Measurements of C:Chl *a*, P_{max}, T_{opt}, and Other Model Parameters in Natural Phytoplankton Populations of the Chesapeake Estuaries

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by

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Disclaimer

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Executive Summary

This report presents new, monitoring-based information about key attributes of natural phytoplankton populations in Chesapeake Bay and its tidal tributaries, including the Potomac River estuary. Phytoplankton rates, constants, and kinetics formulations calculated from the Chesapeake Bay Program monitoring data are compared to their counterparts that are currently used in the Chesapeake Bay Eutrophication Model (Cerco and Noel 2004). Although in general agreement, there are areas where the model's phytoplankton kinetics could be refined.

Total phytoplankton biomass (micro-, nano- and pico size fractions) expressed as carbon was estimated from count data and used to calculate carbon-to-chlorophyll ratios (C:Chl). In high salinity waters, a negative inverse hyperbolic cosine function describes the relationship between C:Chl and light attenuation (Secchi depth) better than an exponential function. The exponential function tends to overestimate winter C:Chl and underestimate summer C:Chl.

The unintended effects of shipboard methodology on productivity were examined, and specifically the length of time collected samples are held prior to C¹⁴ incubation and the light and temperature conditions in which they are held. Maximum productivity rates, or P_{max} , were quantified for 0°C - 30°C from samples least affected by shipboard methodology. Measured P_{max} and T_{opt} , the optimum temperature for algal growth, differ from values currently used in the Eutrophication Model. The model underestimates P_{max} in summer mesohaline waters at all temperatures, and overestimates P_{max} in spring tidal fresh and oligohaline waters at low temperatures.

Phytoplankton photoinhibition is evident as a midday depression in chlorophyll fluorescence (quenching). Data analysis of Chesapeake continuous monitoring data sets suggests the onset of the daytime depression measured with an unshielded fluorometer sensor could be a useful method for directly measuring Ik, the irradiance at which the initial slope of the production versus irradiance (P-I) relationship intersects the value of P_{max} . If Ik can be measured directly with chlorophyll fluorescence, models could use the results to create different P-I curves for high and low light adapted populations. The former are characteristic of shallow environments and the latter, deep environments. The sensitivity of eutrophication models could be improved if Ik is a function of the average local light condition experienced by phytoplankton populations.

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Figure 1-1. Chesapeake Bay and several important tidal tributaries. The biomonitoring station locations of the Chesapeake Bay Program biomonitoring stations (\bullet), Maryland Department of Natural Resources (\blacktriangle), and Mikita (\boxdot) are indicated.



Measurements of C:Chl *a*, P_{max}, P_{opt}, and Other Model Parameters in Natural Phytoplankton Populations of the Chesapeake Estuaries

Claire Buchanan January 2006

1. Introduction

The purpose of this report is to introduce new monitoring information about key attributes of natural phytoplankton populations in present-day Chesapeake Bay and its tidal tributaries (Mid-Atlantic coast, USA). These attributes are integral components of most eutrophication models. Recent analyses of several data sets, some of which have been collected over the past 20 years, have refined our knowledge of estuarine phytoplankton communities. Incorporation of the new information into Chesapeake eutrophication models may improve model sensitivity and calibration efficiency, and build user confidence in the modeled estuarine responses to nutrient and sediment load reductions. It could also improve "dialogues" between water quality and food web/fisheries models.

This report provides empirical information about:

- C:Chl ratios under a range of ambient light conditions;
- T_{opt} , P_{max} , and the effects of short-term light and temperature acclimation prior to phytoplankton C¹⁴ uptake measurements in light-saturated, temperature-controlled incubation chambers;
- daily patterns of phytoplankton photoinhibition measured with chlorophyll fluorescence;

The monitoring results are compared in this report to the rates, constants and kinetics formulations used to model phytoplankton in the Chesapeake Bay Eutrophication Model (Chapter 10 in *Process-based primary production modeling in Chesapeake Bay* by Cerco and Noel, 2004), but they are applicable to other eutrophication models.

Most analyses were performed on data collected since 1984 at Chesapeake Bay Program (CBP) *biological* monitoring stations in the Chesapeake system of estuaries (Figure 1-1). These stations have phyto- and zooplankton taxonomic enumerations and biomass estimates as well as chemical and physical data. Some of the data analyses span shorter or longer time periods due to limitations in the data or when the analyses were done. Analyses were also performed on non-CBP data sets made available to ICPRB, such the continuous monitoring data sets for the Patuxent River collected by Kim Mikita Penn and for the Severn and Magothy rivers collected by Maryland Department of Natural Resources (MDDNR) (Chapter 5).

2. Data sources and general methods

CBP data sets and the methods pertinent to most of the analyses performed in this report are described in this section. Documentation of field and laboratory methods for the CBP biomonitoring data can be found at <u>www.chesapeakebay.net/data/index.htm</u>. More detailed descriptions of specific field and laboratory methods or data analysis methods are described in the individual sections.

2.1. CBP phytoplankton field and laboratory procedures

CBP phytoplankton samples and the associated water quality data, including chlorophyll *a*, are collected with a pump from discrete depths in the water column at each station. The phytoplankton samples are composited into upper and lower layer samples (whole water column samples at some tidal fresh stations) while chlorophyll *a* is measured at discrete depths. The upper layer is in most cases the water above the pycnocline (AP) and the lower layer, water below the pycnocline (BP). Microphytoplankton (>20 μ) and nanophytopankton (2 - 20 μ) species composition and abundance have been monitored in both the Maryland and Virginia portions of Chesapeake Bay since the mid 1980s. Autotrophic picophytoplankton (0.2 - 2 μ) abundance has been monitored in Virginia since 1989, and a comparable program was recently initiated in Maryland in 2002.

In the 1984-2002 Maryland nano-micro-phytoplankton monitoring program, Lugols preserved samples are subsampled and settled directly in an Utermöhl chamber. Random fields are counted at 512X magnification until a minimum cell count (200) or field count (20) is reached. In the parallel Virginia program, Lugols preserved samples are settle for 72 hours in the laboratory, then processed in a three step series of alternating siphoning and settling over the next 72 hours to obtain twenty to thirty-five milliliter concentrate from each sample. Subsamples are settled in an Utermöhl chamber and examined at magnifications of 312X and 500X using a combined random field (10) and minimum cell count (200) procedure. In addition, the Virginia laboratory scans the entire concentrate at 125X for net phytoplankton. Species are identified and counted to the lowest taxonomic category possible in both laboratories, and identifications are based on internationally accepted plankton keys and Laboratory voucher specimens.

Autotrophic picoplankton were only monitored in Virginia after 1989 and in Maryland after 2004. Preserved whole water samples are filtered through a Millipore apparatus onto a 0.20 μ nucleopore filter previously stained in an irgalan black solution and backed with a 0.45 μ nucleopore filter. Picophytoplankton cells fluorescing on the 0.2 μ filter are then counted with a Zeiss Axioskop epifluorescent microscope under a 100X oil immersion objective using a "green" filter set (g546. FT580, LP590).

Only water quality, chlorophyll *a*, and phytoplankton data from the upper, or AP, layer of the water column were used in this analysis, except for a few tidal fresh stations where samples were collected from the whole water column (WC). Secchi depth readings were used instead of light attenuation measurements because they provide the longest and most complete water column transparency record in the monitoring program. On several occasions, phytoplankton samples and chlorophyll measurements were made at different times, and even on different days. They were paired if sampling dates were within three days of each other. More detail about how the data were prepared and merged can be found in Buchanan et al. 2005.

2.2. Count-based phytoplankton biomass estimates

Biomass estimates of natural Chesapeake Bay phytoplankton populations were derived from taxa counts using taxon-specific estimates of cell biovolume and equations for converting cell biovolume to biomass (carbon). Taxa-specific cell biovolumes were quantified with average cell measurements for most phytoplankton species found in Chesapeake Bay by Lacouture (1998) and Lacouture et al. (in prep.). Richard Lacouture and Harold Marshall, the principal investigators of the Maryland and Virginia phytoplankton monitoring programs, respectively, developed taxa-specific equations to convert cell biovolume to cellular carbon content using regressions published by Smayda (1965), Mullin and co-workers (1966) and Strathmann (1967). This approach produces biomass estimates of individual taxa, phylogenetic groups, and total phytoplankton biomass as micrograms carbon per liter (μ g C 1⁻¹) in a relatively inexpensive and technically straightforward manner.

A cell diameter of 1.3μ was used to derived an estimated carbon content of 0.4 picograms (ρg) or 0.4 x 10⁻⁶ μg per cell for autotrophic picophytoplankton. Cell diameters range from 0.5 - 2μ diameter in Chesapeake Bay waters, or ~0.03 - 1.20 ρg cell⁻¹, and average cell size varies with season, light, and species composition of the picophytoplankton community. The 1.3 μ cell diameter was believed to represent an average condition.

3. Chlorophyll Cell Content and the C:Chl Ratio

Water quality models frequently use chlorophyll *a* (Chl) measurements to estimate total phytoplankton biomass, expressed as carbon (C). Chlorophyll *a* measurements are multiplied by a factor that represents the chlorophyll *a* content of an average cell. For example,

$$\mathbf{B} = \mathbf{Chl} * \mathbf{C:Chl}$$

$$=$$
 Chl * $\frac{1}{$ Chl: C

where B is total phytoplankton biomass (μ g C l⁻¹), Chl is the chlorophyll *a* (μ g Chl *a* l⁻¹), Chl:C is the average proportion of cellular chlorophyll (μ g Chl *a* l⁻¹) to cell biomass (μ g C l⁻¹), and C:Chl is the inverse, or "carbon-to-chlorophyll" ratio.

The C:Chl ratio varies from <10 to >100 in phytoplankton cultures (Geider 1997). Mean C:Chl values estimated for Chesapeake Bay include 290 in the upper bay and 70 in the mesohaline and lower reaches of the bay (Malone 1988), and 90 (Harding et al. 1986). C:Chl ratios of 90 (spring) and 75 (summer) were used in earlier versions of the Chesapeake Bay Eutrophication model (Table 3, Cerco 2000). These values were derived from regressions of particulate organic carbon (POC) and chlorophyll *a* measurements in the Chesapeake mainstem (1994 data). In recent model runs, C:Chl has been changed from a constant to an exponential function of water column transparency, and following equation is used (Cerco and Noel 2004):

$$C:Chl = a + b^* e^{-c^* Ke}$$

where a = minimum C:Chl ratio, b = incremental C:Chl ratio at zero light attenuation, c = the effect of light attenuation on the ratio (m), and Ke = 1.4/Secchi depth (m⁻¹).

Chlorophyll-based estimates of phytoplankton biomass that are calculated with a static, or constant, C:Chl ratio can be significantly different from biomass estimates calculated directly from cell counts. This is particularly true for samples collected in turbid estuarine waters (Figure 3-1). Phytoplankton cells acclimate to insufficient light environments by increasing their cellular concentration of chlorophyll, changing the concentrations of enzymes involved in light harvesting pathways, or both (Fogg 1965, Kirk 1975, Day et al. 1989, Lampert and Sommer 1997). Chlorophyll-based estimates of biomass, and related parameters such as algal photosynthesis and growth rates, are more accurate if the C:Chl ratios used in model calculations are a function of water column transparency.

