

Phytoplankton Responses to Localized Climate Patterns in the Potomac River Tributary of the Chesapeake Bay

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Abstract

Climate change and its effects are increasingly documented worldwide. In the mid-Atlantic region there has been an upward air temperature trend in the last several years. In the Chesapeake Bay (hereafter referred to as the Bay), monitoring data has demonstrated distinct and sustained increases in sea surface temperature in some locations. The effect that heightened temperatures will have on the dynamic Bay with its distinct salinity, density, and oxygen gradients and its diverse inhabitants remains to be seen, but is part of a growing body of research. In this project, potential effects of localized temperature changes within the Bay on its phytoplankton communities are explored. The states of Maryland and Virginia, in cooperation with the Chesapeake Bay Program (CBP), have monitored phytoplankton populations since 1984 in the Bay. These long term data lend the ability to determine trends in the Bay over time, and were used in this study to find trends in water temperatures over time as well as chlorophyll *a* and primary productivity over time and with temperature. Current literature indicates an increasing temperature trend in Bay water temperatures as well as increasing trends of phytoplankton biomass; however a firm connection between the two has yet to be established. The connections of temperature and phytoplankton biomass, primary production and community composition are also yet to be explored. This project is the first known effort to incorporate phytoplankton dynamics and Bay temperature trends to determine if there is a direct association which can be attributed to climate change. For the three sampling stations used in the Potomac River, water temperatures above the pycnocline were found to have no net increase over time. There is reason to believe that this is inconsistent with trends for surface temperatures and the Bay as a whole. Chlorophyll *a* and primary productivity demonstrated weak trends with temperature and over time, yet it was apparent other factors are responsible for this variability, such as stream flow. Future studies are needed to determine precise effects of a warming climate on phytoplankton biomass, productivity, and community composition. These effects should be considered in management of the Bay.

Introduction

Background

Eutrophication of the Chesapeake Bay (Bay) and subsequent increases in phytoplankton biomass have long been an area of study and a topic of conversation among residents of the Bay watershed. Traditionally, the growth of phytoplankton in the Bay has been strongly linked with freshwater input from its tributaries (Adolf 2005, Jordan et al. 1991, Kemp et al. 2005, Mulford 1972), however possible other factors are equally responsible, including temperature variability. It has been shown that climate variability can have multiple impacts on marine ecosystems

(Stenseth et al. 2002) including changing the range of temperatures experienced by phytoplankton.

Phytoplankton in the Bay

Phytoplankton dynamics in the Bay have been studied extensively for decades. Mulford (1972) presented a listing of 36 species of Bay phytoplankton including their known distribution, abundance, and seasonal characteristics. At that time, Mulford postulated that the role these organisms play in an ecosystem is directly related to seasonal physicochemical factors (e.g., temperature, salinity). He also postulated that phytoplankton may undergo intra-species morphological changes in response to changes in environmental stresses. However, it was also noted that direct effects (e.g., increased sedimentation and nutrient loading) resulting from human activities in the watershed may also have irreversible effects on the Bay's phytoplankton communities.

Other studies have used techniques to look even farther into the past. Kemp et al. (2005) reported that signs of increased phytoplankton biomass appeared as early as 100 years ago. More recently however, significant increases in chlorophyll *a* surface concentrations (a photosynthetic pigment used as a surrogate for phytoplankton biomass) have taken place in the last 40 to 50 years (Harding and Perry 2005, Kemp et al. 2005). Polyhaline (highly brackish waters with a salinity range of 18-30 PSU) regions of the Bay have shown the largest changes in this time period, increasing 5 to 13 fold. In addition, through a comparison of time-series plots from 1950 to 2000, Kemp et al. (2005) presented an increasing trend in chlorophyll *a* concentrations in the mesohaline (moderately brackish waters with a salinity range of 5-18 PSU) Patuxent River in association with trends of increased fall-line nitrogen loading, decreasing

dissolved oxygen, declining water clarity, and the disappearance of submerged aquatic vegetation (SAV) in the same area.

In contrast, other studies have failed to find temporal trends in chlorophyll *a* concentrations. Adolf et al. (2005) and Jordan et al. (1991) both conducted multi-year studies of phytoplankton concentrations. In the former, a six year study was conducted throughout the Bay and it was found that Susquehanna River flow was the single most important factor affecting chlorophyll *a* and primary productivity measurements. No trend regarding phytoplankton was observed other than what could be explained by Susquehanna flow. Jordan et al.'s (1991) study took place over 18 years, but only involved study sites within the Rhode River subestuary. Contrary to most other studies of the region, they found no clear evidence of increasing eutrophication along with the determination that chlorophyll *a* concentrations did not demonstrate any long-term trends.

Harding and Perry (1997) devised a model with the intent of resolving a long-term trend of increasing chlorophyll *a* from the variability of freshwater flow in the Bay. Through their modeling they determined that a significant increase in chlorophyll *a* had occurred in the Chesapeake Bay since 1950, and that this increase was not accounted for by freshwater flow variability or any of the other variables tested.

Taken together, these studies demonstrate that there is uncertainty as to whether chlorophyll *a* levels are increasing over time, or if they are influenced by temperature changes. As such, this topic warrants further investigation in additional areas of the Chesapeake Bay.

Community Composition of Bay Phytoplankton

The Maryland Department of Natural Resources (MD DNR) reports that waters in the Bay sustain some of the highest phytoplankton biomasses and growth rates observed in estuaries

worldwide (MD DNR 1987). Therefore, community composition and its changes over time are important to understand since each species would have unique biomass and growth rates. In 1972, Mulford determined that 6% of the Bay phytoplankton species accounted for the majority of the biomass throughout the Bay (excluding nanoplankton since it was difficult to identify and preserve these species at that time), and that most of this makeup consisted of diatoms and dinoflagellates. Diatoms are most abundant in the fall, winter, and spring when temperatures are below 20°C, and dinoflagellates became most abundant when temperatures exceeded 20°C. The most abundant species observed during Mulford's study were *Skeletonema costatum*, *Cerataulina pelagica* var. *elongate*, *Asteronella japonica*, and *Rhizosolenia calcar avis*, all diatom species. Patten et al. (1963) found similar trends in the lower Bay with diatoms being dominant in winter and flagellates more significant in warm periods. The dominant organisms found at that time were *S.a costatum* (also found by Mulford 1972), *Chaetoceros affinis*, *C. compressus*, *Cerataulina bergonii*, *Chilomonas* sp., *Massartia rotundata*, *Peridinium triquetrum*, *Gyrodinium* sp., *Gymnodinium* sp. *Pyramimonas* sp. and *Cryptomonas* sp. With the exception of *S. costatum* and two different species of *Cerataulina*, this assemblage of dominant organisms differed from Mulford's study 10 years later. While Mulford (1972) indicated diatoms were the major species, Patten et al (1963) placed equal significance on diatoms and flagellates, noting flagellates were slightly more important in the York River and diatoms more important in the open lower Bay.

In addition to the diatom/dinoflagellate seasonal pattern, others have been reported as well. Silicoflagellates were found to occur primarily in the spring, although their contribution to overall biomass was negligible. Nanoplankton (those too small to collect in a plankton net) were found to be most abundant in the summer. This category would also include blue-green algal

forms which were noted as becoming more abundant in upper Bay tributaries and were common components of spring, summer, and fall assemblages at that time. (Mulford 1972)

Spatial patterns in phytoplankton species composition are also found throughout the Bay. Mean phytoplankton species diversity was found to increase in the seaward direction (Mulford 1972 and Patten 1962). Patten et al. (1963) found that highest mean population size and diversity occurred on the western side of the estuary, which receives significantly greater freshwater delivery than the eastern side of the estuary. However, Patten et al. (1963) were not able to relate nutrient loading to patterns in phytoplankton abundance or diversity.

Trends in phytoplankton community structure have also been reported over time. Sediment stratigraphy records have helped to determine that diatom diversity has steadily decreased since 1760, and that the ratio of centric diatoms (generally planktonic forms) to pennate diatoms (benthic or littoral forms) has increased (Cooper and Brush 1993). This indicates that over time, the Bay has changed from a system dominated by benthic diatoms to smaller free-floating planktonic diatoms. (Brush 2001)

More recent surveys of community composition have found that nanoplankton (microflagellates, small diatoms and cyanobacteria) are responsible for most of the biomass and primary productivity in the Bay, with aperiodic blooms of larger diatoms and dinoflagellates. Small, coccoid cells are predicted to be the largest fraction of cells within the nanoplankton group, likely cyanobacterial forms. (Sellner 1987) Marshall and Lacouture (1986) found that major seasonal growth periods in the summer were dominated by diatomaceous flora and a pico-nanoplankton complex composed mainly of cyanobacteria, chlorophytes and other small cells. This reemphasizes the assertion that diatoms are dominant in the system, but also highlights how cyanobacteria have become an increasingly important component of the community over time.

Dominant diatom species included *S. costatum*, *Leptocylindrus danicus*, and *Asterionella glacialis*. Marshall and Lacouture (1986) concluded *S. costatum* has remained the dominant species in the system, but a more abundant and broader base of small, chain-forming diatoms have become established and that high concentrations of pico-nanoplankton, chrysophyceans and cryptophyceans are also abundant.

Global Climate Change as experienced in the Chesapeake Region

Much of the understanding of the Bay's long-term historical climate has resulted from the ability to interpret sediment cores. Cronin et al. (2002) analyzed cores collected throughout the Bay to determine sea surface temperature (SST) dating back to 200 BC using a magnesium/calcium proxy method as a paleothermometer (from Chivas et al. 1986) and a combination of an age depth model, pollen biostratigraphy, lead-210, and cesium 137 dating . They found dramatic temperature shifts on a centennial-scale related to the North Atlantic Thermohaline Circulation as well as multi-decadal temperature maxima corresponding with the North Atlantic Oscillation. Temperature extremes experienced in the late 19th and 20th centuries however, were found to exceed any experienced in the previous 2000 years, by at least 2-3°C over the mean. This suggests recent anomalous behavior of the climate system. (Cronin et al. 2002)

SST has been increasing during the last century in the Chesapeake Bay region. Preston (2004) studied historical monitoring data of Bay water temperatures both at the surface and in the subsurface (over 15 meters) from 1949-2002. Warming trends for both layers were found: +0.16°C at the surface, and +0.21°C in the subsurface per decade since 1949. This culminates in a total increase of 0.8 to 1.1°C within 53 years. Had Preston's analysis extended into 2005, he

would have been able to incorporate the warmest Bay temperatures ever recorded in the analysis (Chesapeake Bay Program 2007).

Predictions for future temperatures in the region forecast more of the same. As reported in Moore et al. (1997), four global circulation models predicted 3-5°C increases in the New England / Mid-Atlantic region under future conditions of doubled CO₂ concentrations. There is also a predicted shift to generally drier conditions resulting from greater evapotranspiration that suggests a decrease in stream flow values. (Moore et al. 1997). Alternatively, other projections show almost the opposite: rising sea level, heightened temperatures, and increased stream flow (Najjar et al. 2000).

From the literature, it is apparent that concurrent upward trends of both Bay water temperatures and phytoplankton biomass (as measured by chlorophyll *a* concentration) have occurred in the last century, most notably in the last 50 years. While two sources did not observe increasing trends in Bay phytoplankton (Adolf et al. 2005 and Jordan et al. 1991), this discrepancy may be explained. The study by Adolf et al. (2005) only took place over a six year time span. It may be hard to divulge a pattern of increasing biomass in this short of a time period, especially in the shadow of the major effects brought on by Susquehanna River flow. While Jordan et al.'s (1991) research spanned 18 years, only the Rhode River subestuary was tested, which may not be indicative of the Chesapeake as a whole. This is consistent with observations that chlorophyll *a* levels have actually decreased in the upper oligohaline region of the Bay since the 1970's; whereas all other regions showed an increase (Kemp et al. 2005).

Phytoplankton and Temperature

Increased temperatures are often linked with enhanced metabolic rates (Shiah and Ducklow 1994 and Thompson et al. 1992), and a reasonable consideration is that increased water

temperatures will spur increased growth and primary production in phytoplankton species. In addition, species are likely to react differently to changes in environmental conditions, such as temperature thus leading to potential shifts in community composition. Thompson et al. (1992) studied the responses of eight species of marine phytoplankton to temperatures ranging from 10 to 25 degrees C. All eight species were found to have increasing growth rates correlating exponentially with increases in temperature. Chlorophyll *a* concentrations were also found to increase with temperature for all species, and the carbon:chlorophyll *a* ratio decreased with temperature for all species. Because of this, they postulated that a species ability to modify its carbon:chlorophyll *a* ratio increases its ability to regulate its ecological niche. Given that different phytoplankton species will differ in this ability, community composition is likely to be affected. Other studies have indicated that while phytoplankton division rates may increase within defined limits of temperature increase, growth and production dynamics are much more complicated (Goldman 1977, Eppley 1972, Jorgenson 1968). Eppley (1972) developed an equation to calculate the maximum growth rate of phytoplankton, which increased with temperature up to a temperature maximum. This equation, called the Eppley curve, has been improved upon over time (Bissinger et al. 2008), but the temperature/growth rate pattern remains similar.

Linking the Two: Chesapeake Literature

Research on global scale climate patterns and its impact on phytoplankton in the Bay is still in its infancy. Information can be drawn from work that has highlighted climate forcing effects on spring blooms (Miller and Harding 2007) and mesozooplankton dynamics (Kimmel et al. 2006). Spring phytoplankton blooms were found to reach peak biomass farther seaward in the estuary, achieve greater magnitude, occur later in the spring than previously observed, and

were present over a larger area during years with more frequent warm / wet weather patterns as opposed to years with predominately cool / dry weather patterns (Miller and Harding 2007).

