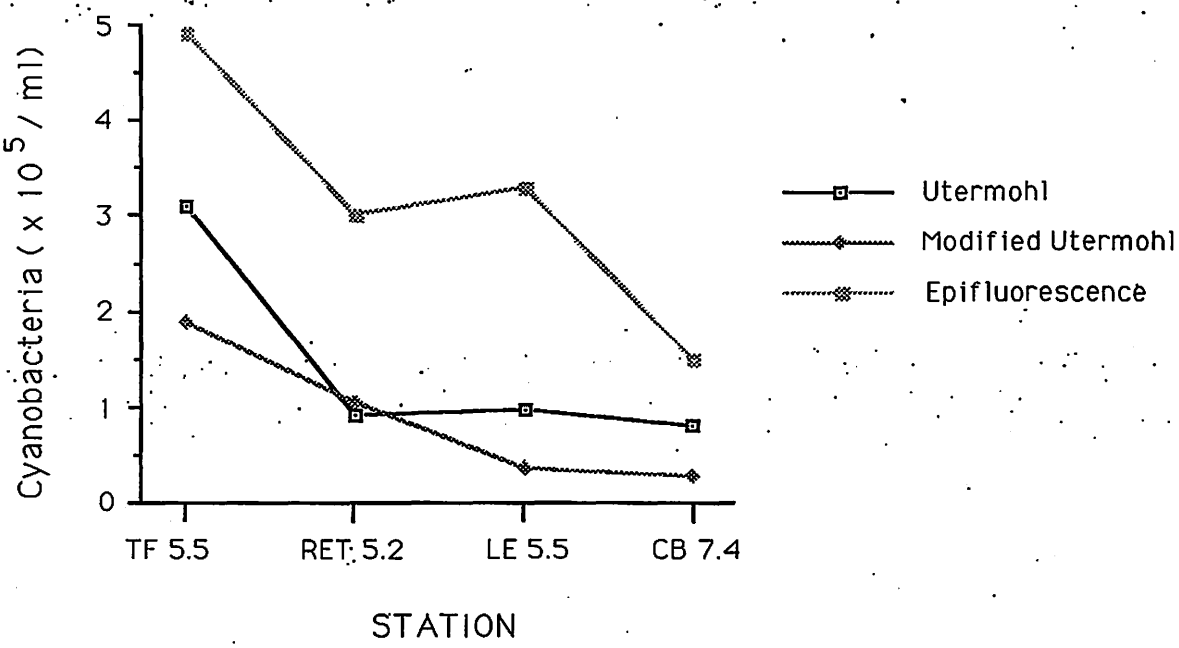

A. Modified Utermohl
B. Utermohl
C. Epifluorescence
Cyanobacteria (x 10⁴ / ml)
1000
100
10
1
.1
O N D J F M A M J J A S O N D
1978
1988
Month / Year
Stations
TF 5.5
RET 5.2
LE 5.5
CB 7.4

FIGURE 4


Cyanobacteria (x 10 5 / ml)
5
4
3
2
1
0
TF 5.5
RET 5.2
LE 5.5
CB 7.4
STATION
Utermohl
Modified Utermohl
Epifluorescence