The experimental results described in Fogg (1965) over four decades ago are still useful in illustrating the effects of light acclimation on phytoplankton Chl:C, and ultimately on maximum photosynthesis rate (P_{max}) and the saturation light intensity for photosynthesis (Ik). The primary benefit of higher cellular concentrations of chlorophyll *a* is a heightened initial positive slope (α) of the productivity-irradiance (P-I) response curve (Figure 3-2). This allows acclimated cells to continue to photosynthesize effectively in low-light environments. Low-light acclimated cells with their higher Chl:C ratios can assimilate carbon faster on a per unit biomass or per cell basis (turnover) than high-light acclimated cells when placed in saturating - but not inhibiting - light levels. Light acclimation also directly alters Ik and P_{max} , changes the proportions of cellular metabolic products such as carbohydrates and fats which ultimately

affects food quality of the phytoplankton, and changes respiratory energy losses which affect cell survival. Hypothetically, the higher production rates per cell of low-light acclimated cells could result in the rapid formation of algal blooms if water currents carry these cells to shallow water column depths and maintain them at intensities nearer their Ik for prolonged periods.

3.1. Data Analysis

C:Chl ratios were calculated for each station-date event by dividing the count-based estimate of phytoplankton biomass (μ g C l⁻¹) from the composited above-pycnocline (AP) sample by the average of the discrete depth chlorophyll *a* measurements (μ g Chl l⁻¹) from the AP layer. Count-based estimates are obtained by multiplying microscope counts of each taxa (cells l⁻¹) by taxa-specific conversion factors. The conversion factors are based on measured cell volume and relationships between cell volume, dry weight, and carbon content (Lacouture et al, in prep.). The calculated C:Chl ratios were parsed into four seasons and four salinity zones to minimize variability due to season and salinity: winter (January - February), spring (March - May) and summer (June - September), and autumn (October - December); and tidal fresh (0.0 - 0.5 ppt), oligohaline (>0.5 - 5.0 ppt), mesohaline (>5.0 - 18.0 ppt), and polyhaline (>18.0 ppt). Within each season-salinity zone, the ratios were further grouped by Secchi depth interval. Secchi depth was used as the measure of water column transparency because its has longest, most complete record for Chesapeake Bay.

3.2. Results

3.2.1. C_{mnp}:Chl, and biomass carbon estimates that include the picophytoplankton

The carbon-to-chlorophyll ratio was initially calculated on a smaller subset of CBP monitoring program data records containing both micro-nano-phytoplankton and picophytoplankton biomass estimates, i.e., the Virginia monitoring data, August 1989 - December 2001. The resulting ratio, C_{mnp} :Chl, represents the pico-, nano-, and micro-phytoplankton size fractions, or total phytoplankton biomass. The proportion of picophytoplankton biomass relative to total phytoplankton biomass ranges from 0.1% and 99% in the data set (Table 3-1). Picophytoplankton are usually a small fraction of total phytoplankton biomass in winter and spring, with seasonal medians ranging between 0.2% and 1.9% in the four salinity zones. The picophytoplankton proportion is highest in summer, with seasonal medians of 4.7%, 10.6%, 28.0%, and 24.2% in the tidal fresh, oligohaline, mesohaline, and polyhaline, respectively.

Neither C_{mnp} :Chl, nor its inverse Chl: C_{mnp} , were normally distributed in any of the 16 season-salinity data groups (Kolmogorov-Smirnoff test of composite normality, $\alpha = 0.01$). Log-transformation normalized the data in 13 of the 16 season-salinity groups, and almost normalized the remaining 3 groups. Therefore, parametric statistics were used on log-transformed data, and non-parametric statistics were used on untransformed data.

Preliminary examination of the untransformed data showed that mesohaline and polyhaline C_{mnp} :Chl values grouped by Secchi depth interval were not significantly different (Mann-Whitney test, $\alpha = 0.05$). Seasonal data from mesohaline and polyhaline salinity zones were therefore combined to increase the number of data points and extend the range of Secchi depths in the regression analyses. Mesohaline Secchi depths ranged from 0.2 - 3.8 meters and

polyhaline Secchi depths ranged from 0.5 - 7.5 meters in the data record. They overlapped strongly between 1.0 and 2.3 meters.

Significant linear regressions between log-transformed C_{mnp} :Chl and Secchi depth are apparent in the combined mesohaline-polyhaline data, with p<0.001 in spring, summer and autumn and p=0.015 in winter. The regression coefficients, r², only range from 0.022 (winter) to 0.144 (spring), however, and the seasonal relationships are better described with non-linear curves. The C_{mnp} :Chl ratios appear to reach an asymptote at Secchi depths above 3 meters, and regressions spanning the entire Secchi depth range had smaller r² than those restricted to 0.1 - 3.0 meters.

Non-linear relationships between C_{mnp} :Chl and Secchi depth were examined with untransformed data grouped by Secchi depth intervals. As Secchi depth increases (water clarity improves), median C_{mnp} :Chl values increase in a pattern best fitted with a negative hyperbolic cosine function (Table 3-2). Correlation coefficients (r²) for these regressions are higher than those for linear, logarithmic, and exponential regressions, which suggests that C:Chl ratio approaches a maximum as Secchi depth increases in the Chesapeake Bay system. The non-linear relationship is accentuated when chlorophyll cell content, or Chl:C_{mnp}, is plotted against Secchi depth (Figure 3-3). Seasonal median chlorophyll cell content is high at Secchi depths < 1 meter, decreases sharply between 1 - 2 meter Secchi depth, and approaches a minimum value at Secchi depths > 2 meter.

Chesapeake tidal freshwater and oligohaline salinity zones currently experience narrow ranges of Secchi depths. This precludes any meaningful regressions between the C_{mnp} :Chl and Secchi depth. The range of Secchi depths in the data record varied from 0.3 - 1.2 meters (summer) to 0.1 - 0.9 meters (winter) in tidal fresh, and from 0.2 - 0.8 meters (autumn, summer) to 0.1 - 0.6 meters (spring) in oligohaline waters. The grand median of C_{mnp} :Chl for tidal fresh and oligohaline salinity zones was compared to the grand median of C_{mnp} :Chl for mesohaline and polyhaline records with less than 1 meter Secchi depth, to determine if C_{mnp} :Chl values in comparable water clarity were similar. The grand median C_{mnp} :Chl for tidal fresh and oligohaline data was 1.33-fold higher than that for mesohaline and polyhaline. In other words, tidal fresh and oligohaline phytoplankton communities presently have a chlorophyll cell content, or Chl: C_{mnp} , equal to about 75% of that observed in mesohaline-polyhaline phytoplankton communities. These differences are significant (Mann-Whitney test, p = 0.01).

3.2.2. C_{mn}:Chl, and biomass carbon estimates that do not include the picophytoplankton

Carbon estimates for the micro- and nano- phytoplankton size fractions, or C_{mn} , are available for most of the Maryland and Virginia 1984 - 2004 biomonitoring records, and constitute a much larger data set than the C_{mnp} data that includes picophytoplankton biomass. Seasonal median Chl: C_{mn} ratios were calculated for each Secchi depth and compared to the hyperbolic cosine regression curves generated for Chl: C_{mnp} vs Secchi depth (Figure 3-4). The Chl: C_{mnp} curves overlap the median Chl: C_{mn} data points closely in winter and spring when the pico- size fraction of total phytoplankton biomass is very small and its effect on the overall population's chlorophyll to carbon relationship is negligible (Table 3-1). Winter and spring values of C_{mn} :Chl derived from the larger Maryland and Virginia 1984 - 2001 data set could be used as surrogates for C_{mnp} :Chl. In summer and autumn, the Chl: C_{mnp} curves are displaced downward reflecting the greater contribution of picophytoplankton biomass during these seasons (Figure 3-4). The Chl: C_{mnp} -Secchi regression curves and the median Chl: C_{mn} data points closely parallel each other despite the displacement, confirming the shape of the regression curve and reiterating the importance of light exposure on phytoplankton chlorophyll cell content.

3.2.3. High variability in Chl:C values at low Secchi depths

Degraded water quality conditions, and specifically those with low ("poor") Secchi depths and excess nutrients, are characterized by extreme chlorophyll *a* concentrations signifying algal blooms and busts. Chl:C in low Secchi depths echo this highly variable, i.e., the range of Chl:C values tends to increase as Secchi depth decreases. Buchanan et al. 2005 found that algal blooms (high biomass group) in degraded conditions were associated with low Chl:C values as well as low turnover rates (amount of carbon fixed per unit of cell biomass as carbon per hour in temperature-controlled, light-saturated conditions) and low proportions of the chlorophyll decomposition product pheophytin relative to total biomass carbon (Pheo:C). Algal busts (low biomass group) were associated with high Chl:C ratios, high turnover rates in incubation chambers, and high Pheo:C (Figure 3-5). The results show that phytoplankton populations in degraded conditions.

3.2.4. Chl:C versus temperature and nutrient concentrations

When sufficient data were available, $Chl:C_{mn}$ and $Chl:C_{mnp}$ values grouped by Secchi depth classes (best, better, poor, and worst, from Table 1, Buchanan et al. 2005) were further grouped by temperature (0-5°C, 5-10°C, etc.) to explore the possibility of an underlying temperature effect on Chl:C ratio. The results indicate there is little, if any, temperature effect on spring and summer Chl:C ratios in natural phytoplankton populations (Figure 3-6).

The variability in the Chl:C values within each light-temperature group could not be explained by differences in DIN and PO_4 concentrations. Under poor and worst light conditions, the most numerous data group, Chl:C ratios in excess DIN and PO_4 were slightly higher than Chl:C ratios in limiting DIN and PO_4 , but the differences could be explained by the generally lower Secchi depths associated with excess DIN and PO_4 samples. Under better and best light conditions in summer, Chl:C ratios in excess DIN and PO_4 were not significantly different from Chl:C ratios in limiting DIN and PO_4 (t-test, p<0.05). No spring samples were available to compare.

3.3. Discussion

3.3.1. Non-linear relationship with light

Chlorophyll *a* measurements and count-based calculations of phytoplankton biomass carbon from the CBP biomonitoring station data produced Chl:C_{mnp} (summer) and Chl:C_{mn} (autumn, winter, spring) values ranging from <0.002 to >0.170. The inverse, or C:Chl, ratios ranged from <5.9 to >500. These values are representative of the Chesapeake system's surface mixed layer, across all salinity zones, for 1984-2004.

The non-linear change in the chlorophyll *a*-carbon relationship as Secchi depth increases represents a general decrease in phytoplankton chlorophyll cell content relative to total phytoplankton biomass as light conditions improve in the mesohaline- polyhaline Chesapeake

Bay (Table 3-2, Figures 3-3, 3-4). Seasonal median $Chl:C_{mnp}$ values are high at Secchi depths < 1 meter, decrease between 1 - 2 meter Secchi depth, and approach a minimum value at Secchi depths > 2 meter. The inverse of $Chl:C_{nmp}$ is the proportion of total phytoplankton biomass per unit chlorophyll *a*, or C_{mnp} :Chl. For the purpose of estimating total phytoplankton biomass from chlorophyll *a*, C_{mnp} :Chl constants of approximately 75 (January-February), 110 (March-May), 130 (June-September), and 60 (October-December) are representative of the combined micro-, nano-, and picophytoplankton size fractions *for Secchi depths greater than 2 m*. Below Secchi depths of 2 m, the use of C_{mnp} :Chl constants produces large over- or underestimates of total phytoplankton biomass.