These findings demonstrate the strong connection between phytoplankton dynamics and weather patterns, and specifically that warmer temperatures appear to result in phytoplankton blooms of a larger magnitude. Related weather connections were also found with mesozooplankton in the Bay, but varied by species (Kimmel et al. 2006). It is likely that phytoplankton species behave differently in response to weather patterns, however this is hard to determine, since most research focuses on biomass as a whole.

Lomas et al. (2002) looked specifically at relationships between various “microbial” processes in the Bay and regional warming. In their analysis they used the Q_{10} index, which represents a change in the rate of a biological process when the temperature is raised by 10°C. Values over 1.0 indicate a faster biological process rate at heightened temperatures until a threshold is reached where increasing temperatures have a deleterious effect. Q_{10} values for phytoplankton biomass in the Bay (as measured by chlorophyll *a*) were slightly less than 1.0 for a Bay wide average, indicating no net biomass increase due to temperature. However, a linear relationship existed between euphotic zone (region where light intensity is greater than 1% of intensity at water surface) chlorophyll *a* and increasing temperatures in the mid-Bay regions during 1989 and 1990, signifying Bay wide averages might not be presenting the entire story. While an overall temperature / phytoplankton biomass relationship was not found, Q_{10} values for primary productivity, photosynthetic rate, and phytoplankton nitrogen uptake (in the southern Bay) were all well over 1.0. This suggests that phytoplankton primary productivity maybe a better indicator for gauging ecological change from climate effects.

Other Estuaries and Marine Systems

While each estuarine system has unique sets of physical, chemical, and biological properties, research on climatic effects upon other systems may provide more insight on Bay processes. Phytoplankton dynamics in the San Francisco Bay area have been well studied. James E. Cloern, a well-known phytoplankton and estuarine expert in the San Francisco area, noted that natural cycles of bloom variability are being altered on a global scale by human activities (Cloern 1996).

Climate variations have been strongly linked with phytoplankton community bio-volume distributions (Lehman 2000). Lehman postulated that this linkage demonstrated the connection between long-term, large-scale global change processes and regional estuarine phytoplankton production. Lehman's study also demonstrated wide variability in the responses of different species to climatically-related environmental variables. This again suggests studying biomass in general may not be sufficient. (Lehman 2000) Anneville et al.'s (2005) study demonstrated that in European lakes, major changes in phytoplankton community composition were found to have occurred over time and were parallel with major shifts in climactic patterns. Phytoplankton blooms have also been found to be more intense and initiate earlier in the season in response to increased water temperatures independent of phosphorous concentrations (Huber et al. 2008).

In addition to annual and seasonal patterns, climate has also been shown to have an effect on exceptional bloom events, such as red tides. In 2004, a dinoflagellate bloom larger than any experienced in the previous three decades occurred in the San Francisco Bay. The bloom coincided with a string of record high air temperatures and calm winds which stratified the water column. This event was evidence that bloom events are responses to changes in local physical dynamics which are driven by large-scale atmospheric processes. (Cloern et al. 2005)

Other estuarine systems have not been studied for climate-driven phytoplankton biomass directly, but some have explored related factors influencing phytoplankton growth. In the Hudson River Estuary, rates of gross primary production increased between the 1970's and 1990's. It was determined this increase was not due to heightened nutrient loading since both inorganic nitrogen and phosphorous concentrations were already high and non-point sources had changed relatively little over this time span whereas point sources may have decreased slightly. Instead, heightened gross primary production was correlated with lower discharge, longer water residence time, increased stratification, and a larger euphotic zone. This shows that climatic variation could have significant effects on the primary production of the estuary. (Howarth et al. 2000) The scenario in the Hudson River Estuary is quite different from the paradigm for the Bay (Hagy 2004) and northern Gulf of Mexico (Justic et al 1996) which states that decreased river flow will result in decreased primary production. It is predicted by general circulation models that freshwater discharge from the Mississippi River into the Gulf of Mexico will increase by 20% if atmospheric CO₂ doubles. Justic et al. (1996) believe that with the increase in discharge and the concentration of limiting nutrients will become at least one order of magnitude higher in the River than in the receiving Gulf, thus enhancing the growth of phytoplankton in the northern region. This may have profound effects on the balance of the salt wedge estuary at the Mississippi's mouth.

Predictions for the Future

With predictions of warmer temperatures and decreased river flow, the future climate of the Chesapeake region may look quite different than that of today. What this means for phytoplankton dynamics and subsequent heightened hypoxia in the Bay is uncertain. It is possible that the forecasted decreases in stream flow and snowpack in the Chesapeake watershed

may result in more nitrogen retention, effectively limiting phytoplankton growth in the future (Moore et al. 1997). Of course if Najjar et al.'s (2000) prediction comes to fruition, the increased stream flow may lead to continued increases in phytoplankton biomass and productivity.

Project scope

While individual trends of increasing phytoplankton biomass and increasing water temperatures related to climate change in the Bay have been documented, the connection between the two is a new area of study. Examining individual trends and available literature from this and other estuarine systems provides clue to the relationship; however research specific to the Bay is needed. In this study I will utilize long-term datasets from the Chesapeake Bay Monitoring Program to attempt to show that water temperatures in the Potomac River basin are increasing over time, indicative of a climate warming pattern. Next, I will look at chlorophyll *a* concentration and primary productivity rates compared against temperature over time to determine if they are associated with warming. Community composition will also be explored.

As the second largest tributary to the Bay, the Potomac River is important to study on its own. However, it may also be regarded as a smaller-scale model of the Bay owing to several similarities. Hamdan and Jonas (2006) point out that like the Bay, the Potomac has a marked salinity gradient (consisting of non-tidal, tidal fresh, oligohaline and mesohaline zones), its watershed and banks have multiple use patterns its basin morphology is similar (deep channel flanked by shallow shores), and like the Bay it also experiences seasonal hypoxia and anoxia in the mesohaline region. I would also add that it is appropriate for this particular study because it lies approximately mid-Bay latitudinally and therefore represents an average climate from upper and lower Bay regions.

Methods

Site Selection

The tidal Potomac River (below the fall line) was chosen as a study area. This was done to simplify the amount of data evaluated within the time constraints of the project, and also to limit interfering signals from spatial variation in climate and salinity throughout the Bay. In addition, it was hoped that the Potomac basin would act as a smaller-scale model for the entire Bay, as described previously.

Although many monitoring sites exist in the Potomac basin (Attachment 1: Monitoring Map of the Chesapeake Bay), only four of sites are sampled for phytoplankton: LE2.2 (formerly MLE2.2), RET2.2 (formerly XDA1177), TF2.3 (formerly XEA6596), and TF2.4 (Attachment 2: Phytoplankton Monitoring Stations). Each site is physically located over the main channel of the river. LE2.2 is located in the mesohaline Potomac approximately 22 km northwest of the river mouth, where it meets the Chesapeake Bay proper. RET2.2 is located in the oligohaline (low salinity waters with a range of 0.5-5 PSU) Potomac approximately 80 km from the river mouth. Both TF2.3 and TF2.4 are located in the tidal fresh (mostly fresh water ranging from 0-0.5 PSU that is still tidally influenced) Potomac approximately 118 and 110 km from the river mouth. If all four of these sites were used in this project, there would be a disproportionate focus on shallow upstream portions of the river. For this reason the middle of the upstream sites, TF2.4, was eliminated from data analysis. Site locations are illustrated in Attachment 3, and photos of the two upstream sites are shown in Attachment 4.

Data Extraction

The primary data sets used in this study were extracted from the Chesapeake Bay Program's (CBP) Data Hub portal (<http://www.chesapeakebay.net/data/index.htm>). The site

serves as a central interface for many individual databases housing years of monitoring data related to the Bay dating as far back as 1949, although most of the datasets commenced in 1984 when the Chesapeake Bay Monitoring Program began. The following databases were used: the CBP Water Quality Database (water temperature, primary productivity, and chlorophyll *a* parameters), the Baywide CBP Plankton Database (community composition and a second chlorophyll *a* parameter), and the USGS Monthly Stream Flow Data Set (stream flow values). A secondary dataset for pycnocline depth was needed to standardize temperature and chlorophyll data sets, and will be discussed in more detail in the results section. This data was acquired directly from Dr. Richard Lacouture at the Morgan State University (MSU) Estuarine Research Center, who was the lead on collecting the field data.

CBP Water Quality data sampling was conducted by the MD DNR. Starting in 1984 phytoplankton data was collected on a separate boat by the Academy of Natural Sciences at the Benedict Estuarine Research Laboratory. In September, 2004 the plankton monitoring program was transferred to the Morgan State University Estuarine Research Center, although many of the same researchers remained. Phytoplankton sampling typically occurs 13 times throughout the year with monthly samples taken in March, June, September, October, and December. Twice monthly samples are taken in April, May, July, and August. In 2005, the phytoplankton monitoring program lost their radiological permit which allowed them to attain primary productivity data, resulting in a data gap for this year.

The time span of the extracted data was determined by the CBP Plankton Database, which had been sampled for the shortest amount of time compared to the other databases. Therefore, the sampling period considered in this paper will span from August of 1984 to June 2007.

Sample Cruise

On April 14, 2008 I participated on one of the two April field sampling trips for the upper Potomac with Stella Sellner and J. Howard Hixson from the MSU phytoplankton monitoring team. This visit enabled me to experience how samples were collected, get a better picture of the entire sampling program, and witness interactions between two sampling agencies (MSU and MD DNR). The MSU cruise launched from Smallwood State Park in Maryland which is approximately 1 KM north east of site TF 2.3. Sites RET2.2 and TF2.3 were sampled. Water conditions on the ride down to RET2.2 (approximately 45 minute duration) were choppy and the MSU team was doubtful the MDDNR boat, which typically samples water quality parameters simultaneously, would be out. Upon arriving at RET2.2, the MD DNR boat was present and provided the MSU team with their water quality data (e.g., temperature, salinity, dissolved oxygen). For phytoplankton sampling, we navigated around the sample area until a depth of 30 feet or greater was found (33ft in this instance). Ten sample depths are used in collection; in this case depths were set at 0.5, 1, 2, 3, 5, 6, 7, 8, 9, and 10 meters. One end of a weighted hose connected to a deck impeller pump was lowered to each depth so that water from each could be brought up for collection. At each depth, the pump was run for 30 seconds prior to collection to flush out water from previous depths. Samples at each depth were collected and homogenized in large (25 L) carboys and used as field composite samples for primary productivity measurements. One composite sample included depths from the top half of the water column) and a separate composite sample was collected for the bottom. One small Nalgene bottle was filled with water from the top water column composite for species identification. Smaller individual samples were collected for each depth in one liter Nalgene bottles to measure chlorophyll *a* concentrations. Bottles were stored in a cooler filled with ambient Potomac River

water to maintain sample temperature and sample integrity for live productivity measurements. All three parameters, chlorophyll *a*, primary productivity, and community composition would be analyzed later that day in the laboratory. Light attenuation was measured following the sample using a Licor PUV meter. At RET2.2, light dissolution was measured at 1.5m, while the DNR Secchi depth was 0.2m. At TF2.3, an identical procedure was followed. At this site, the water was less turbid and the light dissolution depth was at 3m. The MDDNR boat had not reached this site yet and therefore no Secchi depth was available.

Data Formatting

All data obtained from the CBP Data Hub were downloaded as text files in five year increments. Data was collected individually for each parameter (temperature, chlorophyll *a*, primary productivity, and species composition). With each, 5 year files were converted to Microsoft Excel spreadsheet files and manually merged to create single sheets for 1984-2007. Each sheet contained data for all three stations. Sheets were sorted primarily by station, then by sample date, then depth to create functional rows of data. All data, across parameters were collected essentially simultaneously with the exception of some samples which were taken within two days of one another. Rare instances also occurred where data was not taken within two days of one another. In these cases the data was excluded from further analysis. Irrelevant columns were discarded as were rows containing incomplete data. Data were further stratified by season (Spring: March 20-June 19, Summer: June 20-September 21, Fall: September 22-December 20, Winter: December 21-March 19) to normalize trend analyses from year to year. The result of the effort was a complete data matrix which lends itself to trend analyses (see Attachment 6).

Phytoplankton data originated from the Baywide CBP Plankton Database. The Phytoplankton and Zooplankton Monitoring Database: Version 2.0, Database Design and Data Dictionary (1998), the 2000 User's Guide to Chesapeake Bay Program Biological and Living Resources Monitoring Data, and the Chesapeake Bay Program Phytoplankton Monitoring Survey Data Dictionary were used as guides in understanding the data sets. Phytoplankton community composition, primary productivity and chlorophyll *a* concentrations were obtained from this database. Personal communication with CBP contractors (Lacouture and Sellner) also greatly assisted with understanding the data.

Chlorophyll *a* concentrations in the CBP Water Quality Database were recorded in μL^{-1} at varying depth intervals (both between sample sites and at one station over time) for each station. Temperature data (also from the CBP Water Quality Database, and reported in degrees Centigrade) were recorded similarly; however primary productivity (MSU) and community composition (MSU) were recorded as field composites for depths above the pycnocline. This presented a problem for making direct comparisons between parameters. Because it is not possible to separate surface depths from a field composite, all discrete data (temperature and chlorophyll *a*) were standardized to represent the area above the pycnocline. Pycnocline depths (obtained from MSU) were compared to chlorophyll *a* (CBP) and temperature data (CBP). Data from depths between 0.5 m below the surface to 0.5 m of the recorded pycnocline depth were mathematically averaged for each row of data. If more than one data replicate was present at one depth point, one replicate was chosen at random so that the resulting average was not biased toward that depth. For any given CBP cruise date, either a pycnocline depth or temperature or chlorophyll *a* data may not have been available. In such cases, the remaining data were not considered in analysis.