The leveling-off of the Chl:C curves at Secchi depths greater than 2 m (Figures 3-3, 3-4) suggests the underwater light gradient in Chesapeake Bay is becoming sufficient for photosynthesis and does not prompt an acclimation response. The inflection point approximates the classification criteria separating "poor" and "better" light classes in Tables 1 and 2 of Buchanan et al. (2005). The inflection point denotes a threshold for adequate average light attenuation at about 2 m in Chesapeake Bay mesohaline-polyhaline salinities. Local thresholds will likely vary depending on estuary morphology and circulation patterns.

3.3.2. Season and salinity

Seasonal differences in phytoplankton chlorophyll cell content, and the C_{mnp} :Chl ratio are evident in the results. Autumn and winter curves for Chl: C_{mnp} (Figures 3-3, 3-4) tend to be higher than the spring and summer curves, i.e., phytoplankton communities in these seasons generally have higher chlorophyll cell contents (lower C_{mnp} :Chl ratios). This likely is a result of the lower insolation levels and shorter day lengths of autumn and winter in the northern hemisphere. The slope of the winter curve is flatter than those of the other seasons, i.e., winter communities are apparently less responsive to changes in light attenuation. This may be caused by the colder winter temperatures, which slow phytoplankton cell metabolism and population growth rates, thus making cell responses to light conditions and their ability to modify chlorophyll cell content slower.

The similar Chl:C ratios in the Virginia Chesapeake mesohaline and polyhaline data with equivalent Secchi depths is not unexpected. The two salinity zones have comparable water clarity characteristics relative to depth. Specifically, Secchi depths as percent of the above-pycnocline (upper mixed layer) depth during the summer stratification period, and as percent of total depth during the other, less stratified seasons, are similar in the mesohaline and polyhaline salinity zones baywide, regardless of the large differences in total depth. In contrast, Secchi depth relative to total depth in the more turbid, tidally-mixed tidal fresh and oligohaline salinity zones is generally smaller (Table 3-3). The phytoplankton community itself is another reason for the similarity in mesohaline and polyhaline Chl:C. While polyhaline waters have a sizeable representation of neritic species, seasonally dominant and sub-dominant taxa are often the same in the two salinity zones (Marshall 1994, Marshall and Alden 1988, Marshall and Alden 1993).

3.3.3. Variability

The greater variability in chlorophyll cell content of cells in poor/worst light conditions is coincidental with a greater variability in other phytoplankton physiological characteristics. Specifically, algal busts or low biomass groups have high Chl:C and high Pheo:C ratios, with

high turnover rates in saturating light. High biomass groups or algal blooms have low Chl:C and low Pheo: C ratios, with low turnover rates in saturating light (Figure 3-5). Mesozooplankton biomass data indicate the busts are not the result of high grazing pressure. The combination of traits suggests the low biomass groups are nascent blooms or populations stressed by low light levels. The high biomass groups appear to be algal blooms at or near their peaks, in situations where recent light conditions have been adequate and nutrient resources are becoming limited. The nutrient data support this thesis, i.e., the DIN and sometimes PO₄ concentrations associated with high biomass groups are usually lower than those associated with the low biomass groups (Figure 3-5). Similarity between Chl:C_{mnp} values in the reference community and the high biomass-poor light class group suggests that the cells in algal bloom populations were exposed to favorable light conditions recently and for long enough to adjust their chlorophyll cell concentrations. This occurs at the cell-division stage. Stratification after weather events, surface laminar flow, upwelling on shoals, and other estuarine features are able to maintain discrete water masses in high light micro-habitats despite overall turbid conditions (Boicourt et al. 1999). Hypothetically, the higher production rates of low light acclimated cells would result in the rapid formation of algal blooms if water currents maintain the cells at intensities nearer their Ik for prolonged periods.

The greater variability in Chl:C and other phytoplankton light-related traits of populations in poor light conditions attests to the importance of light, and particularly light acclimation, in the turbid Chesapeake system. To a lesser degree, variability in Chl:C values may also be due to species composition. Chesapeake Bay algal blooms are at times dominated by "bloom-formers that rely on heterotrophic as well as autotrophic pathways. Many dinoflagellates are considered facultative heterotrophs. Examination of the Virginia 1989 - 2001 mesohaline-polyhaline data indicates that phytoplankton samples consisting of more than half dinoflagellate biomass occurred in 25% of the records with Chl:C_{mnp} <0.005 (low), as compared to 10% in the records with Chl:C_{mnp} >0.050 (high). There appears to be little, if any, variability in the Chl:C ratios of natural phytoplankton populations that can be explained by ambient water temperature (Figure 3-6) or nutrient concentration, although the data are sparse in many instances. Temperature and nutrient concentration of the photosensitive pigment chlorophyll *a*.

3.3.4. Models

C:Chl constants ranging from 10 - 112 have been used in models of surface water quality (e.g., Bowie et al. 1985, Cerco 2000). An exponential function of light attenuation is presently used in the Chesapeake Bay Eutrophication Model to account for the influence of light environment on chlorophyll cell content (p.197, Cerco and Noel 2004):

$$C:Chl = a + b \bullet e^{-c \bullet Ke}$$

A comparison of the exponential functions and the negative inverse hyperbolic cosine functions fitted to the observed seasonal data indicates the results of the two approaches are similar in present-day spring and autumn conditions but different in winter and summer conditions (Figure 3-7). The exponential function appears to overestimate winter C:Chl and underestimate summer C:Chl at Secchi depths greater than 1.5 m.

Comparisons of monthly C_{nmp} :Chl in Secchi depths ≥ 2 m versus ratios in Secchi depths \leq

1 m suggests the annual pattern in C_{nmp} :Chl will probably change as water clarity improves in above-pycnocline waters (Figure 3-8). A rehabilitated open water environment in Chesapeake Bay will hypothetically have better water clarity due to lower sediment inputs from runoff and resuspension, even during the winter-spring freshet period. Figure 3-8 indicates C_{nmp} :Chl could track the annual cycle in solar irradiance, with values near the winter solstice ~ 60% of those near the summer solstice. Presently, winter and summer solstice C_{nmp} :Chl are approximately the same, and spring has the highest C_{nmp} :Chl. Some seasonal variability related to species composition could still be evident in the rehabilitated bay, especially during spring stratification and autumn turnover. Populations may be less influenced by dinoflagellate and cyanobacteria biomass in summer.

3.4. Summary

- C:Chl ratios of approximately 75 (January-February), 110 (March-May), 130 (June-September), and 60 (October-December) are representative of the combined micro-, nano-, and picophytoplankton size fractions for Secchi depths greater than 2 m in mesohaline and polyhaline waters of Chesapeake Bay and its tidal tributaries.
- Below Secchi depths of 2 m, the use of C:Chl constants can produce over- or underestimates of total phytoplankton biomass. This is because chlorophyll cell content (Chl:C) and other phytoplankton physiological traits are more variable in generally poor light environments.
- C:Chl ratios in tidal fresh and oligohaline waters, where Secchi depths rarely exceed 1 m, are typically 1.33-fold higher than ratios in mesohaline and polyhaline waters at equivalent Secchi depths.
- In generally poor light environments, high C:Chl values tend to coincide with algal blooms and low C:Chl values tend to coincide with algal busts. Algal populations in good light environments (reference communities) have consistently high C:Chl values.
- Cellular chlorophyll *a* concentrations in natural Chesapeake populations are not affected by either nutrient concentrations or ambient temperature.
- Chlorophyll *a* can more accurately estimate phytoplankton biomass when the C:Chl ratio multiplier is a variable dependent primarily on light attenuation, and secondarily on season and salinity zone.
- A negative inverse hyperbolic cosine function of light attenuation describes the relationship between C:Chl and light attenuation better than an exponential function.
- Annual fluctuations in C_{nmp} : Chl will probably track the annual pattern in solar irradiance more closely in a rehabilitated Chesapeake Bay with improved water clarity.

Table 3-1. Seasonal frequency of picophytoplankton biomass relative to total phytoplankton biomass (as %) in present-day Chesapeake Bay tidal waters for four seasons and four salinity zones, based on the August 1989 - December 2001 Virginia sampling events containing chlorophyll, and nano-, micro- and pico phytoplankton size fraction counts. Total biomass is the estimated biomass of all size fractions summed. Descriptive statistics indicate the picophytoplankton frequency distribution is rarely normal or even log-normal within each season-salinity regime, and the median is the most representative measure of central tendency. Skewness values indicate the frequency distributions have asymmetric tails extending towards high frequencies, except in summer mesohaline and polyhaline when asymmetric tails extend towards low frequencies. Kurtosis values indicate the frequency distributions are strongly peaked, except in summer mesohaline and polyhaline when the peaks are slightly flattened.

1 2	1	6,		
	Tidal Fresh	Oligohaline	Mesohaline	Polyhaline
Winter (Jan - Feb)	n = 67	n = 28	n = 99	n = 181
Mean <u>+</u> SE	2.3% <u>+</u> 0.6%	3.1% <u>+</u> 1.1%	1.6% <u>+</u> 0.3%	3.0% <u>+</u> 0.4%
Geometric Mean	0.5%	0.8%	0.4%	0.9%
Median (IQR)	0.4% (2.3%)	0.8% (2.1%)	0.4% (1.0%)	2.0% (2.9%)
Min-Max	0.01% - 26.7%	0.05% - 27.4%	0.01% - 24.3%	0.01% - 41.0%
Kurtosis	13.33	10.90	24.08	18.48
Skewness	3.34	3.17	4.35	3.82
Spring (Mar - May)	n = 79	n = 27	n = 181	n = 112
Mean	4.1% <u>+</u> 1.3%	3.4% +1.0%	2.2% <u>+</u> 0.4%	4.0% <u>+</u> 0.8%
Geometric Mean	0.9%	1.1%	0.3%	0.8%
Median	0.8% (3.2%)	1.9% (2.1%)	0.2% (1.2%)	0.8% (2.2%)
Min-Max	0.01% - 99.9%	0.01% - 23.4%	0.00% - 44.0%	0.02% - 45.5%
Kurtosis	55.56	8.53	26.36	9.77
Skewness	6.99	2.74	4.58	3.03
Summer (Jun - Sep)	n = 87	n = 60	n = 204	n = 311
Mean <u>+</u> SE	13.5% <u>+</u> 2.0%	15.3% <u>+</u> 1.8%	32.5% <u>+</u> 1.7%	29.0% <u>+</u> 1.3%
Geometric Mean	5.4%	9.9%	19.4%	17.7%
Median	4.7% (15.1%)	10.6% (16.6%)	28.0% (42.6%)	24.2% (35.0%)
Min-Max	0.03% - 80.0%	0.31% - 67.9%	0.01% - 90.4%	0.01% - 86.3%
Kurtosis	3.35	2.86	-0.99	-0.69
Skewness	1.96	1.64	0.45	0.64
Autumn (Oct - Dec)	n = 40	n = 28	n = 63	n = 166
Mean	2.8% <u>+</u> 1.0%	8.7% <u>+</u> 1.9%	13.0% <u>+</u> 1.6%	10.2% <u>+</u> 1.0%
Geometric Mean	1.9%	3.9%	7.2%	4.5%
Median	1.4% (6.7%)	4.5% (9.3%)	9.2% (15.2%)	5.2% (11.7%)
Min-Max	0.01%-37.9%	0.05%-37.4%	0.25%-49.8%	0.06%-59.7%
Kurtosis	31.38	2.05	0.89	4.28
Skewness	5.34	1.68	1.28	2.00

Table 3-2. The ratio of total phytoplankton biomass carbon to chlorophyll *a* (C_{mnp} :Chl) in the mixed upper layer waters of Virginia Chesapeake Bay and tidal tributaries (based on the 1989 - 2001 monitoring data). Total phytoplankton includes the micro-, nano-, and pico- size fractions. The Secchi depth ranges in tidal fresh-oligohaline salinities were too narrow for a meaningful relationship between Secchi depth and C_{mnp} :Chl to be described (0.2 - 1.3 m in tidal fresh, 0.2 - 0.8 m in oligohaline). Seasonal relationships between Secchi depth and median C_{mnp} :Chl can be described for mesohaline-polyhaline salinities. They are best characterized with a negative hyperbolic cosine function, i.e. y = a - (b/cosh(cx)), where a, b, and c are constants and cosh is the hyperbolic cosine of Secchi depth, x, in meters.