Primary productivity data and a second set of chlorophyll *a* concentration data (found after averaging of CBP data) were taken from the MSU Phytoplankton Database. As discussed previously, primary productivity data was recorded as a field composite above the pycnocline, as was this second set of chlorophyll *a* data. The area of the water column for this composite consisted of depths from 0.5 m below the water surface to 0.5 m above the pycnocline depth. Primary productivity is recorded in units of $\mu\text{g Carbon fixed L}^{-1} \text{ h}^{-1}$ (here after $\mu\text{g C L}^{-1} \text{ h}^{-1}$). Two replicates of an above pycnocline field composite were recorded and averaged. The second set of chlorophyll *a* data differed slightly from values taken from the DNR samples. This dataset was more complete than the MSU data and also reported in μL^{-1} . As with primary productivity values, two above pycnocline replicates were recorded for each sample date. Replicates for primary productivity and this second set of chlorophyll *a* data were mathematically averaged. Because this set of chlorophyll data had replicates (MSU), it was considered a more robust data set and was used in subsequent analyses. Nevertheless, a strong degree of covariance between the data sets was evident (Pearson's correlation coefficient: 0.82; $p < 0.000$; $n = 970$).

USGS stream flow data were obtained from the Chesapeake Bay River Input Monitoring Program (URL: <http://va.water.usgs.gov/chesbay/RIMP/loads.html>). Daily stream flow data collected as total discharge (cubic feet per second) from the USGS river gauging station 01646500, located 1.2 mi upstream from Chain Bridge, Washington DC were obtained for 1985-2007. Daily Stream flow values were averaged monthly.

Community composition data was obtained as field depth composite samples. On each date, at each station, phytoplankton species data were reported as the number of each cell type per liter. Composite samples at stations LE2.2 and TF2.3 were collected as described above. Only above pycnocline data was considered in this analysis. Station RET2.2 was evaluated both

above and below the pycnocline until 1989, after which time an entire water column composite was taken. There are 999 different species codes identified in the data. Because of the large breadth of data, plotting histograms of species compositions over time was not possible. However, for station TF2.3, data had previously been displayed in this manner by the MDDNR. Histograms were prepared annually showing the dominant species makeup for each month. These plots were used in a qualitative assessment of species composition changes over time and intra-annually. In the future, we plan to quantitatively assess the species composition data using a Biota and Environmental Analysis test which would compare species compositions over time and determine the abiotic factors (e.g., temperature, salinity, water clarity) which structure community composition. (Clarke & Ainsworth 1993). The analysis generates a Spearman rank correlation coefficient (ρ), which explains the degree of covariance between compositional data and abiotic data variables, and attributes covariance to the most important abiotic variables (Clarke & Ainsworth 1993).

Statistical Methods

Microsoft Excel was used to plot trends in the data and to provide guidance on statistical analysis. Temperature, chlorophyll *a*, and primary productivity were plotted annually. Subsequently, data were subdivided according to season using astronomical divisions. Temporal trends for each data parameter were assessed using single component linear regression analysis using SPSS v. 12. Likewise, single component linear regressions were conducted individually for primary productivity, and chlorophyll regressed on temperature.

SPSS v. 12 was also used to analyze the data using the Pearson's Product-Moment Correlation. The Pearson's correlation was used to demonstrate relationships between variables which are not truly independent of each other. This analysis measures the degree of correlation

between two variables and does not imply causation as in regression analysis. Pearson's correlations were run between each of the variables, both seasonally and annually but only the correlations between time and temperature, and for chlorophyll *a* and primary productivity are displayed in the results section.

The null hypothesis for all tests is that there is no association between water temperature and phytoplankton primary productivity and abundance, or between time and these variables. The alternate hypothesis would then be that these variables are related. The null hypotheses will be considered to be rejected at p-values less than 0.05, and a relationship between variables will be considered highly significant at p-values less than 0.01. Chlorophyll *a* and primary productivity on the other hand, are expected to have a positive linear relationship, but because these parameters are not mutually independent of each other, this relationship will be explored using the Pearson's correlation. Thus, the null hypothesis for these variables is that they are significantly and positively related.

It was not possible to directly analyze community composition data statistically because of the large number of possible genera and species. Anneville et al.'s paper (2005) represented phytoplankton community composition in European Lakes in a tabular form where each genus constituted a certain percentage of the total population per year. This was not possible in this scenario, given the 999 species groups that existed. In the future I hope to categorize them by genera and analyze the most prevalent groups over time. Also, as indicated in the previous section, in the future I plan to do a Biota and Environmental Analysis test to quantitatively analyze the data. Histograms of community structure of TF2.3 from 1995 to 2007 (from MDDNR) were examined qualitatively. The major groups of species for each year were noted, along with any seasonal trends.

Results

Figure 1: Plot of average annual water temperatures

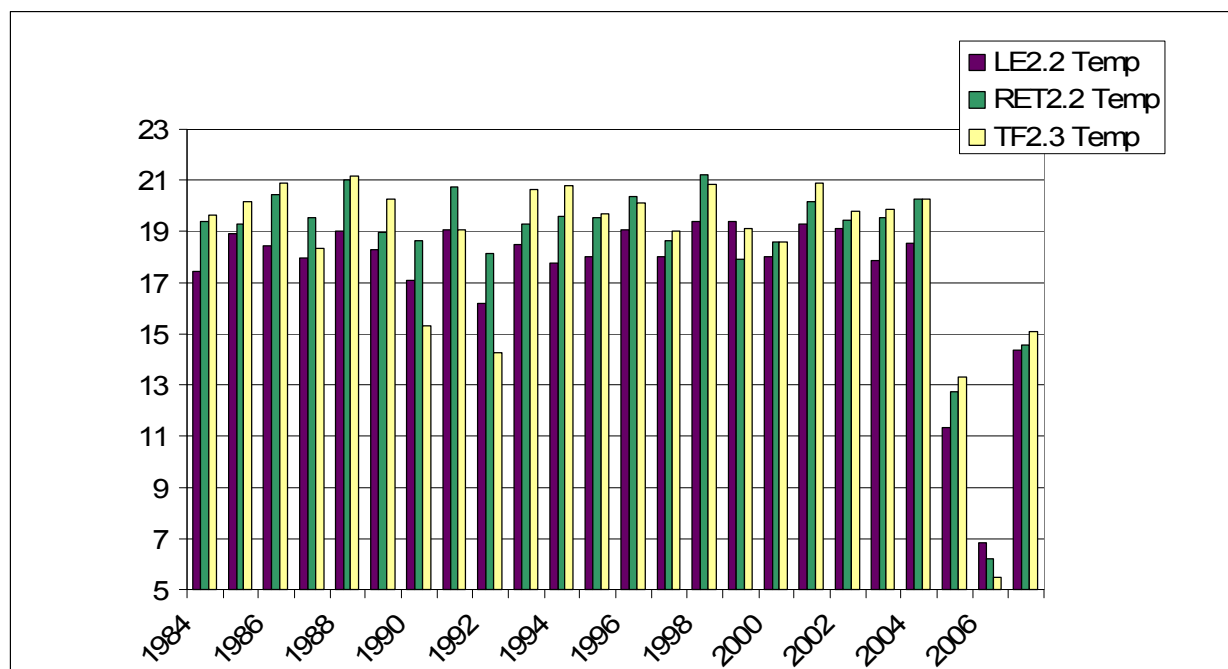


Figure 1 portrays annual averages of water temperatures (°C) above the pycnocline for each station in the 1984-2007 study period. At station LE2.2 annual average temperature ranged between 16.17°C and 19.39°C during the study period and was lowest in 1992 and highest in 1999. At station RET2.2 annual average temperature ranged between 17.94°C and 21.21°C during the study period and was lowest in 1999 and highest in 1998. At station TF2.3 annual average temperature ranged between 14.28°C and 21.17°C during the study period and was lowest in 1992 and highest in 1988. It is important to note that data in years 2005 to 2007 are incomplete, thus these years were not considered to be representative of temperatures in those years. For this reason, the three year span of data was omitted from this trend analysis. Station TF2.3 had the highest annual temperatures for 13 of the 20 year (1984 and 2004) data set. Station LE2.2 had the lowest annual temperatures for 18 of the 20 years. In 1998 and 2001 temperatures were comparatively warmer at all three stations. 1990 and 1992 were cooler years

for all three stations. The experimental averages for the three stations were 18.10 °C (LE2.2), 19.29 °C (RET2.2), and 19.17 °C (TF2.3). The comparison of mean temperature at the three stations (using a standard t-test: paired two sample for means) revealed that temperatures at both RET2.2 and TF2.3 were significantly elevated compared to LE2.2. Experimental maximum temperatures were observed in July at LE2.2 and TF2.3 and late June for RET2.2. Experimental minimum temperatures were observed in January for LE2.2 and RET2.2 and in October for TF2.3.

Figure 2: Plot of annual average chlorophyll *a* concentrations

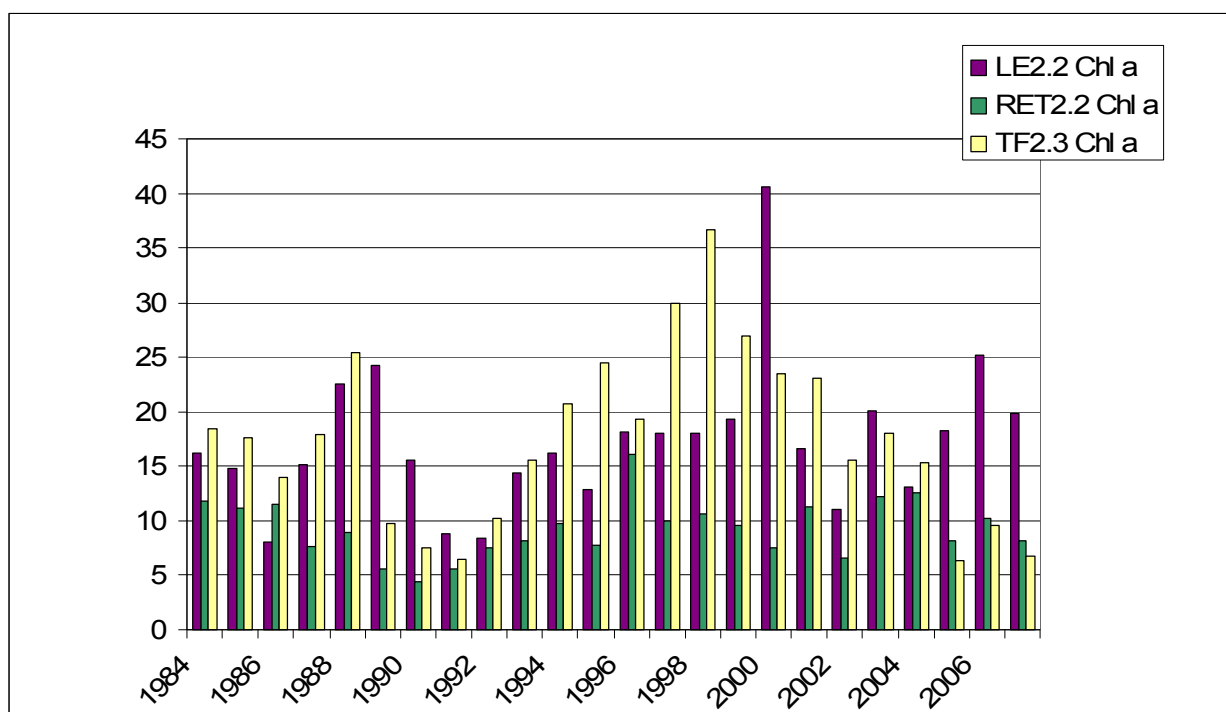


Figure 2 portrays annual averages of chlorophyll *a* concentrations ($\mu\text{g L}^{-1}$) above the pycnocline for each station in the 1984-2007 study period. At station LE2.2 annual chlorophyll *a* average concentrations ranged between $8.04 \mu\text{g L}^{-1}$ and $40.65 \mu\text{g L}^{-1}$ during the study period and were lowest in 1986 and highest in 2000. At station RET2.2 annual chlorophyll *a* average

concentrations ranged between $4.47 \mu\text{g L}^{-1}$ and $16.06 \mu\text{g L}^{-1}$ during the study period and were lowest in 1990 and highest in 1996. At station TF2.3 annual chlorophyll *a* average concentrations ranged between $6.45 \mu\text{g L}^{-1}$ and $36.68 \mu\text{g L}^{-1}$ during the study period and were lowest in 1991 and highest in 1998. Although the minimum annual average for LE 2.2 was observed during 1986, it is important to note that at each location, consistently low annual averages in chlorophyll *a* concentrations were observed during the years spanning 1989 and 1992. Following this period, the annual averages for each station generally rose until 2000. The experimental averages for the three stations were $16.44 \mu\text{g L}^{-1}$ (LE2.2), $9.07 \mu\text{g L}^{-1}$ (RET2.2), and $17.90 \mu\text{g L}^{-1}$ (TF2.3). The comparison of mean chlorophyll *a* concentrations at the three stations revealed that concentrations at LE2.2 and TF2.3 were significantly elevated compared to RET2.2.

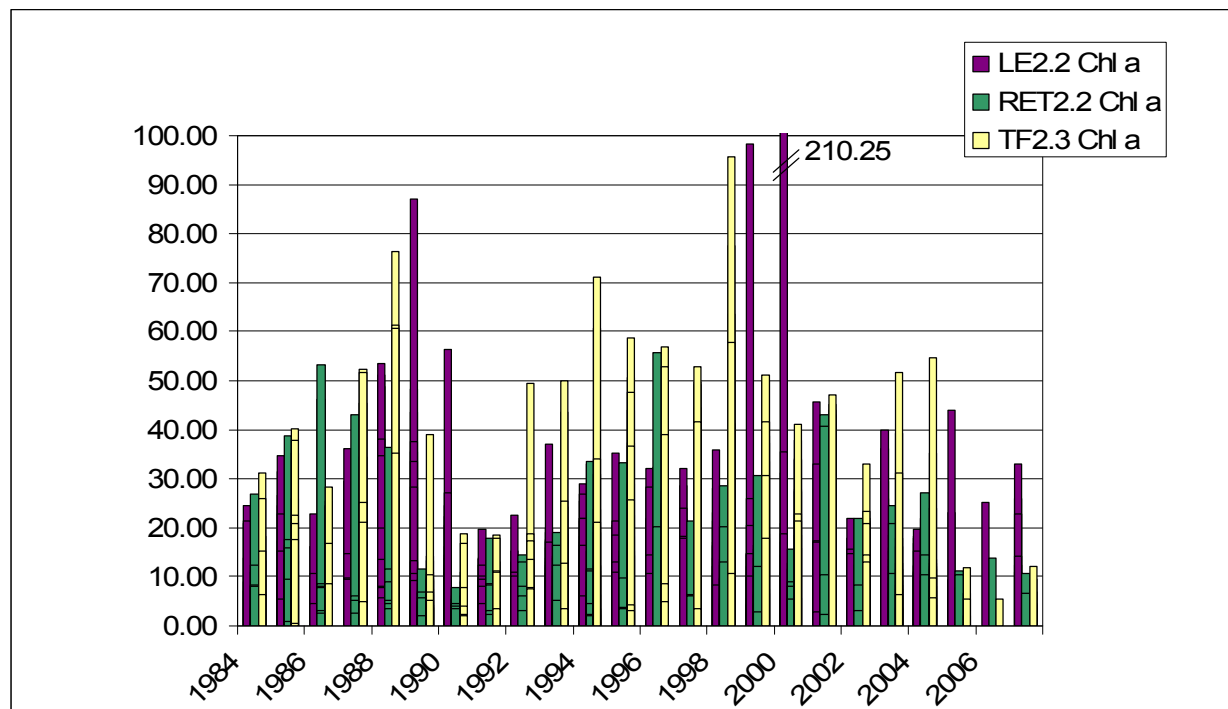
Figure 3: Plot of annual maximum chlorophyll *a* concentrations

Figure 3 shows actual chlorophyll *a* concentrations for each composite sample collected during a particular year (horizontal bars within the columns). The upper most bar in each column portrays the maximum chlorophyll *a* concentration for that year. At station LE2.2 sample chlorophyll *a* concentrations ranged between $<0.01 \mu\text{g L}^{-1}$ and $210.25 \mu\text{g L}^{-1}$ during the study period and were lowest in June 1987 and highest in May 2000. The maximum observed at LE 2.2 during that year was the highest concentration recorded at any station during the study period.

At station RET2.2 chlorophyll *a* concentrations ranged between $<0.01 \mu\text{g L}^{-1}$ and $55.80 \mu\text{g L}^{-1}$ during the study period and were lowest in May 1990 and highest in August 1996. At station TF2.3 daily chlorophyll *a* concentrations ranged between $<0.01 \mu\text{g L}^{-1}$ and $95.70 \mu\text{g L}^{-1}$ during the study period and were lowest in October 1995 and highest in September 1998. As observed for the annual averages consistently low maximums in chlorophyll *a* concentrations

were observed during the years spanning 1989 and 1992. Following this period, the annual averages for each station generally rose until 2000. There was also a steady increase in concentrations in the period between 1984 and 1989. At TF2.3 a third peak was also present surrounding 1994. Also of note is that peaks for LE2.2 seem to lag behind peak years for the two upstream stations.

Figure 4: Plot of annual average primary productivity rates

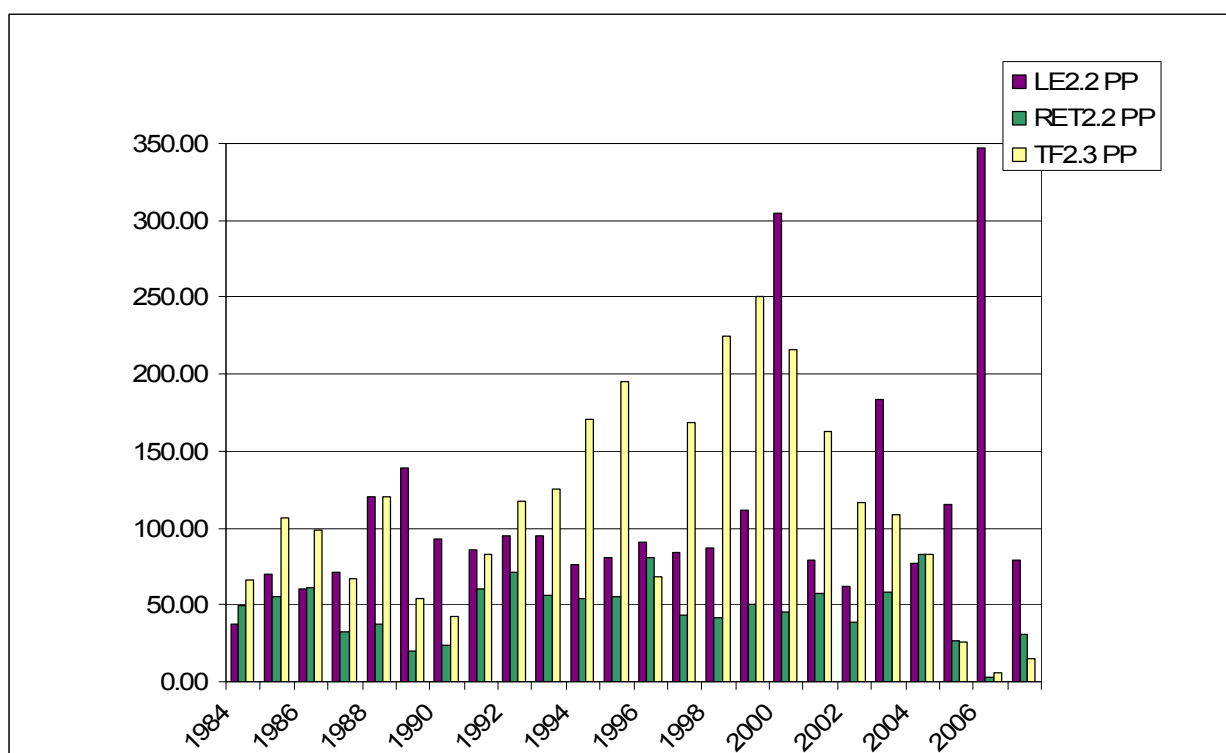


Figure 4 portrays annual averages of primary productivity rates ($\mu\text{g C L}^{-1} \text{ h}^{-1}$) above the pycnocline for each station in the 1984-2007 study period. At station LE2.2 annual primary productivity rates ranged between $37.13 \mu\text{g C L}^{-1} \text{ h}^{-1}$ and $304.51 \mu\text{g C L}^{-1} \text{ h}^{-1}$ during the study period and were lowest in 1984 and highest in 2000. The highest annual average for primary productivity coincided with the year when highest chlorophyll *a* concentrations were observed. At station RET2.2 annual primary productivity rates ranged between $20.14 \mu\text{g C L}^{-1} \text{ h}^{-1}$ and 83.03

$\mu\text{g C L}^{-1} \text{ h}^{-1}$ during the study period and were lowest in 1989 and highest in 2004. At station TF2.3 annual chlorophyll *a* concentrations ranged between $41.99 \mu\text{g C L}^{-1} \text{ h}^{-1}$ and $249.93 \mu\text{g C L}^{-1} \text{ h}^{-1}$ during the study period and were lowest in 1990 and highest in 1999. At station TF 2.3 the annual average for primary production increased linearly from the minimum value observed in 1990 through 1995, and then decreased to an average in line with that observed during the previous decade in 1996. Following this, primary productivity rates increased nearly exponentially each year until the all time high was observed in 1999. Station TF2.3 had the highest annual primary productivity rates for 12 of the 20 years (between 1984 and 2004). Station RET2.2 had the lowest annual primary productivity rates for 17 of the 20 years. 1984, 1987, and 2004 were low productivity (relative to the experimental mean) years for all three stations. The experimental averages for the three stations were $110.15 \mu\text{g C L}^{-1} \text{ h}^{-1}$ (LE2.2), $47.36 \mu\text{g C L}^{-1} \text{ h}^{-1}$ (RET2.2), and $112.15 \mu\text{g C L}^{-1} \text{ h}^{-1}$ (TF2.3). The comparison of mean primary productivity at the three stations revealed that rates at LE2.2 and TF2.3 were significantly elevated compared to RET2.2. Experimental maximum rates were observed in August at RET2.2 and TF2.3 and in May for LE2.2. Experimental minimum rates were observed in the month of December for all three stations.

Table 1: Pearson's table of primary productivity and chlorophyll *a*

Station			chlorophyll <i>a</i> (ug/L)
LE2.2	primary productivity (ug/L/h)	Pearson Correlation	0.784**
		N	322
RET2.2	primary productivity (ug/L/h)	Pearson Correlation	0.644**
		N	317
TF2.3	primary productivity (ug/L/h)	Pearson Correlation	0.800**
		N	320

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

The Pearson's Product-Moment Correlation was used to describe the degree of covariance between primary productivity data ($\mu\text{g C L}^{-1} \text{ h}^{-1}$) and chlorophyll *a* concentration ($\mu\text{g L}^{-1}$). The reason this analysis was selected instead of the single component linear regression analysis is because the two parameters are not completely independent of each other. For each station, primary productivity and chlorophyll *a* were highly significantly ($p < 0.001$) correlated, with the strongest correlation at upstream TF2.3 and the lowest at RET2.2.

Table 2: Statistical analysis of chlorophyll *a* vs. water temperature

Station	Season	R-Square	Degrees of Freedom (total)	P-Value
LE2.2	Fall	0.000	59	0.996
	Spring	0.001	112	0.716
	Summer	0.008	106	0.344
	Winter	0.037	34	0.266
RET2.2	Fall	0.099	54	0.019
	Spring	0.000	106	0.913
	Summer	0.018	106	0.165
	Winter	0.107	30	0.073
TF2.3	Fall	0.085	54	0.031
	Spring	0.063	105	0.009
	Summer	0.151	105	<0.001
	Winter	0.001	30	0.879

Table 2 describes the results of the single component linear regression analysis for chlorophyll *a* concentration regressed on water temperature above the pycnocline at all three stations. In order to observe meaningful trends at each location, the analysis was stratified by season. Water temperature predicted between 0% and 15% of the variability in chlorophyll *a* data. In few cases the regression was significant, however, during summer at station TF 2.3, when the highest r^2 was observed, a highly significant relationship between temperature and chlorophyll *a* was present. It is important to note that the statistics for fall and winter may have been greatly impacted by the fact that significantly fewer sampling events occurred during these periods. However, the degrees of freedom were within 7 observations of each other among stations for a particular season thus rendering between-station comparisons possible.

Table 3: Statistical analysis of chlorophyll *a* vs. time

Station	Season	R-Square	Degrees of Freedom (total)	P-Value
LE2.2	Fall	0.153	60	0.002
	Spring	0.010	114	0.284
	Summer	0.005	110	0.441
	Winter	0.002	35	0.819
RET2.2	Fall	0.131	60	0.004
	Spring	0.004	112	0.528
	Summer	0.006	111	0.418
	Winter	0.082	34	0.095
TF2.3	Fall	0.038	59	0.135
	Spring	0.001	112	0.744
	Summer	0.058	111	0.010
	Winter	0.078	34	0.103

Table 3 describes the results of the single component linear regression analysis for chlorophyll *a* concentrations regressed on time at all three stations. This analysis was conducted to determine if chlorophyll *a* concentrations demonstrated a measurable trend over the study period. As above, the analysis was stratified by station and season to elucidate meaningful

trends. Time predicted between 0% and 15% of the variability in chlorophyll *a* data. During spring, summer and winter, generally there was no trend in chlorophyll *a* concentration over time. However at stations LE2.2 and RET 2.2, a highly significant, albeit weak regression was observed during fall. These data indicate that during the fall period, chlorophyll *a* concentrations are increasing.

Table 4: Statistical analysis of primary productivity vs. temperature

Station	Season	R2	DF	P
LE2.2	Fall	0.007	60.000	0.516
	Spring	0.003	111.000	0.582
	Summer	0.002	108.000	0.632
	Winter	0.015	34.000	0.487
RET2.2	Fall	0.259	54.000	0.000
	Spring	0.034	106.000	0.056
	Summer	0.054	107.000	0.015
	Winter	0.091	30.000	0.099
TF2.3	Fall	0.133	55.000	0.006
	Spring	0.086	106.000	0.002
	Summer	0.077	105.000	0.004
	Winter	0.002	30.000	0.792

Table 4 describes the results of the single component linear regression analysis for primary productivity rates regressed on water temperature above the pycnocline at all three stations. In order to observe meaningful trends at each location, the analysis was stratified by season. Temperature predicted between 0% and 26% of the variability in primary productivity data. Although there was a highly significant relationship between chlorophyll *a* and phytoplankton primary productivity, the analysis of PP vs. temperature did exhibit the same trends as was observed for chlorophyll *a*. During fall, at stations RET2.2 and TF2.3, there were highly significant trends between primary productivity and time. While chlorophyll *a* concentrations at these stations in the fall also had significant correlations with temperature,

regressions were weak ($r^2 < 0.10$). At RET2.2 water temperature predicted 26% of the variability in primary productivity rates, which was the strongest relationship observed during this study found for any parameter. TF2.3 demonstrated a slightly weaker, although still highly significant, association ($r^2 = 0.13$). This trend was not observed at LE2.2 in the fall.