				-												
Secchi Depth		Winter	(Jan - Feb)	Spring (Mar - May)		Summer (Jun - Sep)			Autumn (Oct - Dec)						
(meters)	n	Median	25th%	75th%	n	Median	25th%	75th%	n	Median	25th%	75th%	n	Median	25th%	75th%
Tidal Fresh (<0.5 ppt)																
all	67	67.8	27.5	194.7	79	67.7	25.0	153.3	87	62.2	38.2	118.0	40	49.0	28.6	96.4
Oligohaline (0.5 - 5 ppt)																
all	28	59.2	30.2	177.4	27	56.1	24.7	132.6	60	88.7	48.3	145.2	28	44.8	24.4	74.9
Mesohaline - Polyhaline (5 - 32 ppt)																
0.3 - 0.4 (0.35)	10	58.57	32.18	90.87	16	30.42	18.85	102.45	22	38.66	22.11	64.66	too fe	W		
0.5 - 0.7 (0.6)	27	47.98	22.93	107.60	35	54.90	30.41	77.96	56	38.32	24.07	79.90	26	32.57	16.51	89.62
0.8 - 0.9 (0.85)	20	38.52	27.08	60.23	20	42.19	31.46	88.94	48	31.52	20.95	46.66	12	29.89	22.88	59.30
1 - 1.2 (1.1)	34	55.38	37.17	76.10	42	64.04	37.29	91.71	105	54.97	36.49	90.38	34	57.07	33.86	91.00
1.3 - 1.4 (1.35)	32	63.03	40.04	100.63	22	56.91	31.20	78.60	72	66.00	40.78	95.87	28	45.60	23.48	83.74
1.5 - 1.7 (1.6)	33	70.92	49.52	107.39	39	80.61	42.06	118.15	94	74.37	48.47	106.20	33	64.17	34.82	112.68
1.8 - 1.9 (1.85)	23	63.55	39.29	97.43	22	52.67	38.00	81.17	43	71.01	49.59	117.73	32	56.17	37.11	98.44
2 - 2.2 (2.1)	33	81.49	44.42	114.47	34	114.16	67.50	197.46	25	151.90	78.66	201.46	24	57.77	30.55	92.88
2.3 - 2.4 (2.35)	15	73.64	41.87	114.40	14	81.31	55.32	138.45	12	161.68	92.92	228.82	10	76.35	53.52	99.83
2.5 - 2.7 (2.6)	20	72.82	48.10	140.84	18	115.72	93.85	205.47	12	119.13	90.49	137.14	8	45.71	27.58	70.40
2.8-3.8 (3.1,3.5)	23	71.33	53.21	135.04	22	127.90	72.32	208.16	13	113.62	80.40	178.91	12	61.53	40.46	110.22
>4 (5)	too	few			too f	ew			6	103.25	54.20	150.08	too fe	W		
hyperbolic cosine regression of C_{mnp} :Chl medians		median (35.96/c (r ² = 0.6	f(y) = 82.04 osh $(0.85x)$ 3, p < 0.0	4 - .) 1)		median ($235.59/c$ ($r^2 = 0.7$	y) = 274.1 cosh(0.34x 9, p < 0.0	6 - x) 1)		median (y 116.24/c $(r^2 = 0)$	y) = 131.0 cosh(1.05x 0.66, p < 0	2 - :) .01)		median (y 55.59/cc $(r^2 = 0.56)$	y) = 61.76 osh(1.87x) b, p < 0.05	; -) 5)

Table 3-3. Average Secchi depth as percent (%) of total station depth or summer pycnocline depth (^a). (Derived from 1997 - 2001 Virginia monitoring data.)

Average Total Depth (m)	Tidal Fresh 8.7m	Oligo- haline 7.4m	Meso- haline 7.3m	Poly- haline 13.1m
Autumn	7%	7%	13%	15%
Spring	8%	6%	14%	14%
Summer	7%	7%	21% ^a	21% ^a
Winter	6%	6%	15%	16%

Figure 3-1. Chlorophyll-based (Δ) and count-based (\bullet) estimates of phytoplankton biomass as carbon, across the range of Secchi depths experienced in the four salinity zones of Chesapeake Bay from 1984-2002 (see text for details). Chlorophyll-based estimates were derived with a C:Chl constant of 50. Tidal fresh, 0.0-0.5%; oligohaline, >0.5-5.0%; mesohaline, >5.0-18.0%; polyhaline, >18.0%.



Figure 3-2. A comparison of chlorophyll-specific and cell density-specific production rates as a function of light intensity for *Chlorella vulgaris* grown at 3 or 30 kilolux (Klux). From Fogg (1965), after E. Steemann Nielsen et al. 1962. Cells grown in the 3 Klux light level had higher concentrations of chlorophyll than cells grown in the 30 Klux level. Since this was an algal monoculture, cell density-specific production rates are comparable to biomass-specific production rates. Upper Panel: the cell density-specific production rates of low-light acclimated (3 Klux) cells are greater at low-light intensities than those of the high-light acclimated (30 Klux) cells. The cell density-specific production rates of the 30 Klux cells eventually catch up to and surpass the rates of the 3 Klux cells at higher light intensities. Cells grown in 3 Klux reached a maximum cell density-specific production rate or P_{max} , of 1.4 mg C h⁻¹ 10⁹ cells⁻¹ at ~5 Klux. Cells grown at 30 Klux reached a P_{max} of >1.4 mg C h⁻¹ 10⁹ cells⁻¹ at light intensities >30 Klux. At very high light intensities, cell density-specific production rates decreased due to photoinhibition. This portion of the curve was not shown in the original graphs. Lower Panel: the chlorophyll-specific production rates of the 3 Klux cells are the same as those of the 30 Klux cells in low-light intensities (<3 Klux), but level off at ~5 Klux. Rates of the 30 Klux cells continue to climb past 30 Klux. Notice that Ik, the saturation light intensity, remains similar to the one in the upper panel.



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Figure 3-3. Median chlorophyll cell content (Chl: C_{mnp}) by Secchi depth interval in the mesohaline-polyhaline upper mixed layer of Chesapeake Bay and its tidal tributaries, for winter (January-February), spring (March-May), summer (June-September), and autumn (October-December) (see text for details). C_{mnp} is the estimated biomass carbon of the micro-, nano-, and pico- phytoplankton size fractions. The equations for the best-fitting hyperbolic cosine curves through the median carbon-to-chlorophyll ratios (C_{mnp} :Chl), which are the inverse of the Chl: C_{mnp} curves shown in this figure, are given in Table 2.



Figure 3-4. The seasonal, hyperbolic cosine regression curves for median Chl:C_{mnp} versus Secchi depth (black line) from Figure 2, and the medians and interquartile ranges for Chl:C_{mn} , by Secchi depth (black dots, gray bars, $n \ge 8$). C_{mnp} and C_{mn} are count-based estimates of phytoplankton biomass carbon. The C_{mnp} estimates include picophytoplankton biomass, and the Chl:C_{mnp} are derived from 1,281 data records of the Virginia monitoring program, 1989-2001. The C_{mn} estimates do not include picophytoplankton biomass, and the Chl:C_{mn} are derived from 4,480 data records of the combined Maryland and Virginia monitoring programs, 1984-2004. The Chl:C_{mnp} curves parallel the Chl:C_{mn} medians closely, and the apparent downward displacement of the Chl:C_{mnp} curves reflects the contribution of picophytoplankton biomass, which varies seasonally. Graphs are for mesohaline-polyhaline salinities only (5-32‰). See Fig. 2 heading for seasons.



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Figure 3-5. Phytoplankton and habitat characteristics associated with the high biomass group in the poor light class (includes worst), the low biomass group in the poor light class, and the reference community from the better light class (includes best), for spring and summer mesohaline (from Figure 3, Buchanan et al. 2005). High and low biomasses are concentrations $>95^{\text{th}}\%$ and $<50^{\text{th}}\%$ of reference community concentrations (Table 4, Buchanan et al. 2005), respectively; poor light classes have Secchi depths <1.8 m (spring) or <1.45 m (summer). The interquartile range (25^{th}) - 75^{th}) of each parameter is shown as percentages of the overall median in the poor light classes. Abbreviations: MD, Maryland; VA, Virginia; PicoBiomass, picophytoplankton biomass as µg carbon liter⁻¹; NMBiomass, nano-micro-phytoplankton biomass as µg carbon liter⁻¹; Pheo:C ratio, µg pheophytin liter⁻¹ divided by total phytoplankton biomass expressed as μg carbon liter⁻¹; turnover, $\mu g C$ fixed liter⁻¹ h⁻¹ in saturated light conditions divided by total phytoplankton biomass expressed as μg carbon liter⁻¹; Mesozoop., mesozooplankton biomass estimated from abundance data. *, only data records with count-based carbon biomass estimates for nano-micro- and pico- phytoplankton were used in calculating Chl:C; **, picophytoplankton biomass estimates derived from Virginia results for each seasonsalinity-habitat category were added to Maryland nano-micro phytoplankton biomass data to calculate turnover.



Figure 3-6. The spring (March - May) and summer (July - September) Chl:C ratios in natural Chesapeake Bay populations do not appear to be affected by ambient water temperature. Chl:C is plotted against light condition rather than Secchi depth in order to increase the number of data records, and the statistical certainty of the result, within each temperature group. Data groups with n < 7 are excluded. Chl:C_{mn} is evaluated in spring and Chl:C_{mnp} is evaluated in summer. Spring data (March-May): Virginia, 1986-2004; Maryland, 1985-2004. Summer (July-September) data: Virginia, 1989-2004; Maryland, 2002-2004. Light conditions: season- and salinity-specific light classes described in Table 1, Buchanan et al. 2005.