Table 5: Statistical analysis of primary productivity over time

Station	Season	R-Square	Degrees of Freedom (total)	P-Value
LE2.2	Fall	0.178	61	0.001
	Spring	0.015	113	0.195
	Summer	0.001	110	0.734
	Winter	0.107	35	0.051
RET2.2	Fall	0.005	60	0.584
	Spring	0.000	110	0.853
	Summer	0.014	110	0.210
	Winter	0.097	34	0.069
TF2.3	Fall	0.033	60	0.162
	Spring	0.011	114	0.255
	Summer	0.107	110	<0.001
	Winter	0.146	34	0.024

Table 5 describes the results of single component linear regression analysis for primary productivity rates regressed on time at all three stations. This analysis was conducted to determine if primary productivity rates demonstrated a measurable trend over the study period. In order to observe meaningful trends at each location, the analysis was stratified by season. Time predicted between 0% and 18% of the variability in primary productivity data. Although there was a highly significant relationship between chlorophyll *a* and phytoplankton primary productivity, the analysis of primary productivity vs. time did not follow the same trend as was observed for chlorophyll *a*. During fall, at station LE2.2, there was a highly significant trend between primary productivity and time ($r^2 = 0.18$). However this trend was not observed at either of the other stations. Nevertheless, during winter at TF 2.3 a significant trend was

observed ($r^2 = 0.15$). However, as mentioned above, these analyses are likely skewed by the fact that there were significantly fewer sampling events during winter and fall as compared to spring and summer. A highly significant, although weak, regression was also present at TF2.3 in the summer.

Table 6: Pearson's correlation of temperature vs. time

Station	Season			Date
LE2.2	Fall	Temperature	Pearson Correlation	-.003
			N	61
	Spring	Temperature	Pearson Correlation	-.070
			N	113
	Summer	Temperature	Pearson Correlation	.105
			N	109
	Winter	Temperature	Pearson Correlation	.139
			N	35
RET2.2	Fall	Temperature	Pearson Correlation	-.051
			N	56
	Spring	Temperature	Pearson Correlation	-.060
			N	109
	Summer	Temperature	Pearson Correlation	.111
			N	109
	Winter	Temperature	Pearson Correlation	.336
			N	31
TF2.3	Fall	Temperature	Pearson Correlation	-.030
			N	56
	Spring	Temperature	Pearson Correlation	-.039
			N	108
	Summer	Temperature	Pearson Correlation	.173
			N	107
	Winter	Temperature	Pearson Correlation	-.105
			N	31

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

The Pearson's Product-Moment Correlation was used to describe the degree of covariance between water temperatures above the pycnocline (°C) over time. The reason this analysis was selected instead of the single component linear regression analysis is because the

two parameters are not completely independent of each other. An additional reason that this method of analysis was selected over regression analysis is related to the assumptions associated with this study. It was initially hypothesized that water temperatures in the Potomac were increasing over time, in-line with trends of observed air temperature. Therefore, since the two were considered to be related, linear regression analysis (where variables are considered independent) was not appropriate for this comparison. Analyses were stratified by station and season. Interestingly, the correlation coefficient values did not deviate significantly from 0, indicating that no trend was evident in these data. Thus, it is concluded that no statistically significant trend in temperature data is observed over time.

Figure 5: Plot of USGS stream flow data

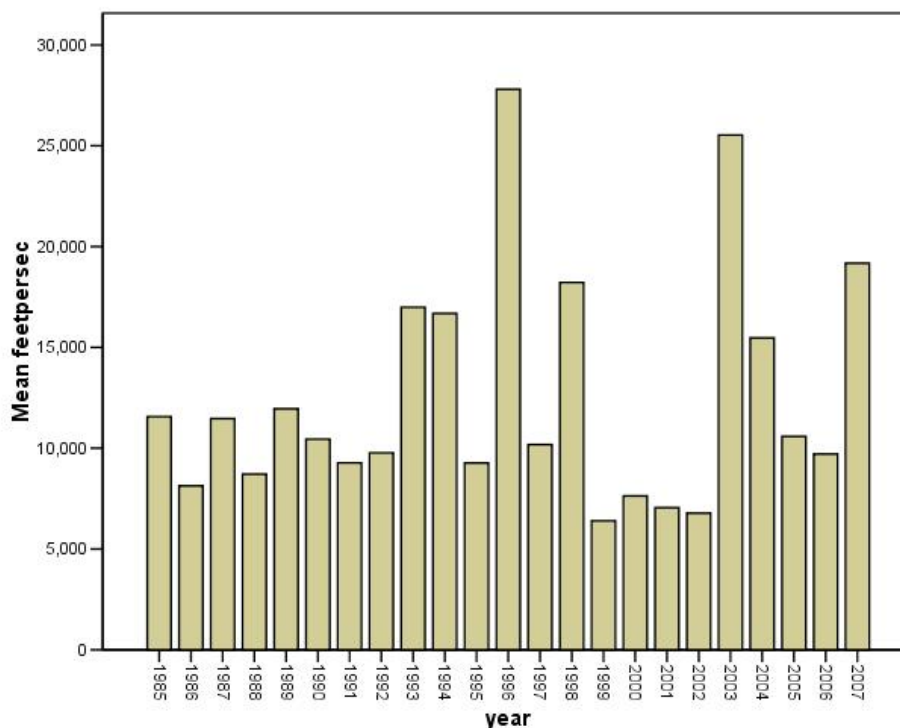


Figure 5 illustrates the annual mean stream flow values for the Potomac from 1985 to 2007 in cubic feet per second. Flow was at a minimum in 1999 and a maximum at 1996. This

chart is displayed because regression analyses demonstrated weak trends for chlorophyll *a* and primary productivity against water temperatures. Likewise, no temporal trend (in general) was observed during the study period. Because temperature only accounted for a small portion of variability within these variables (as described above), the influence of other environmental factors, in this case stream flow, was explored.

Discussion

Temperature over time

A basic assumption of this study was that *in situ* water temperature in the Bay was increasing over time, and that trend would be apparent in the 23 year Potomac River data set. However, the data revealed that this assumption was not true. Specifically, the plot of annual water temperatures over the 23 year study period (Figure 1) indicated temperature at all stations tended to vary around a single average with no prominent peaks or troughs (with the exception of years 2005-2007 which were omitted from discussion due to incomplete data sets). Likewise, the absence of a clear trend was verified by the Pearson's Product-Moment Correlation analysis (Table 6), wherein no statistically significant relationship between temperature and time was found for any of the stations seasonally or annually (data not shown). This supports the null hypothesis that temperature did not significantly vary during the course of the time frame of the study. It is possible that this is due in part to the way in which temperature samples were collected and averaged. In this dataset, temperatures above the pycnocline were averaged to create a 100% data match between all variables. Doing otherwise was unavoidable as parameters of primary productivity, chlorophyll *a* (MSU set), and community composition were analyzed as above pycnocline field composites. However, if it were possible to make

comparisons of surface phytoplankton data to surface temperatures, there is potential that a trend would have been observed. Lomas et al. (2002) observed this was the case in the mid-Bay where chlorophyll *a* levels had a positive linear relationship with water temperatures in the euphotic zone, while the Bay overall did not show trends with chlorophyll *a* utilizing the Q_{10} index. In addition, there was inconsistency in the depths which were sampled at each station. This inconsistency may have also contributed to masking any trends in temperature over time. The location in which sample stations are positioned also may have contributed to masking any potential temperature trends. As noted in the methods section, all three stations, as well as nearly all CBP monitoring stations throughout the Chesapeake Bay are located over the deepest part of the navigation channel. It is likely that significant temperature trends would be more apparent in shallow waters near the flanks of the Bay due to the fact that a greater portion of the total water depth is generally found in the euphotic zone. It is common to find temperatures near the flanks in excess of what is observed in the main channel. Thus, due to the sampling design, any trends near shore or in shallower, warmer waters are not accounted for in this analysis. There exists some data that may support this assertion (Hamdan 2000). Overall, average temperatures were highest at TF2.3, followed by RET2.2 and lowest at LE2.2 (Figure 1 means). This pattern may reflect bathymetry of the basin. TF2.3, RET2.2 and LE2.2 are approximately 9 m, 12 m and 18 m deep respectively. Because TF2.3 is found in the most shallow and most narrow portion of the tidal Potomac River, it is prone to the greatest heating effects as described above. Likewise, this site as well as RET2.2 are found near greater population densities and consequently, near areas of the watershed which have a greater proportion of impervious surfaces (Hamdan 2000). Impervious surfaces increase surface runoff to the river, and oftentimes, the surface runoff is of higher temperature than would be found in forest dominated portions of the watershed (Paul and

Meyer 2001), as is typical of the watershed surrounding LE2.2. Likewise, LE2.2 is more saline and composed of a greater percentage of cooler seawater.

It would be interesting to determine if temporal trends in temperature are present in other tributaries or the main stem of the Bay. However, if composite samples are utilized as were in this study, the same sampling issues as well as complications arising from using samples collected over the main channel would likely also be present.

Chlorophyll *a*

Unlike water temperature, chlorophyll *a* concentrations were more variable over the study period. Two overall peaks were present, one in the late 1980's and one in the late 1990's with a period of comparatively lower concentrations between during the years of 1989-1992 (Figures 2 and 3). While 1990 was a relatively cooler year at all three stations, a significant reduction in chlorophyll *a* was not observed as was hypothesized. This lack of a visual trend was confirmed by regression analysis (Table 2) which found that only occasionally did chlorophyll *a* concentration covary with temperature. During summer months, at station TF2.3 chlorophyll *a* did covary with temperature. However, despite the statistically significant regression, temperature only accounted for 15% of the variability in this chlorophyll *a* concentration. Regression analysis also showed that chlorophyll *a* concentration increased throughout the study period during fall at LE2.2 and RET2.2. However, once again, temperature only accounted for less than or equal to 15% of the variability in chlorophyll *a* data (Table 3). In Figures 2 and 3, these seasonal patterns are largely masked by the annual averages. TF2.3 had its peak chlorophyll *a* concentration in 1998, yet it steadily decreased from 1999 to 2004, which could explain why trends were not evident at this station. At LE2.2 chlorophyll *a* concentration peaked in 2000. However instead of a steady decrease from that time forward, chlorophyll *a* levels

dropped markedly in 2001 to less than half of the 2000 level and varied only slightly in the next 3 years.

Regardless of the fact that temperature did not explain a majority of the variability in chlorophyll *a* data, as noted above, in select cases, the parameters co-varied significantly. Thus, the null hypotheses that no correlation between chlorophyll *a* concentrations and temperature exists is rejected. However the majority of site / season combinations were not able to demonstrate a significant relationship. Importantly, during this study neither temperature, nor time (as a proxy for possible increasing water temperatures) proved to be strong predictors of chlorophyll *a* concentrations.

Another noted visual pattern in Figures 2 and 3 was that peak chlorophyll *a* concentration at LE2.2 lagged a year behind the TF2.3 study period maximum. Although any meaning derived from this observation would need to be substantiated, it raises the question whether climate effects are observed at all stations simultaneously, or if there is a significant delay in effects from an upstream to downstream area, or in shallow vs. deep locations.

Primary Productivity

Primary productivity and chlorophyll *a* concentrations were found to be highly correlated in this study at all stations (Table 1), thus supporting the null hypothesis that the two have a significant positive relationship. Despite this association, similar trends were not observed for both variables with respect to temperature and time. Considering Figure 4, it appears that primary productivity rates at TF2.3 gradually rose from 1990 to peak in 1999 (with the exception of 1995) and have decreased since. Primary productivity at LE2.2 peaked one year later in 2000. Station RET2.2 had the lowest rates of all the stations, and while annual averages did not vary much here over the 23 year period, a generally low period was observed between the years of

1987 and 1990. In agreement with the visual trends seen at TF2.3, the regression against temperature (Table 4) was highly significant in the fall ($r^2 = 0.13$; $p = 0.006$), spring ($r^2 = 0.09$; $p = 0.002$) and summer ($r^2 = 0.08$; $p = 0.004$). Likewise, a highly significant positive trend was also present in the fall at RET2.2 ($r^2 = 0.26$; $p < 0.001$).

There is limited data to support the assertion that primary productivity varies as a function of time (Table 5). Only in three cases was a significant relationship between primary productivity and time observed (LE2.2, fall period, TF2.3, summer and winter). Taken together, these findings indicate that temperature does at times exert control over primary productivity rates, however the data indicate primary productivity rates are not increasing, or decreasing over time. The null hypotheses that no relation between primary productivity rates and temperature or time was rejected in select cases. As noted above, there is little evidence to support any temporal trends in primary productivity for these three stations. However, there is evidence to support that temperature moderately influences primary productivity rates in the upper portions of the tidal Potomac River during fall months.

As was the case for chlorophyll *a* concentrations, neither temperature, nor time (as a proxy for possible increasing water temperatures) was able to explain a majority of the variance in primary productivity data. However, it is possible that phytoplankton could be responding to very minor changes in temperature over time. Preston (2004) reported decadal temperature increases at the surface of 0.16C per decade since 1949. Such a gradual increase may not be immediately apparent in the 23 years of monitoring data used in this study. The impact of temperature seems to exert greater control over primary productivity than biomass production (as evidenced by chlorophyll *a*). This finding demonstrates that microplankton populations may respond to changes in to *in situ* conditions in different and unpredictable ways, and may respond

to even minute changes in their environment. A follow up study to this would be to determine changes in primary productivity rates in response to small changes in temperature in a laboratory setting. This would provide a better prediction of the changes that may take place under real-world conditions. Also, as with chlorophyll *a* concentrations, peaks in primary productivity at LE2.2 each had a one year lag behind those for upstream TF2.3. This visual trend again brings into question the temporal scale of effects on phytoplankton. Nevertheless, it indicates that during 2000, the marked increase in primary productivity and biomass production at LE2.2 was in response to factors not identified during this study.