Figure 3-7. Comparison of the best-fit line for the seasonal median C:Chl, or the inverse of Chl:C, observed at different Secchi depths in mesohaline and polyhaline salinities, and the line used in Cerco and Noel (2004) to describe the C:Chl relationship with light attenuation. Line equations are shown for each season. Best-fit line (solid line): negative inverse of the hyperbolic cosine function, or C:Chl = $a - b/cosh(c \cdot z)$; Cerco and Noel (2004) equation (dashed line): exponential function, or C:Chl = $a + b \cdot e^{(-c \cdot 1.4/z)}$; z: Secchi depth in meters; winter: December - February; spring: March - May; summer: June - September; autumn: October - November (see text for further detail).



Figure 3-8. Monthly C_{nmp} :Chl for Secchi depth ≥ 2 m in mesohaline and polyhaline salinities. Only data with nano-, micro- and pico-phytoplankton biomass estimates are used (Virginia 1989-2004, Maryland 2002-2004). Bars indicate the monthly 95th% (-), 75th% (--), median (o), 25th% (--), and 5th% (-) values. Dark grey line: polynomial regression through medians (y = 0.0514x⁴ - 1.2636x³ + 8.4611x² - 10.55x + 90.333). For comparison purposes, the polynomial regression through the medians of C_{nmp} :Chl for Secchi depth ≤ 1 m is shown as a light grey line (y = -0.0312x⁴ + 0.8623x³ - 7.7099x² + 22.798x + 41.094).



4. P_{max} (Light-Saturated Photosynthesis) and T_{opt} (Optimum Temperature)

Chlorophyll-specific C¹⁴ uptake rates under nutrient-replete, light-saturated conditions are used to quantify a phytoplankton population's P_{max} , or maximal production rate. Plots of P_{max} against ambient water temperatures are used to identify T_{opt} , or the optimum temperature associated with the highest values of P_{max} , identified here as P^*_{max} . Both P^*_{max} and T_{opt} are used in water quality models to estimate the phytoplankton photosynthesis and growth under various conditions. P^*_{max} is multiplied by light, nutrient, and temperature coefficients to account for the effects of these factors on phytoplankton photosynthesis and growth. T_{opt} is incorporated into functions that describe the effect of temperature on P_{max} above and below T_{opt} .

 P_{max} and T_{opt} for natural Chesapeake phytoplankton populations were determined from approximately 5,900 volume-specific primary production measurements ($\mu g C^{14}$ fixed $l^{-1} hr^{-1}$) normalize to the amount of chlorophyll *a* present ($\mu g C^{14}$ fixed $\mu g chl a^{-1} hr^{-1}$). The production measurements were made by the Maryland (1984-2004) and Virginia (1995-2004) CBP monitoring programs in light-saturated chambers at ambient water temperatures. A recent analysis of the1984-2002 CBP primary production monitoring data suggests that P_{max} estimates are affected by shipboard methodology, specifically the length of time collected samples are held prior to C¹⁴ incubation and the light and temperature conditions in which they are held (Buchanan et al. 2005). This chapter further explores the unintentional but valuable experiment performed by the Maryland and Virginia laboratories, and describes how pre-incubation history altered C¹⁴ uptake rates of natural Chesapeake phytoplankton populations in light-saturated incubation chambers.

4.1. Maryland and Virginia methods

Water samples for productivity measurements are collected with comparable methods in the field by Morgan State University Estuarine Research Center, formerly the Academy of Natural Sciences Estuarine Research Center ("Maryland") and Old Dominion University ("Virginia"). Both laboratories acclimate their productivity samples to saturating light intensities at ambient water temperatures for approximately one hour in the incubation chambers prior to C^{14} inoculation, and their incubation methods are comparable. There are two major differences: the size of the water samples from which the subsamples are taken and the conditions under which the samples are held prior to incubation. The Maryland samples are 15 liters, and they are held in varying light and temperature conditions during the transition from field to incubation chamber. The Virginia samples are 1 liter, and are held in iced coolers in the dark between field and incubation chamber. Other differences in the two laboratories' techniques are relatively minor. Lacouture (2003), Lacouture (2001), Marshall (2001), and Marshall and Nesius (1996) have described the two protocols. The following descriptions are taken from these sources and from conversations with MSU staff Richard Lacouture and Ann Marie Hartsig.

4.1.1. Maryland field protocol

At each station, samples are pumped from five discrete depths in the surface layer above the pycnocline (or top half of the water column if a pycnocline is not evident) and composited in a 20-gallon carboy on shipboard. Two composite samples are collected at each station. Carboys are held in varying conditions, for <1 to >6 hours, depending on station, but procedures are consistent for each station. Pre-incubation history can be classified into four groups:

- **Group A** Samples are immediately put in a shipboard incubator chamber.
 - TF1.5 Patuxent River, tidal fresh
 - CB1.1 mouth of the Susquehanna River, tidal fresh
 - CB2.2 upper Bay mainstem, oligohaline
 - CB3.3 middle Bay mainstem, mesohaline
 - WT5.1 Baltimore Harbor, mesohaline
- Group B Unprotected samples are taken immediately to shore, then transported ~1 2 h in a van to the laboratory where they are placed immediately in the laboratory incubator. Air temperature in the van is somewhat warmer than ambient in winter. The van was not air conditioned in summer until recently, and summer samples could experience very warm temperatures. The samples also experience extremes in light conditions, from direct sunlight on shipboard to the interior darkness of a van.

TF2.3 (~2 h) - upper Potomac River, tidal fresh

- LE2.2 (~1 h) lower Potomac River, mesohaline
- CB5.2 (~1 h) middle Bay, mesohaline
- **Group C** Samples are kept on shipboard in an unshaded, ambient temperature water bath for $\sim 1 5.5$ h, then put in a shipboard incubator.
 - TF1.7 (~1 h) middle Patuxent River, oligohaline
 - LE1.1 (~2.5 h) lower Patuxent River, mesohaline
 - CB4.3 (~5.5 h) middle Bay mainstem, mesohaline
 - **Group D** Samples are held unprotected on shipboard & van (~5 h) or in the van (~3-4 h), then placed in the incubator. Samples experience temperature conditions that differ from the ambient, i.e. typically colder in winter and hotter in summer. The van is heated in winter but not air conditioned in summer until recently. The samples also experience extremes in light conditions, from direct sunlight on shipboard to the interior darkness of a van.

ET5.1 (~ 3 h) - upper Choptank, oligohaline ET5.2 (~ 4 h) - lower Choptank, mesohaline RET2.2 (~ 5 h) - middle Potomac River, oligohaline

4.1.2. Maryland incubation protocol

Productivity incubation is performed either on shipboard, on a dock, or immediately upon returning to the laboratory. The following description is from Lacouture (2003). Four 100 ml subsamples per station are decanted from the two surface-layer composite samples into sample-rinsed Pyrex milk dilution bottles (or polycarbonate bottles after July, 1989). One is used for a time-zero C¹⁴ blank (t₀), one for an alkalinity determination, and one from each composite for C¹⁴ incubation. The incubation samples are placed in a constant light incubator (>250 μ E m⁻² sec⁻¹) receiving running water maintained at ambient temperature for an acclimation period of more than 0.5 h, and usually about 1 h. A 1-ml innoculate of 1-2 μ Ci labeled NaHCO₃ is added to each sample, and the samples are returned to the incubator for >1 h. After incubation, 15 ml is filtered through a 0.45 μ m Millipore membrane filter, rinsed with filtered sample water, and fumed over concentrated HCl. Fifteen ml of the t₀ sample is similarly filtered and fumed, immediately following the addition of the radioisotope. The filters are placed in scintillation vials and stored in a freezer. Scintillation cocktail (Aquasol from August 1984 to October 1994;

Cytoscint from October 1994 to present) is added to the scintillation vials and the samples are run on a Packard Tri-Carb 2500TR Liquid Scintillation Analyzer equipped with internal quench standards and serviced once a year by the Packard technician.

Field stock solutions of radio-labeled NaHCO₃ are obtained from mixing portions of 25 mCi C¹⁴ NaCO₃ stock solutions with pH of 10-10.2 de-ionized water. Final field stock activities approximate 2 μ Ci C¹⁴ per ml, determined from liquid scintillation counting of field stocks in phenethylamine and Biofluor. Field stock activities for each dilution are then recorded in a laboratory log and are assigned a date interval corresponding to the period that the field stock is employed in the program.

Carbon fixation rates are calculated according to a formula in Strickland and Parsons (1972):

 $\begin{array}{l} mgC \ m^{-3} \ h^{-1} = \left[(dpm1 - dpm0) * (100/V) * 1.05 * CA \right] / (Rs/t_1) \\ \ where \ dpm1 = \ dpm \ of \ replicate \ 1 \ sub-sample \\ \ dpm0 = \ dpm \ of \ time \ zero \ sub-sample \\ V = volume \ of \ sub-sample \ filtered \\ CA = \ carbonate \ alkalinity \ in \ mg \ C \ m^{-3} \\ Rs = \ total \ C^{14} \ dpm \ in \ 100 \ ml \ sample \ (dpm \ of \ stock \ solution) \\ t_1 = \ incubation \ time \ (hrs) \ for \ replicate \ 1 \ sub-sample \end{array}$

Chlorophyll-normalized rates are determined from the ratio of the carbon fixation rate and chlorophyll *a* concentration measured for each composite sample.

4.1.3. Virginia field protocol

Water is pumped from five discrete depths above the pyncoline (or from top half of water column if pynocline is absent) and composited in a carboy. Two composite samples are collected at each station. Two 1-liter water samples are obtained from each of the two carboys (total of 4 per station) and placed in an iced cooler until their return to the laboratory. The Virginia protocol holds all its samples for <1 to >6 hours on shipboard. The Virginia samples were put into a single category, **Group E**, for purposes of comparing short-term acclimation effects.

4.1.4. Virginia incubation protocol

Productivity incubation is performed immediately upon returning to the laboratory. Two 100 ml subsamples are obtained from each station's two 1-liter composite samples (four from each station). One is used for an alkalinity determination, one is used for a time-zero C^{14} blank (t_0) after acclimation, and one from each composite is used for the C^{14} incubation. Samples are placed in a water bath equipped with a bottle holder which rotates between banks of cool-white fluorescent lights. The light levels exceed the light saturation point of the phytoplankton. Temperature of the water bath is the same as the temperature at each station when the samples were taken. After one hour of acclimation, the bottles are inoculated with 2-5 Ci C¹⁴ NaHCO₃. One of the samples is analyzed for C¹⁴ activity immediately (time 0 sample). Two samples are returned to the water bath for approximately one hour. All inoculated samples are filtered through a 25 mm 0.45 pore-size Millipore filter under a vacuum pressure < 5 cm Hg pressure. The Millipore filters are fumed over concentrated HCl for 30 seconds and placed in scintillation

vials. Scintillation fluid is added to each vial and C^{14} activity is determined using a Beckman Model LS 1701 scintillation counter.

An estimation of the hourly carbon fixation rates is calculated using formulas from Strickland and Parsons (1972). Chlorophyll concentration is determined from the composite samples or from surface layer fluorometric chlorophyll readings at the station.

The amount of C^{14} in the stock bottle is determined by placing 20-50 µl of stock solution in scintillation vials containing 0.5 ml phenethylamine. Scintillation fluid is added to the vials, set in the dark overnight, and analyzed for C^{14} activity.