Other environmental drivers

In most cases, temperature and time were only able to explain 15% or less of the variability in chlorophyll *a* concentrations or primary productivity rates. This indicates that there are likely other, more influential factors driving variation in phytoplankton measurements. A reasonable factor to consider is stream flow data (Figure 5). Trends in Figure 5 seem to follow the inverse of chlorophyll *a* concentrations, as portrayed in Figures 2 and 3. While 1998-2002 were peak years for chlorophyll *a* concentrations, Figure 5 displays a trough in stream flow during these years which was lower than the 1985-2007 average. Figure 5 also illustrates a peak in stream flow for the years 2003 and 2004, which were lower years for chlorophyll *a* concentrations at all stations. This same trend follows for primary productivity rates (Figure 4), which peaked during the 1999-2002 trough in stream flow data. Captured within this time frame was the all time peak in chlorophyll *a* concentration at one station (LE2.2, Figure 2) and primary productivity rates at two stations (TF2.3 and LE2.2, Figure 4). This observation is based upon qualitative, visual analysis only, but is a trend which warrants further investigation. Others have found stream flow to be the main determinant in chlorophyll *a* concentrations and primary

productivity in the past (Adolf et al. 2005), however it was a direct relationship meaning that increased stream flow was associated with heightened chlorophyll *a* concentrations, and not an inverse relationship as is the case with this study. Conversely, Harding and Perry (1997) found an overall increasing trend in the Bay since 1950, yet their models indicated that freshwater flow could not account for this increase. It may be intuitive that greater stream flow would result in increased nutrient input into the system and subsequently heightened phytoplankton biomass and productivity levels. The results of this study bring that paradigm into question. Perhaps in this case, dilution proved to be a larger factor than nutrient input, or possibly increased stream flow created turbulent conditions which impacted the light requirements for phytoplankton. This observation agrees with findings in Howarth et al.'s (2000) Hudson River study where primary production was correlated with lower discharge and a larger euphotic zone. Nonetheless, nutrient input and in-stream concentrations, particularly of nitrogen and phosphorous are likely strong indicators of phytoplankton biomass and productivity. These would need to be explored further to determine the degree of their relation with Potomac phytoplankton dynamics.

Spatial trends

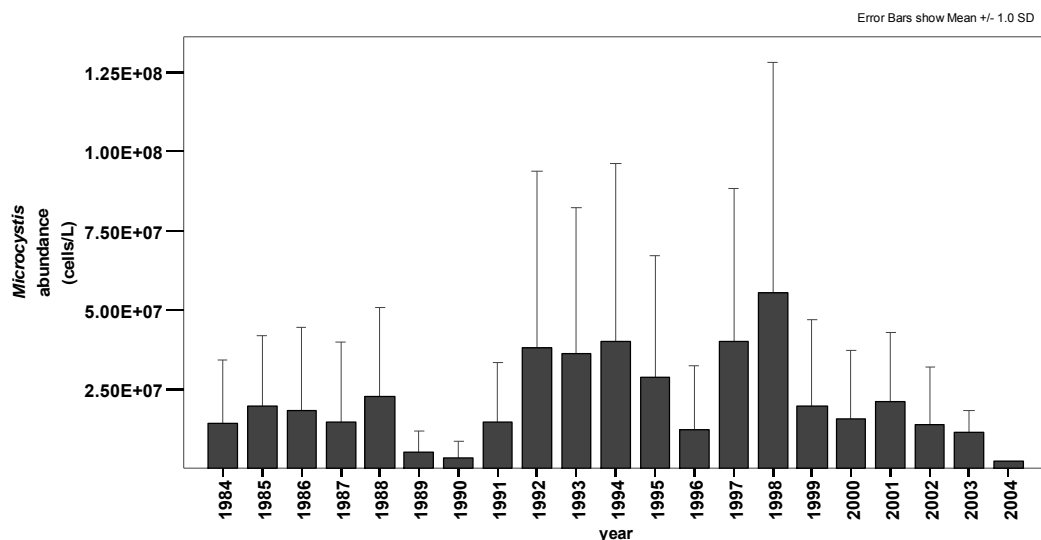
LE2.2 had the least variation in average annual temperatures, yet the highest variation in annual average primary productivity and chlorophyll *a* (annual and yearly maximums). It also had higher measurements of primary productivity and chlorophyll *a* than at RET2.2, yet the lowest average temperatures. As mentioned above, the influence of higher salinity waters entering the estuary from the south likely play an important role in regulating extremes in temperature at this location. Station RET2.2 had the least variation in chlorophyll *a* and primary productivity. It also had the lowest chlorophyll *a* and primary productivity levels, as well as the weakest correlation between primary productivity and chlorophyll *a*. TF2.3 had the most

variation in annual average temperatures and most frequently held the highest annual temperatures. This station also had the highest average chlorophyll *a* and primary productivity levels and had the strongest correlation between primary productivity and chlorophyll *a*. Hamdan and Jonas (2006) also noted all-time high levels of chlorophyll *a* at this station, and attributed this to *Microcystis aeruginosa* blooms. A key observation at this station is that it had the highest annual average temperatures, chlorophyll *a* levels, and primary productivity rates. Thus, while trends between these variables were not that strong, these data suggest there is still a relationship between biomass and productivity with increasing temperatures.

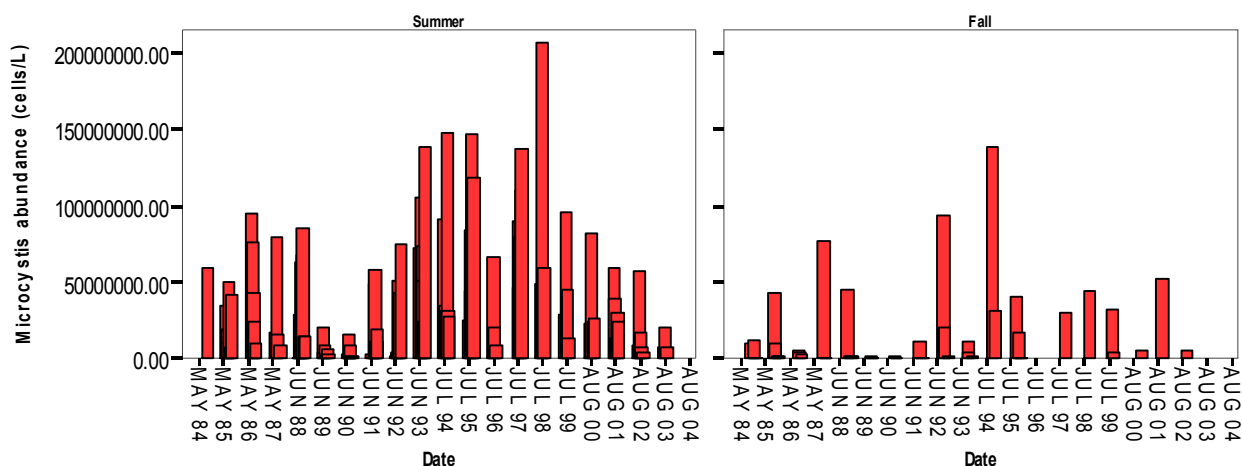
Also of note is that the majority of the significant regression trends occurred in the fall yet were distributed among stations. This could potentially be attributed to longer summers in a warming climate in which heightened temperatures extend their effects into the fall. Additionally, the late summer / early fall period is when the largest numbers of *Microcystis* sp. are found in the upper Potomac. Others have demonstrated an increase in *Microcystis* sp. since 1972 (Mulford 1972), and prevalence of cyanobacterial forms in taxonomic studies (Sellner.1987, Marshall and Lacouture 1986). It is possible that trends are being driven by the response of one genus to climate signals to a greater effect than the others. Lack of statistically significant relationships in the winter period were not surprising. Winter had the lowest amount of sampling events (typically one or two per year) resulting in lower degrees of freedom. Additionally, one sample event is not likely to represent typical conditions over this three month period and therefore any trends may be misleading. RET2.2 had the strongest regression trend by far, between primary productivity and temperature.

Community composition

With the vast amount of community composition data obtained for the study period, statistical and qualitative assessments were not possible in the timeframe of this study. Histograms of community structure of TF2.3 from 1995 to 2007 (from MDDNR) (Attachment 5) portray fairly consistent annual cycles of diatoms followed by the appearance of blue-green (cyanobacterial) species in the spring and summer. From these histograms, it appears that blue-green prevalence has steadily increased in abundance over the years. Mulford (1972) noted an increased prevalence in blue-green species three decades ago. This increasing trend is particularly relevant because along with an increase in blue-green species may come an increase in occurrence of harmful nuisance blooms, such as *Microcystis*. Data on the abundance of this particular species at TF2.3 was previously mined from the CBP data base in accordance with the methods described above (Hamdan and Donato, in prep) (Figure 6). The trend in the abundance of this species is a general increase between the years of 1984 and 1998 to a peak of a approximately 5.5×10^7 cells L^{-1} in 1998. Following this year, abundance has steadily decreased through 2004.

Figure 6: Plot of *Microcystis* abundance

The time in the year when the highest concentrations of *Microcystis* sp. are observed are during late summer and early fall. Figure 7 illustrates the seasonal trends in these data over time. It is clear that during the 1990s in summer months *Microcystis* sp. abundance steadily increased until 1998 (with the exception of 1996). Following this year, abundance has steadily decreased through 2003. A single component linear regression of whole year *Microcystis* sp. abundance regressed on temperature indicated that temperature has a significant, positive influence on the abundance of this single group of phytoplankton ($r^2 = 0.12$; $p < 0.000$; $n = 189$). This finding illustrates temperature may not have a unilateral impact on all phytoplankton, but may in fact have a significant impact on specific genera, during key periods of the year.

Figure 7: Seasonal plots of *Microcystis* abundance

These data indicate that it is important to consider not just the bulk abundance of phytoplankton but also temporal and spatial patterns in the abundance of key species. Each species has unique characteristics of biomass and primary productivity, so a shift in community structure could be responsible for predicting these variables as well.

Mulford (1972) and Patten (1962) found that mean species diversity increased seaward. Comparing the three Potomac stations used in this study, it would be interesting to see if the same trend held true, with lower diversity at TF2.3 and greater diversity at LE2.2. All of these trends warrant further study on community composition.

Conclusions

Summary

Above pycnocline water temperatures in the Potomac did not increase over time in the 23 year study period at the stations sampled. This is not in line with current assumptions on climate

warming in the mid-Atlantic region and in the Bay itself. However, as previously discussed, due to the methods employed to obtain data used in this study, a trend may be present but is not represented by these data. The presence of significant (although somewhat weak) covariance between both phytoplankton biomass and productivity with temperature and time seems to indicate a connection. Also, the co-occurrence of highest productivity, most biomass, and highest temperatures at station TF2.3 seems to indicate an association as well. Inner-station variation was present, and generally in-line with bathymetry characteristics. With the exception of *Microcystis* sp., in the absence of statistical analysis, it is not possible to comment in-depth on any changes on phytoplankton community composition over time. There is a notable visual increase in proportions of blue-green bacterial species compared to diatom species over time. In line with this, data from Hamdan and Donato (in prep) do demonstrate a distinct increase in *Microcystis* sp. abundance in the 1990's. Overall, while water temperature was not shown to be increasing, and not demonstrated to be a strong predictor of phytoplankton dynamics, it is still a factor which may influence some phytoplankton groups in the Potomac. If temperatures warm in the future in accordance with global climate change, it will be imperative to know what factors will be expected to influence phytoplankton in the Bay. As was noted in the discussion, other environmental factors are likely responsible for variation in phytoplankton biomass and productivity, such as stream flow and in stream nutrient load. While not directly linked to increasing water temperature, stream flow is a characteristic of climactic patterns and would also fluctuate in response to climate warming. It is important to understand the link between increasing temperature, and stream flow, nutrient delivery and how altered stream flow signatures may impact phytoplankton communities in the Bay.

Future Directions / Policy Implications

In order to further evaluate whether temperature invokes physiological responses phytoplankton, and if these responses are affected by climate warming, the first step necessary is to prove that their estuarine environment is connected to temperature change. This study did not effectively accomplish this due to using an above pycnocline data average and sampling only the deepest point in the River. Studying strictly surface temperatures instead of above pycnocline data averages may be a better way to establish these trends. Ideally, the sampling design would be randomly stratified throughout the basin so that shallow areas are accounted for as well. This would mean additional data collection beyond the scope of current monitoring activities over a span of multiple years, an unlikely feat without substantial funding.

In addition, chlorophyll *a* concentrations, primary productivity rates, and community composition were chosen as variables in part because they are easily measurable parameters for gauging phytoplankton dynamics. It would be advantageous to use laboratory studies to first determine the effects of environmental changes on different species of phytoplankton. Environmental changes in this case could be slight to moderate increases in temperature, a rise in CO₂, or a simulated increased or decreased nutrient concentration based on rainfall and stream flow. A laboratory environment would be more appropriate to divulge very slight modifications in phytoplankton dynamics, and would define the specific parameters to look for in a field study.

It would also be prudent to determine if stream flow is, in fact, a strong predictor for phytoplankton biomass and productivity, or if some other factor is responsible. This could be done fairly simply by following the pattern of data extraction and analysis laid out here for USGS stream flow data, rainfall, euphotic zone, and nutrient loading / concentrations regressed against chlorophyll *a* concentrations and primary productivity rates.

Phytoplankton community composition is certainly an area which needs further research. Environmental variables, such as water temperature could potentially have profound impacts on community structure, yet be unnoticed in overall growth and metabolism. As mentioned previously, the Biota and Environmental Matching Analysis (as described by Clarke and Ainsworth (1993)) would be a good tool to determine the abiotic factors (e.g., temperature, salinity, water clarity) which structure community composition. Also, determining ratios of dominant species over time would provide information on temporal shifts which have been made and may allow us to speculate on the causes of them. Determining spatial patterns of phytoplankton diversity in the Potomac would also be of interest. Knowing general spatial and temporal trends for phytoplankton community structure may also help environmental managers to better predict and deal with nuisance algal blooms in the future.