4.2. Analysis

The large number of productivity records (n ≈ 5900) available in the 1984 - 2004 Chesapeake monitoring database makes it possible to explore P_{max} across a range of ambient water temperatures and salinities, by month or season, and to examine the short-term effects of several temperature and light exposure regimes prior to incubation. Samples were collected throughout the year, so an ambient water temperature range of 0 °C to >30 °C is found in the data. An abbreviated range of estuarine salinities is experienced in Maryland samples because the Maryland portion of Chesapeake Bay usually does not experience polyhaline salinities (>18 ‰). Virginia experiences the full range of estuarine salinities.

The data were grouped by season, salinity, ambient temperature, and short-term exposure conditions. Dividing the data into two seasonal groups helped to account for taxonomic differences between the diatom-dominated winter and spring communities (January-May) and the more mixed summer and autumn communities (June-December). This division also matches two of the three phytoplankton data groups in the Chesapeake Water Quality Model (Cerco 2000, Cerco and Noel 2004). The salinity (tidal fresh, <0.5 %; oligohaline, >0.5 - 5.0 %; mesohaline, >5 - 18 %; polyhaline, >18 %) and temperature (≤ 2.5 °C, >2.5 - 5.0 °C, >5.0 - 7.5 °C, etc.) groupings reduce variability caused by the natural environment. The data were further sorted into five exposure groupings according to their short-term temperature and light exposure prior to incubation: Group A, B, C, D, and E (above). The 5th%, 25th%, median, 75th%, and 95th% within each seasonal-salinity-temperature-exposure group were calculated and the medians compared using the Wilcoxon Sign Rank test.

4.3. Results

4.3.1 Neighboring stations

A straightforward comparison of monitoring results from the adjacent Maryland station CB5.2 (1984 - 2001) and Virginia station CB6.1 (1989 - 2001) illustrates how the different shipboard methodologies prior to incubation could be affecting productivity measurements. CB5.2 is a Group B station and CB6.1 is a Group E station. Measured carbon fixation (volume-specific C¹⁴ assimilation rate) and productivity (chlorophyll-specific C¹⁴ assimilation rate) are significantly higher at the Maryland station between late spring and early autumn (Figure 4-1). Maryland productivity parallels the ambient surface layer water temperature changes while the Virginia productivity shows no sensitivity to ambient temperature. The two stations have similar water quality conditions, with average ambient chlorophyll *a* concentrations that track each other closely.

4.3.2. Group A

The phytoplankton samples of Group A are put immediately into incubation chambers and have little or no exposure to conditions other than ambient temperatures and saturating light intensity after they are drawn from the water column. Group A is considered to have productivity rates that most closely represent P_{max} , and the data are used as a reference against which all other groups are compared and short-term exposure effects are assessed. The 1984-2004 data in Group A span the range of salinities experienced in Maryland: TF1.5 (Patuxent) and CB1.1 (upper Bay) are tidal fresh, CB2.2 (upper Bay) is oligohaline, and CB3.3 (middle Bay) and WT5.1 (Baltimore Harbor) are mesohaline. There are few polyhaline results for Maryland, so Maryland mesohaline results are used as the polyhaline surrogate for now. Figure 4-2 compares the data distributions in Group A with those in the other season-salinity-temperatureexposure groupings. The T_{opt} ranges and the median and maximal assimilation ratios at T_{opt} determined from Group A results are given in Table 4-1.

Season and salinity-related effects are apparent in Group A. In tidal fresh waters, median rates of the January-May seasonal group level off between 12.5 and 25 °C with T_{opt} probably at 17.5-20 °C. Median rates of the June-December group level off between 25 and 30 °C with T_{opt} at 25-27.5 °C. In oligonaline waters, Group A rates show no leveling off at higher temperatures, and peak in the 17.5-20 °C interval in January-May and in the 25-27.5 °C interval in June-December. In mesohaline waters, rates of the January-May seasonal group in Group A level off between 12.5 and 20 °C with T_{opt} apparently in the 15-17.5 °C interval. Rates of the June-December group level off between 20 and 27.5 °C with T_{opt} probably at 25-27.5 °C.

4.3.3. Group B

The Group B samples experience warmer than ambient temperatures in winter and sometimes in summer for ~1 h (mesohaline stations LE2.2 and CB5.2) or ~2 h (tidal fresh station TF2.3) while enroute in the van to a laboratory incubation chamber. Group B samples tended to have higher productivity rates than Group A at the tidal fresh station TF2.3, where samples were held for a longer period in the van. Specifically, Group B rates were higher than those in Group A at 5-7.5 °C (p=0.078), 10-12.5 °C (p<0.01), and 15-17.5 °C (p=0.096) in the January-May season, and at 22.5-25 °C (p<0.01) in the June-December season. At the mesohaline stations, Group B rates were not significantly different from the Group A rates (p>0.05).

4.3.4. Group C

Group C samples experience either direct solar radiation levels or partial shading while being held in ambient temperature water baths for $\sim 1 - 5.5$ h prior to shipboard incubation. There were typically no significant differences between Group A and C productivity rates across the range of temperatures. There was one exception in January-May when Group C was somewhat higher (5-7.5 °C mesohaline, p=0.08) and one exception in June-December when Group C was somewhat lower (17.5-20 °C mesohaline, p=0.03). These exceptions are probably not ecologically significant since they produce weak changes in opposite directions.

4.3.4. Group D

The Group D samples are stored unprotected on shipboard and in a van for up to 5 hours prior to incubation. They are not kept in ambient temperature water baths, and they experience a wide range of light conditions. The oligohaline upper Choptank station ET5.1 has the shortest exposure to surface light and temperature conditions (~ 3 h) while the oligohaline middle Potomac station RET2.2 has the longest exposure (~ 5 h). The January-May Group D rates were not significantly different from the Group A rates except for the tidal fresh rates at 12.5-15°C, which were significantly lower (p<0.01). In the June-December season, there were many significant differences between the two groups, especially at higher temperatures. Group D rates were significantly lower in the tidal fresh at 25-27.5 °C and 27.5-30 °C (p<0.01), in the oligohaline at 22.5-25 °C (p=0.04), 25-27.5 °C (p<0.01), and 27.5-30 °C (p=0.09), and in the mesohaline at 15-17.5 °C (p=0.07), 17.5-20 °C (p=0.08), 20-22.5 °C (p=0.045), and 25-27.5 °C (p<0.01).

4.3.5. Group E

The Group E samples are placed immediately in an iced cooler in the dark after collection, and are held there for about 1- 6 hours prior to incubation. The productivity rates of these samples showed the largest and most numerous differences from the Group A rates (Table 4-2). Group E rates are sometimes higher than Group A rates when ambient temperatures are below 10°C, and they are consistently lower than Group A rates when ambient temperatures are greater than 20°C.

4.3.6. Water quality effect

A water quality effect on P_{max} of natural Chesapeake Bay populations was not evident in groups A and C, the samples least disturbed by shipboard methodology. Very degraded conditions have excess nitrogen (>>0.07 mg DIN 1⁻¹), excess phosphorus (>>0.007 mg PO₄ 1⁻¹), and poor water clarity. Least impaired conditions have limiting nitrogen, limiting phosphorus, and adequate light. Data within narrow temperature ranges near T_{opt} were examined, i.e. 15-20 °C winter-spring seasonal group, >20°C summer-autumn seasonal group. The P_{max} values of groups A and C samples obtained from very degraded water quality conditions were not significantly different from those obtained from the least degraded conditions (p<0.05). Buchanan et al. (2005) also observed no significant differences in P_{max} that could be related to water quality condition.

4.4. Discussion

Group A productivity measurements are least disturbed by pre-incubation temperature and light exposure, and therefore are most representative of P_{max} . T_{opt} are seen in the Group A data at ~18.8 °C (tidal fresh, oligohaline) and ~16.3 °C (mesohaline) in the January-May season and at ~26.3 °C (tidal fresh, oligohaline, mesohaline) in the June-December season (Table 4.1). The spring T_{opt} values are slightly higher than the Chesapeake Bay Eutrophication Model's 16 °C spring T_{opt} ; the summer T_{opt} value is slightly higher than the model's 25 °C summer T_{opt} (from Cerco and Noel 2004, Table 10-1).

Maximal (95th%) rates of Group A's chlorophyll-specific productivity tended to be highest in mesohaline salinities. Spring maximal (95th%) productivity at or near T_{opt} were ~14.6

 μ g C μ g Chl⁻¹ h⁻¹ in tidal fresh, ~13.9 μ g C μ g Chl⁻¹ h⁻¹ in oligohaline, and ~19.4 μ g C μ g Chl⁻¹ h⁻¹ in the mesohaline. Summer maximal rates were ~13.8 μ g C μ g Chl⁻¹ h⁻¹ in tidal fresh, ~17.7 μ g C μ g Chl⁻¹ h⁻¹ in oligohaline, and ~18.1 μ g C μ g Chl⁻¹ h⁻¹ in the mesohaline. These values are used to represent P*_{max}. They are equivalent to average daily rates of about 336, 384, and 456 μ g C μ g Chl⁻¹ d⁻¹ for tidal fresh, oligohaline, and mesohaline, respectively. The tidal fresh daily rate is roughly comparable to the 300 and 350 g C g Chl⁻¹ d⁻¹ P*_{max} values used for spring and summer, respectively, in the Chesapeake Bay Eutrophication Model for all salinities (Table 10-1, Cerco and Noel 2004). However, the monitoring-based oligohaline and mesohaline P_{max} values are higher.

The observed effects of temperature on P_{max} above and below T_{opt} are somewhat different from the modeled effects in the Chesapeake Bay Eutrophication Model. Cerco (p.5-6, 2000) and Cerco and Noel (p.189, 2004) quantify temperature effects above and below T_{opt} as follows:

$$f(T) = e^{-KTg_{loc}(T-T_{opt})^2}$$
 when $T \le T_{opt}$

$$= e^{-kTy_{2\bullet}(T_{opt}-T)^2} \quad \text{when } T > T_{opt}$$

T = temperature (°C) $T_{opt} = optimal temperature for algal growth (°C)$ $KTg1 = effect of temperature below T_{opt} on growth (°C⁻²)$ $KTg2 = effect of temperature above T_{opt} on growth (°C⁻²)$

The following values for these variables were used in the earlier version of the Eutrophication model (Table 2, Cerco 2000):

Spring (January - May): $T_{opt} = 20 \text{ °C}$; KTg1 = 0.0025, KTg2 = 0.012Summer (June - December): $T_{opt} = 25 \text{ °C}$; KTg1 = 0.0025, KTg2 = 0.01

 P_{max}^* is assigned a value of 300 g C g Chl⁻¹ d⁻¹, or 12.5 g C g Chl⁻¹ h⁻¹. The variable values were changed in the 2002 version of the Eutrophication model (Table 10-1 in Cerco and Noel 2004):

Spring (January - May): $T_{opt} = 16 \text{ °C}$; KTg1 = 0.0018, KTg2 = 0.006; $P*_{max} = 300 \text{ g C g}$ Chl⁻¹ d⁻¹, or 12.5 g C g Chl⁻¹ h⁻¹. Summer (June - December): $T_{opt} = 25 \text{ °C}$; KTg1 = 0.0035, KTg2 = 0; $P*_{max} = 350 \text{ g C g}$ Chl⁻¹ d⁻¹, or 14.6 g C g Chl⁻¹ h⁻¹

The P_{max} vs temperature curves generated from these equations and values are shown in Figure 4-3 where they are compared to curves generated from the monitoring results. In the January-May season, tidal fresh and oligohaline P_{max} values at temperatures below ~12.5 °C are overestimated by both the Cerco 2000 and Cerco and Noel 2004 curves, and the mesohaline P_{max} values at temperatures below ~15 °C are overestimated by the Cerco and Noel 2004 curve. In the June-December season, tidal fresh and oligohaline P_{max} values are fairly well represented by any of the curves; however, mesohaline P_{max} values at all temperatures are very noticeably underestimated.

Earlier comparisons of Maryland and Virginia data from the adjacent stations CB5.2 and CB6.1 (Figure 4-1), and chlorophyll-specific C^{14} fixation measurements grouped by state-season-

salinity-habitat category (Buchanan et al. 2005, Figure 2) indicated that Maryland and Virginia protocols can yield significantly different production rates. In this analysis, all variations of the Maryland protocol (Groups A-D) produce significantly higher (p<0.05) volume-specific and chlorophyll-specific C¹⁴ fixation rates when ambient temperatures were >15 °C (Figures 4-2). Maryland productivity parallels the ambient surface layer water temperature changes while the Virginia productivity shows no sensitivity to ambient temperature. The sharp decrease in temperature experienced by Virginia samples in the iced cooler during spring, summer, and autumn are apparently sufficient to depress productivity, even after the samples are returned to ambient temperatures in incubation chambers. Virginia rates in the January-May groups were at times significantly higher than Maryland's when ambient temperatures were below 7.5 °C. We have no hypothesis to explain this.

Station-related differences in the Maryland protocol (Group A-D) were sufficient to introduce variability in Maryland's productivity measurements. Samples held for 1-2 hours in a van with heating but no air-conditioning prior to incubation (Group B, or stations TF2.3, LE2.2, CB5.2) tended to have higher rates than Group A in the hottest months of the year. Samples kept on shipboard in ambient temperature water baths (Group C, or stations TF1.7, LE1.1, CB4.3) had productivity rates similar to those in Group A, even when the samples were exposed to incident solar radiation for up to 5 hours before incubation. Samples that experienced varying temperatures in the van for more than 2 hours (Group D, or stations ET5.1, ET5.2, RET2.2) sometimes had higher productivity rates in the cold months (van heated) and almost always had higher productivity rates in the hot months (van without air-conditioning). Exposure to changing temperatures for more than 2 hours prior to incubation seems to be the proximal cause of the observed variability in productivity. Increasing temperatures prior to incubation tends to heighten productivity in the incubation chambers when ambient water temperatures are <10 °C while increasing temperatures tends to depress productivity when ambient water temperatures are >20 °C.

4.4 Summary

- Large temperature fluctuations in the hours preceding C^{14} incubation in light-saturated, ambient-temperature chambers appears to cause significant variability in the CBP productivity data. Exposure to direct solar radiation prior to incubation does not appear to be a cause of variability. A water quality (nutrient and water clarity) effect on the P_{max} of natural Chesapeake Bay populations was not evident.
- Exposure to increasing temperatures for more than 2 hours prior to incubation heightens productivity when ambient water temperatures are <10 °C and depresses productivity when ambient water temperatures are >20 °C. Exposure to iced conditions prior to incubation depresses productivity at ambient temperatures >20 °C.
- Group A productivity measurements are least disturbed by pre-incubation temperature and light exposure, and therefore are most representative of P_{max} . T_{opt} are seen in the Group A data at ~18.8 °C (tidal fresh, oligohaline) and ~16.3 °C (mesohaline) during the January-May season and at ~26.3 °C (tidal fresh, oligohaline, mesohaline) during the June-December season.

- Group A P_{max}^* (P_{max} at T_{opt}) are about 336, 384, and 456 µg C µg Chl⁻¹ d⁻¹ in the tidal fresh, oligohaline, and mesohaline salinity zones, respectively.
- The observed temperature effects on P_{max} above and below T_{opt} are somewhat different from the modeled temperature effects in the Chesapeake Bay Eutrophication Model (Cerco 2000, Cerco and Noel 2004). This is particularly true in the summer mesohaline where the model underestimates P_{max} at all temperatures.

Table 4-1. The temperature interval with the apparent optimal temperature (T_{opt}) , and the highest median and maximal (95th%) values of chlorophyll-specific productivity (μ g C fixed μ g Chl⁻¹ h⁻¹) observed in that interval.

Salinity Zone	Tidal Fresh	Oligohaline	Mesohaline
Seasonal Group	(<u><</u> 0.5 ‰)	(>0.5 - 5 ‰)	(>5 - 18 ‰)
January - May	17.5 - 20 °C	17.5 - 20 °C	15 - 17.5 °C
	median 4.9	median 6.4	median 5.9
	maximal 14.6	maximal 13.9	maximal 19.4
June - December	25 - 27.5 °C	25 - 27.5 °C	25 - 27.5 °C
	median 6.9	median 8.1	median 7.9
	maximal 13.8	maximal 17.7	maximal 18.1

Table 4-2. Significant differences between Group A and Group E productivity rates (Wilcoxon Sign Rank test). Key: ns, no significant difference; *, p<0.05; **, p<0.01; -, insufficient data in one or both groups (n \leq 5); \uparrow , Group E is higher than Group A; \downarrow , Group E is lower than Group A. Group A samples are put immediately in a shipboard incubation chamber; Group E samples are held in an iced cooler for <1 to >5 hours prior to incubation.

Seasonal Group	Temp (°C)	Tidal Fresh	Oligohaline	Mesohaline
January - May	0 - 2.5	-	-	ns
	2.5 - 5	** ↑	-	p=0.06↑
	5 - 7.5	** ↑	-	ns
	7.5 - 10	p=0.10↑	-	ns
	10 - 12.5	ns	-	ns
	12.5 - 15	-	-	ns
	15 - 17.5	p=0.08↓	-	p=0.03↓
	17.5 - 20	-	-	ns
	20 - 22.5	-	-	-
June - December	2.5 - 5	-	-	-
	5 - 7.5	-	-	ns
	7.5 - 10	-	-	ns
	10 - 12.5	-	-	ns
	12.5 - 15	-	**↓	**↓
	15 - 17.5	ns	-	p=0.07↓
	17.5 - 20	ns	ns	**↓
	20 - 22.5	**↓	**↓	**↓
	22.5 - 25	**↓	**↓	**↓
	25 - 27.5	**↓	**↓	**↓
	27.5 - 30	**↓	**↓	**↓
	>30	-	-	-

Figure 4-1. Comparison of CB5.2 and CB6.1, neighboring Chesapeake Bay mainstem stations in Maryland and Virginia, respectively, between 1984 and 2001. Top panel: monthly average insolation (light), surface layer temperature, and production ($\mu g C^{14}$ fixed $\mu g Chl^{-1} h^{-1}$) in light-saturated ambient temperature incubation chambers. Bottom panel: monthly average chlorophyll *a* ($\mu g Chl^{-1} l^{-1}$) and carbon fixation ($\mu g C^{14}$ fixed $l^{-1} h^{-1}$) at each station. Monthly average water temperature for the surface mixed layer was calculated from the water quality monitoring data base and monthly average insolation was obtained from Fisher et al. 2004.



Figure 4-2. Chlorophyll-specific C¹⁴ assimilation rates ($\mu g C^{14}$ fixed $\mu g Chl a^{-1} h^{-1}$) in samples collected at CBP biomonitoring stations and exposed to various light/temperature conditions prior to incubation in light-saturated chambers maintained at ambient temperatures. The 5th%, 25th%, median, 75th%, and 95th% in each 2.5-°C interval are indicated. If n≤5, data not shown; if n=6 or 7, only medians are shown. Dashed line is for ease of comparison and indicates the highest median value in each season-salinity zone's Group A. The polyhaline dashed line is set equal to the mesohaline line. See text for further details.



Figure 4-2 (cont.).



Ambient Water Temperature (oC)

Figure 4-3. Temperature effects on P_{max} . See Figure 4-2 heading for details. Solid gray line: P_{max} temperature response curves used in Cerco (2000); solid black line: P_{max} temperature response curves used in Cerco and Noel (2004); dashed line: "envelope" around Group A's observed P_{max} values in each season-salinity group, as determined by a best-fit line through the 95th percentiles of the productivity values of each 2.5°C temperature interval (0-2.5°C, 2.5-5°C, etc.).



5. Photoinhibition in *in situ* phytoplankton populations

based on the Master's Thesis work of Kim Mikita Penn, with contributions from Deborah Tipton (formerly ICPRB) and Cherie Schultz (ICPRB)

Information about photosensitivity is potentially useful to water quality models that strive to predict oxygen production and plant growth. Phytoplankton photosensitivity, or the ability of algal cells to harvest light energy, directly impacts the cellular oxidation of H_2O to form O_2 and the creation of high-energy chemical bonds in adenosine diphosphate (ADP) and nicotinamideadenine dinucleotide phosphate (NADP), also known as the electron carriers. These compounds mediate the production new algal biomass from CO_2 and H_2O elsewhere in the cell. The photosensitivity of most algal taxa can be measured with fluorometers, which use chlorophyll fluorescence to measure the amount of light-reactive chlorophyll molecules in cells (e.g., Kolber and Falkowski 1993, Falkowski and Raven 1997, others). Fluorometers induce light-reactive chlorophyll molecules to fluoresce by shining 470 nm (blue) light into the water and then measuring the 670 nm (red) light that is returned. Fluorescence signifies the capture of light photons by "open" Photosynthesis II (PSII) reaction centers, which are clusters of photopigments and proteins in the thylakoid membranes of chloroplasts. Both phytoplankton biomass and photosensitivity can be accurately estimated from fluorescence measurements, despite some method limitations (http://www.ysi.com/environmental.htm).

Phytoplankton photosensitivity is suppressed by irradiance levels greater than Ik, the saturation light intensity (photoinhibition). A large bombardment of photons temporarily inactivates PS II reaction centers and/or damages them by denaturing their proteins (Falkowski and Kiefer1985, Kirk 1994). Recovery from temporary inactivation can take nanoseconds while recovery from photodamage can take hours. A number of studies in shallow water environments where irradiance levels become high during the day have observed large mid-day depressions in chlorophyll fluorescence, signifying photoinhibition (Falkowski and Kolber 1995, Behrenfeld et al. 1998, Kinkade et al al. 1999). Evidence of photoinhibition is found in the Mikita (2002) *in situ* measurements in surface waters of the Patuxent River (Figure 5-1). The frequency of depressed mid-day fluorescence chlorophyll values suggests phytoplankton photoinhibition is common in Chesapeake surface waters.

In situ surveys of chlorophyll fluorescence have been, or are being, done recently in Chesapeake Bay using moored data sondes. These include Mikita (2002), the Chesapeake Bay Observing System (<u>http://www.cbos.org/index.php</u>), the Virginia Institute of Marine Science Real Time Data Buoy (<u>http://www.vims.edu/realtime/</u>), and the Maryland Eyes on the Bay (<u>http://mddnr.chesapeakebay.net/eyesonthebay/index.cfm</u>). Although the data were collected for the purpose of estimating phytoplankton chlorophyll *a* concentrations and phytoplankton biomass, some can be used to quantify phytoplankton photosensitivity. A comparison of day-time fluorescence levels with night-time levels, when the cells are maximally photosensitive, indicates the degree to which phytoplankton cells are photoinhibited. Phytoplankton photosensitivity can then be tracked in different natural environments, over the course of a day.