One final trend which warrants further exploration is the short-term temporal scale at which different areas of the water body are affected by environmental signals. The apparent one-year lag of maximums for chlorophyll *a* and productivity between TF2.3 and LE2.2 is an important coincidence to address. One would need to determine if it really does take one year for changes in phytoplankton dynamics realized upstream to reach downstream locations? This has important implications for the ways in which current environmental monitoring studies and management plans for eutrophication and subsequent phytoplankton response are conducted. Essentially, conditions in-stream could be actually responding to a signal from a previous year, or conversely current responses to an environmental event may not yet represent the entire range of response that will occur in the future.

This study proved to be a prime example of the challenges in managing a large monitoring program which spans across several state and agency jurisdictions. Different

agencies may sample the same location, such as the case at the two upstream sites on the Potomac at roughly the same time yet at slightly different coordinates. If weather conditions are adverse, one agency may not sample on the same day, and in some cases not within 2 days of one another. In addition, sample depths differ between sites. Beyond sample collection, technology for evaluating water quality and biotic parameters has evolved over time, and as such laboratory analyses methods have changed as well. These factors prevent the compilation of a single, consistent data set. Such uncertainty in the data brings into question the relevance of found trends and subsequently their ability to support management decisions. This is not to say that the monitoring effort in the Bay is without merit. The Chesapeake Bay Program's data hub is a valuable resource that brings together decades of data in a central location. Singular, intermittent studies could not give such a comprehensive picture of these variables over this amount of time.

Results of this study have important policy implications. It is important to know the physiological effects phytoplankton will undergo in the face of a changing climate. The entire ecosystem will be affected by increased or decreased biomass and productivity by phytoplankton. We must better understand the climactic processes by which phytoplankton biomass, productivity, and community composition will be altered in order to anticipate effects farther down the line, such as increased seasonal hypoxia or harmful algal blooms. Having such an understanding will allow managers to be proactive in the future. While this study weakly demonstrated that phytoplankton growth parameters are affected by temperature, it is important to find the major determinant of variation among them. The need for accurate climate predicting models which tell us how air and water temperatures, rainfall, and CO₂ levels is paramount. It is only after having this information that we can begin to make predictions on phytoplankton

processes in the future. It is also an important policy note that primary productivity rates demonstrated no net increase or decrease over time.

Chlorophyll *a* concentrations are one of the water quality parameters which have established Total Maximum Daily Loads (TMDLs) as established by section 303(d) of the Clean Water Act (Federal Water Pollution Control Act amendments, 1972). Each state sets its own limits for acceptable chlorophyll *a* TMDL's. If chlorophyll *a* levels will change over time in response to climate warming, then perhaps current TMDLs will need to be re-examined in the future. Since chlorophyll *a* levels are a proxy for phytoplankton biomass, TMDLs are actually setting limits on phytoplankton biomass, which may or may not be living. Primary productivity may be a better indicator for water systems since it gives us information on how much carbon is being fixed, and how productive a system is. In this study, the two variables shared a strong positive correlation; however each demonstrated different trends with temperature and over time. Therefore, it cannot be assumed that one is an indication of the other. In the future, perhaps primary productivity levels should be considered as a criterion for TMDLs. Research by Lomas et al. (2002) supports this, as primary productivity was shown to increase along with photosynthetic rate, and phytoplankton nitrogen uptake while chlorophyll *a* levels did not.

As primary producers, phytoplankton are at the base of any aquatic food web and thus an integral part of its functioning. While countless studies over the years have looked at phytoplankton dynamics in the Bay and other estuarine systems, the connection between these dynamics and global climate change requires further development. Studies addressing altered ecological dynamics as a result of climate change are composing an increasing body of research in the Mid-Atlantic region. Policy and management initiatives will need to be adaptive in order to respond to these changes effectively. Existing criteria such as TMDL's and setups of current

long-term monitoring programs should be reexamined in the face of science which continues to evolve in a changing climate.

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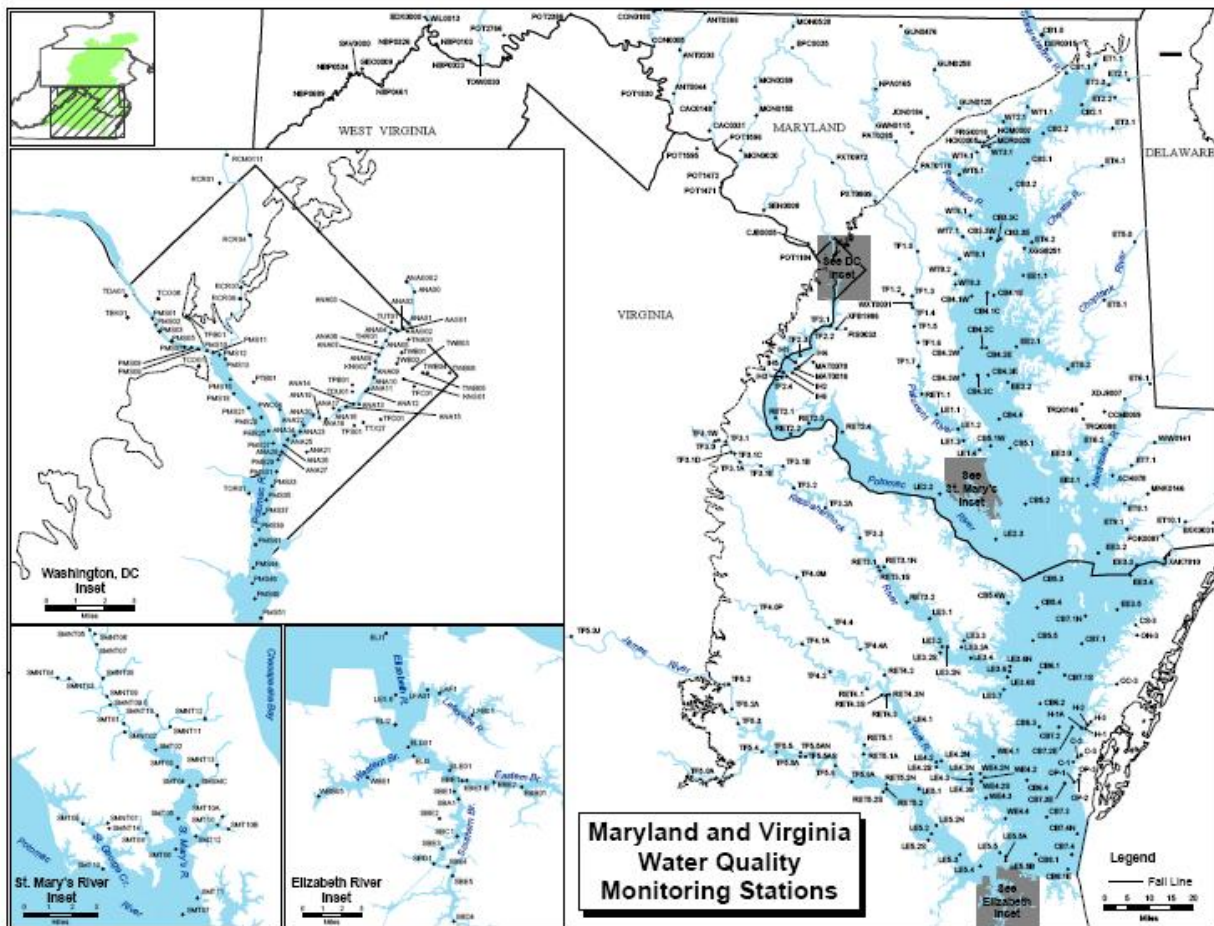
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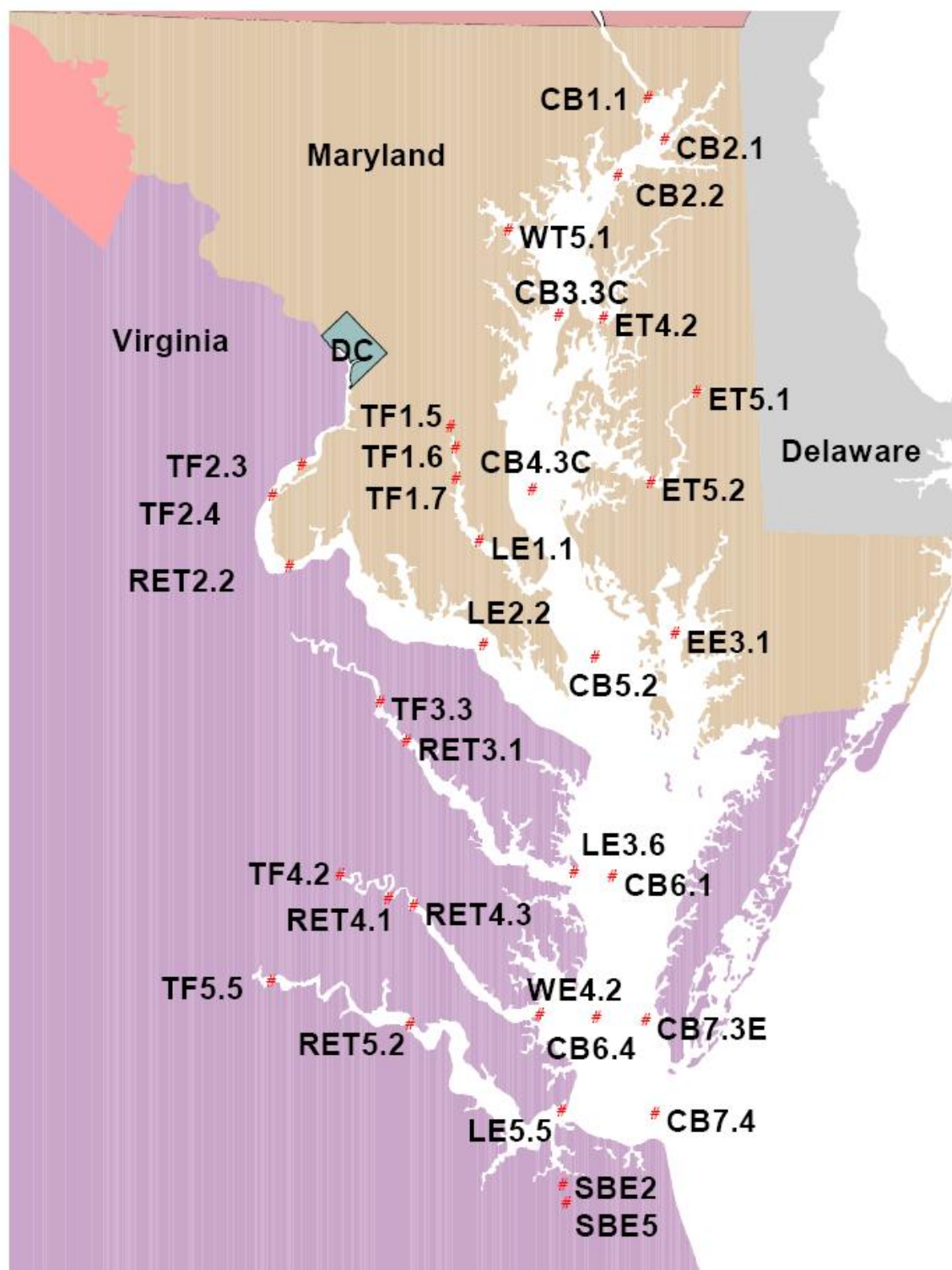
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Attachment 1: Chesapeake Bay Monitoring Map

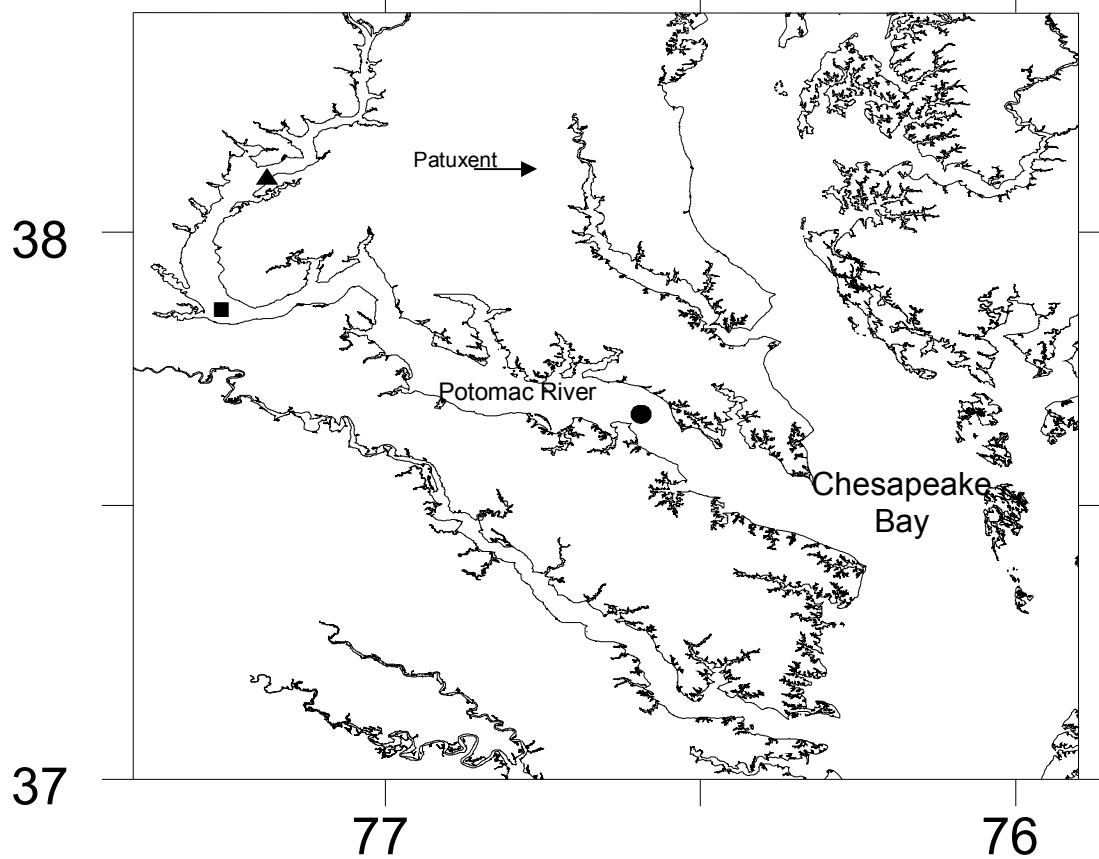


Attachment 2: Phytoplankton Monitoring Map



Plankton and Vertical Fluorescence Monitoring Stations

Attachment 3: Locations of sample stations along the Potomac River



(▲) TF2.3, (■) RET2.2 and (●) LE2.2

Attachment 4: Photos of upstream sample sites



Station RET2.2 with MD DNR boat in distance



Station TF2.3



MSU sample boat



Pumping from 0.5m – Very turbid Potomac

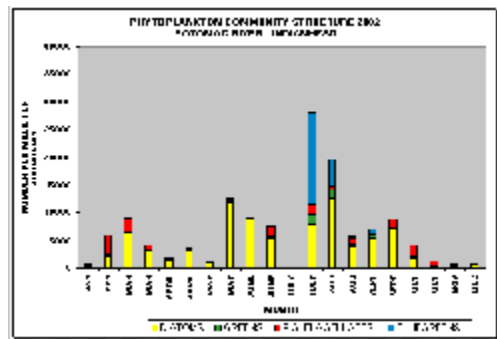
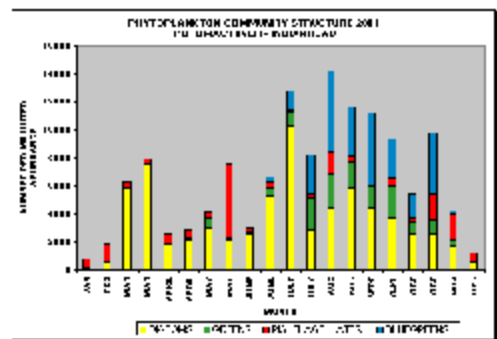
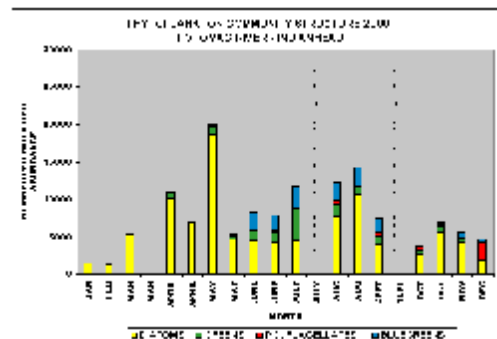
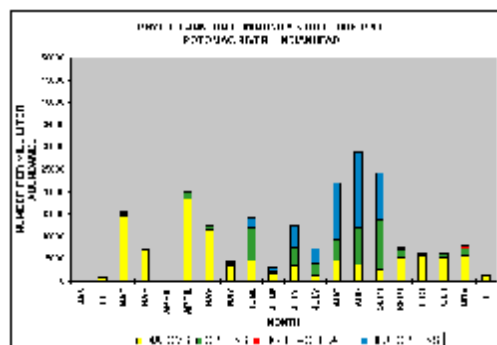
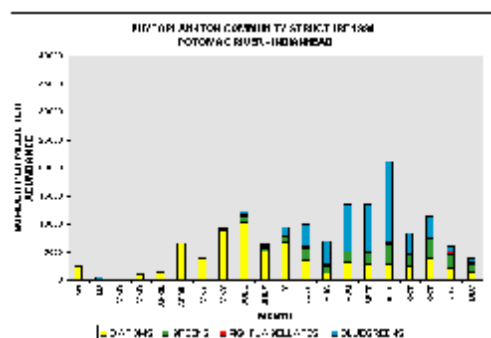
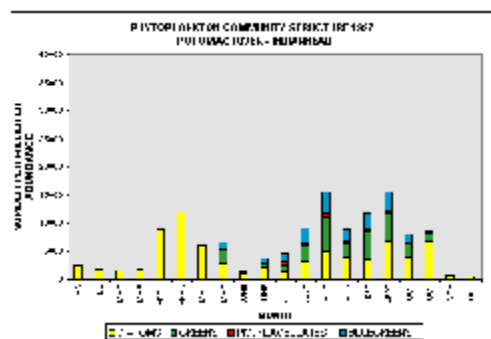
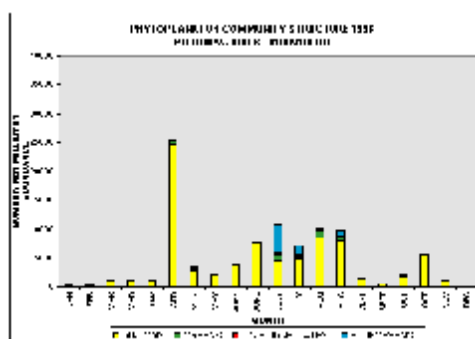
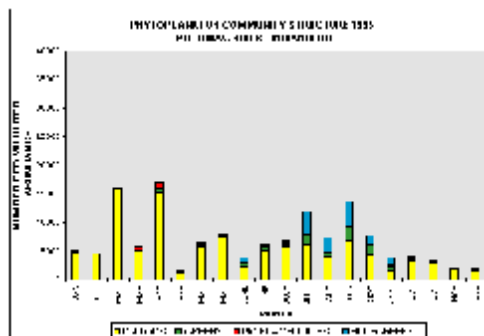


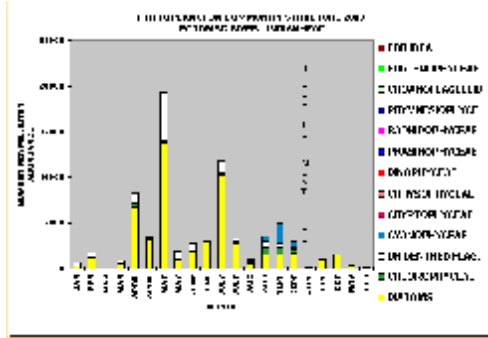
Chlorophyll *a* samples



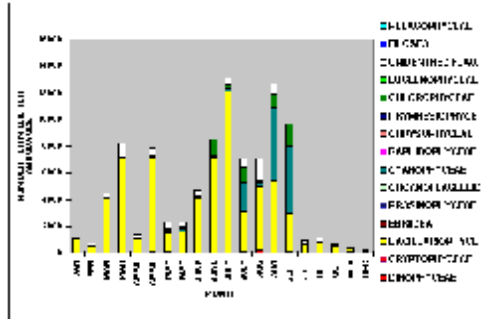
Filling carboy - primary productivity sample

Attachment 5: TF2.3 Community Structure Plots (MD DNR)

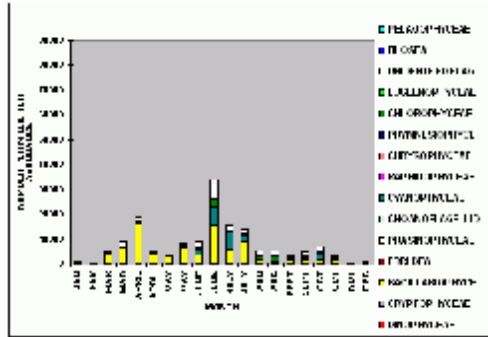




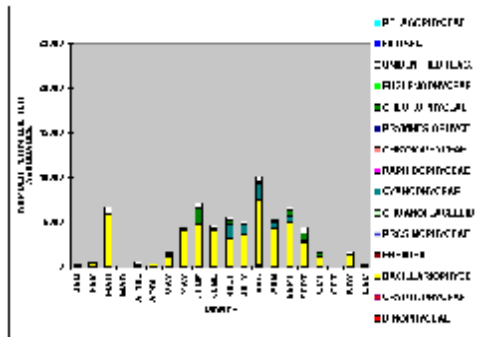
2004



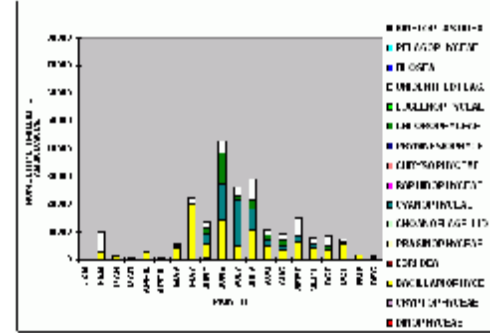
2005



2006



2007



Attachment 6: Final data set used in analysis

This is page one of my finalized data set, which represents a culmination of downloading each parameter in 5 year increments, merging years, formatting text files to MS Excel, deleting unnecessary information, matching data 100% to sample date, and averaging where necessary. The final data set consists of 971 rows of data spanning years 1984-2007.

Station	season	Date	PP mean	PP stdev	Chla mean (MSU)	Chla stdev (MSU)	Avg Chla (data average)	Avg Temp (DNR)
LE2.2	Summer	8/6/1984	51.00	10.75	20.80	0.00		25.95
LE2.2	Summer	9/4/1984	35.50	0.42	14.00	1.27	11.87	25.15
LE2.2	Summer	9/17/1984	44.30	6.51	24.55	0.35		
LE2.2	Fall	10/15/1984	28.90		7.30			17.90
LE2.2	Fall	11/19/1984	25.90	22.06	8.95	2.05	5.78	10.62
LE2.2	Fall	12/17/1984	37.15	9.12	21.45	0.92	7.98	7.75
LE2.2	Winter	2/26/1985	59.35	10.39	24.50	3.25		6.37
LE2.2	Winter	3/19/1985	6.30	1.70	26.55	0.64		7.05
LE2.2	Spring	4/15/1985	67.05	42.78	9.50	0.00		11.32
LE2.2	Spring	4/30/1985	110.40	7.07	31.60	0.00	42.10	17.36
LE2.2	Spring	5/13/1985	22.55	9.12	7.45	0.35	5.17	18.25
LE2.2	Spring	5/27/1985	53.90		34.73	24.94	52.37	21.44
LE2.2	Spring	6/10/1985	108.45	2.47	11.90	0.00	10.52	23.07
LE2.2	Summer	6/25/1985	72.55	10.54	9.05	1.20	5.29	23.69
LE2.2	Summer	7/8/1985	134.55	10.25	17.45	2.19	6.34	24.09
LE2.2	Summer	7/24/1985	103.65	18.31	22.75	17.61	9.73	25.77
LE2.2	Summer	8/6/1985	71.10		4.85	2.20		25.10
LE2.2	Summer	8/22/1985	108.55	12.66	11.00	1.27	7.49	25.12
LE2.2	Summer	9/9/1985	45.95	56.07	3.95	4.60		27.53
LE2.2	Fall	9/23/1985	29.35	5.73	6.60	1.27	3.86	23.20
LE2.2	Fall	10/7/1985	22.45	4.03	8.00	3.11	6.59	19.82
LE2.2	Fall	11/19/1985	149.20	7.07	15.20	1.27	7.94	14.77
LE2.2	Fall	12/9/1985	32.75	14.78	5.45	1.77	2.96	7.90
LE2.2	Winter	3/4/1986	20.95	5.44	4.55	0.78	6.10	2.58
LE2.2	Spring	4/1/1986	37.15	8.13	6.85	0.49	8.23	11.48
LE2.2	Spring	4/28/1986	82.95	6.15	16.20	1.13	18.39	13.43
LE2.2	Spring	5/12/1986	44.60	0.14	9.65	4.31	12.41	16.17
LE2.2	Spring	5/27/1986	77.05	22.98	5.40	1.13	3.89	19.41
LE2.2	Spring	6/2/1986	48.60	7.78	5.65	0.78	4.73	24.24
LE2.2	Spring	6/16/1986	126.80	0.14	22.70	3.54	7.78	24.19
LE2.2	Summer	7/14/1986	91.25	12.09	3.75	0.21	7.70	27.68
LE2.2	Summer	7/28/1986	52.30	3.82	5.80	0.57	6.06	27.90
LE2.2	Summer	8/25/1986	62.90	20.79	6.20	0.71	4.78	24.47
LE2.2	Summer	9/15/1986	82.30	1.56	8.35	0.78	12.06	22.73
LE2.2	Fall	9/29/1986	52.75	7.57	5.95	1.48	2.89	22.86
LE2.2	Fall	10/14/1986	75.30	2.55	10.55	1.06	8.68	19.83
LE2.2	Fall	11/17/1986	20.25	8.56	4.30	0.42	6.95	11.63
LE2.2	Fall	12/8/1986	25.75	1.48	4.70	0.00		8.08
LE2.2	Winter	1/5/1987	36.10	1.56	7.15	0.21	9.20	4.25
LE2.2	Winter	3/9/1987	26.40	11.31	15.40	2.97	5.46	5.88
LE2.2	Spring	4/9/1987	77.45	6.58	12.65	3.04		10.82
LE2.2	Spring	4/21/1987	186.00	14.57	25.20	0.00	14.21	13.13
LE2.2	Spring	5/4/1987	143.95	81.81	25.60	0.00	20.99	15.23
LE2.2	Spring	5/18/1987	77.70	6.36	11.45	0.21	17.50	17.76
LE2.2	Spring	6/1/1987	41.80	0.00	10.55	0.21	5.91	23.24
LE2.2	Spring	6/15/1987	69.50	3.11	8.85	0.21	4.30	23.06
LE2.2	Summer	7/6/1987	107.70	55.01	7.25	1.34	11.67	26.12
LE2.2	Summer	7/20/1987	83.40	2.26	29.55	3.18	22.68	26.43
LE2.2	Summer	8/3/1987	67.55	8.56	15.30	4.24	16.37	27.70
LE2.2	Summer	8/17/1987	58.90	13.15	36.25	4.31	15.26	27.08
LE2.2	Summer	9/1/1987	69.55	4.60	11.20	0.00	8.00	24.91