5.1. Data collection

The following analysis is based in large part on the chlorophyll fluorescence data collected by Kimberley Mikita and colleagues, for her master's thesis with Dr. Walter Boynton, Chesapeake Biological Laboratory, Solomons Island, MD (Mikita 2002). The data were

collected at a station (38° 23.396N, 76° 31.985W) located off Broome's Island in the Patuxent River, a moderately sized, western sub-estuary of the Chesapeake Bay, in Maryland (Fig. 1-1). The data were collected during the spring bloom period, between March 2 and May 31, 2001, using a moored sonde equipped with an unshielded fluorometer sensor, i.e., exposed to direct irradiance. Mikita's results are compared to spring 2002 results of two Maryland Department of Natural Resources (MDDNR) moored sondes with shielded sensors: the Severn River near Sherwood and the Magothy River near Stonington.

5.1.1. Mikita data (from Mikita 2002)

Fluorometer chlorophyll readings were collected every 15 minutes by two moored Yellow Spring Instrument (YSI) model 6600 data sondes positioned at 0.5 meter depth ("surface") and 1 m above the river bottom ("bottom"). The sensors in the sonde were not shielded from the ambient light environment (Mikita, pers. comm.). The fluorescence sensor was calibrated regularly using rhodamine WT dye (2 point calibration method). Other parameters measured included date, time, water temperature, specific conductance, salinity, dissolved oxygen concentration and percent saturation. Parameters were also measured at weekly intervals at the buoy location using a 5 liter Niskin bottle. They included Secchi depth, water column light flux (measured at 3 depths with a LiCor LI-192SA meter), total suspended solids, nutrients, chlorophyll *a*, dissolved oxygen, water column respiration rates, phytoplankton and mesozooplankton species composition and abundances. High frequency (15 minute) measurements of incident photosynthetically active radiation (PAR) were made with a LiCor Quantum sensor model LI-190SZ at the Chesapeake Biological Laboratory's weather station in Solomons, MD, 10 km south of the buoy.

Mikita's station is in a region of the sub-estuary that has a maximum depth of 16 m, an average depth of 4 m, and a width of 2.5 km at the surface. It is near the Chesapeake Bay Program (CBP) monitoring station LE1.2, which was characterized by high mesohaline salinities (>10 ppt) and a median Secchi depth of 0.9 m (range: 0.05 - 2 m) in the spring of 2001. The estimated depth of the euphotic zone (1% incident light) at Mikita's station ranged from 2.3 to 5.5 m, indicating the surface senor located at 0.5 m was consistently in the euphotic zone. The mean amount of light received by phytoplankton population in the station's well mixed water column was estimated to be approximately 22 μ E m⁻² s⁻¹.

This analysis focused on the spring bloom initiation and peak periods, which occurred between March 2 and 28. A highly significant (p<0.001, $r^2 = 0.985$) linear regression between daytime chlorophyll measurements determined with acetone extraction and the average of the previous nights' chlorophyll fluorometric measurements indicated the latter were good surrogates for the former in daytime. This was possible because the nighttime fluorescence readings indicated total chlorophyll concentrations did not vary dramatically from day-to-day at the station.

5.1.2. Maryland Department of Natural Resources

MDDNR deployed several YSI 6600 data sondes at Chesapeake Bay locations in the spring of 2002. Most were positioned at a constant depth of 1 meter below the surface of the water. This suspension was achieved by hanging the meters from a float *inside* PVC pipes that had 3" holes drilled along their length to allow for water exchange. The sensors, therefore, were

shielded from both direct and most indirect lighting and phytoplankton cells had an undetermined amount of time inside the PVC pipe to begin recovery from high light intensities before they were stimulated to fluoresce. Parameters were measured every 15 minutes and included water temperature, salinity, dissolved oxygen (DO) saturation, DO concentration, pH, turbidity, and chlorophyll fluorescence. These data can be downloaded from the MDDNR web site www.http://mddnr.chesapeakebay.net/eyesonthebay/index.cfm. The sondes were retrieved weekly to clean the sensors, recalibrate the data loggers and download stored data. The fluorometric sensors were recalibrated regularly. Weekly water samples were also taken at each location and the results used to calibrate the meters and to check the YSI data for accuracy. Parameters included total and dissolved fractions of nitrogen and phosphorus, dissolved organic carbon, total suspended solids, and chlorophyll *a*. The extractive measurements were made in the Magothy between 7:45 and 11:00 with one exception, and in the Severn between 11:00 and 14:15. The weekly data sets were obtain through William Romano, MDDNR.

MDDNR spring 2002 chlorophyll fluorescence data for the Severn River near Sherwood and the Magothy River near Stonington were analyzed. The 15 min data points were timematched to the hourly averages of incident PAR values measured at the nearby Smithsonian Estuarine Research Center (SERC) as follows: 1:30, 1:45, 2:00, and 2:15 fluorescence chlorophyll data were matched with the 2:00 average PAR value, etc. Both stations had salinities between 7 and 11 ppt in the spring of 2002. The Severn station is 3.2 m deep at mean low tide, and had a Secchi depth of ~1.05 m in spring 2002. The Magothy station is 1.9 m deep at mean low tide, and had a mean Secchi depth of ~0.95 m. The Severn and Magothy river segments at these stations have mean depths of 3.86 m and 2.88 m, respectively (CBP 2004).

5.2. Data analysis

5.2.1. Mikita - surface data

Extractive chlorophyll *a* measurements are considered more reliable than *in situ* fluorometric chlorophyll measurements because they are performed under controlled laboratory conditions. However, high frequency fluorometric measurements can be made with little effort in the field for prolonged periods. A close correspondence between nighttime fluorometric chlorophyll measurements and the extractive method chlorophyll measurements the following day in Mikita's data (Figure 5-2) confirms that the former approximate the latter at the Patuxent location, despite sampling time differences as large as half a day. Daily changes in phytoplankton photosensitivity were examined by expressing daytime fluorometric chlorophyll estimate, i.e. 00:00 - 5:30 of the previous night and 20:30 - 24:00 of the following night.

Chlorophyll fluorescence at the 0.5 m sonde depth began to deviate from the nighttime average when incident PAR exceeded ~ 100 μ E m⁻² s⁻¹. It reached levels as low as 20% during peak mid-day intensities on clear days (Figure 5-3a), indicating phytoplankton cells were strongly photoinhibited. Chlorophyll fluorescence ranged widely on partly cloudy days (Figure 5-3b), and was nearly 100% throughout overcast days (Figure 5-3c). On most days, chlorophyll fluorescence was lower in the morning than in the afternoon, indicating the cells were acclimating to their light environments throughout the day and as a result were able to capture photon energy more effectively in the afternoon at equivalent irradiances (Figure 5-3a-c).

The diel response was consistent enough that surface layer phytoplankton

photosensitivity, expressed as the percent of nighttime chlorophyll *a* fluorescence, could be modeled based on time after dawn and incident PAR irradiances (Figure 5-4). When incident PAR remained low throughout the day (<100 μ E m⁻² sec⁻¹), the fraction of chlorophyll *a* fluorescing recovered almost linearly from a post-dawn minimum of 80% and reached 100% shortly after solar noon, which occurs approximately 6 hours after sunrise in March. At somewhat higher PAR irradiances (100 - 500 μ E m⁻² sec⁻¹), the fraction of chlorophyll *a* fluorescing begins to recover shortly after the post-dawn minimum, and reaches 100% in the late afternoon. At relatively high irradiances (>500 μ E m⁻² sec⁻¹), average % fluorescence remains at minimum levels of 30% - 40% prior to solar noon, when irradiances are increasing, and then begins to increase after solar noon, when irradiances are decreasing.

5.2.2. Mikita - bottom data

Phytoplankton chlorophyll fluorescence at the 11 m sonde depth was approximately 100% throughout the day, and typically did not drop below 90% (Figure 5-5), indicating the phytoplankton cells were not photoinhibited at this depth. A slight pattern of variability is evident in the data, which may be the result of a circadian rhythm of cell photosensitivity or a residual response of the cells circulating down from surface waters. Assuming an average extinction coefficient of 1.6 m⁻¹ (derived from a mean Secchi depth of 0.9 m), light intensities at 11 m rarely exceeded an estimated 2.4 x 10⁻⁵ μ E m⁻² s⁻¹ during the study period and cell at this depth were most likely light limited.

5.2.3. MDDNR data

Daytime depressions in fluorometric chlorophyll are seen in the MDDNR spring 2002 data for the Magothy and Severn rivers (Figure 5-6). Both nighttime and daytime fluorescence measurements are more variable in these systems than in the lower Patuxent, and night-time fluorescing chlorophyll *a* could vary significantly within and between consecutive nights. This is probably a result of Chesapeake Bay tidal influences since the Magothy and Severn stations are fairly exposed to the bay mainstem. For consistency, we continued to analyze daily changes in phytoplankton photosensitivity by expressing daytime fluorescence as a percent of nighttime fluorescence on that date (0:00 - 4:00 and 21:00 - 23:15).

The correspondence between nighttime fluorometric chlorophyll measurements and daytime extractive method chlorophyll measurements (Figure 5-7) is not as tight as that in Mikita's data (Figure 5-2). Unlike Mikita's data, the MDDNR's daytime fluorometric chlorophyll also shows a strong correspondence to daytime extractive chlorophyll measurements, and the regression line is close to the 1:1 line (Figure 5-7).

Midday depressions in phytoplankton photosensitivity occur in spring, summer and autumn although the results vary considerably (Figure 5-8). Most of the variability appears to be caused by the highly variable nighttime fluorescent chlorophyll measurements. When sections of the time series with minimal nighttime variation in night-to-night chlorophyll readings are analyzed, a more consistent pattern appears (Figure 5-9, Figure 5-10). The extent of the midday depression isn't as large as Mikita's, the depression is centered around solar noon, and chlorophyll fluorescence at dawn and dusk tend to exceed the average nighttime fluorescence. Chlorophyll fluorescence at the 1.0 m sonde depth began to deviate from the nighttime average when incident PAR exceeded ~ 900 $\mu E m^{-2} s^{-1}$.

5.3. Discussion

The daytime reduction in phytoplankton photosensitivity is the result of both photochemical and nonphotochemical changes in phytoplankton cell chloroplasts. When a chlorophyll molecule of a PSII reaction center absorbs a photon, the center "closes" for a few nanoseconds and will not absorb additional photons. The proportion of open PSII centers decreases as irradiance increases and is evident as "photochemical fluorescence quenching," or a drop in % chlorophyll fluorescence. Cells recover from this form of photoinhibition rapidly when light is dimmed.

At high irradiances, other mechanisms affecting photosynthesis and fluorescence become important and cells take much longer to recover from these forms of photoinhibition. They include photoinhibitory damage (e.g., denatured proteins in the PSII reaction center) and a variety of photoadaptation mechanisms. Photodamage results in "nonphotochemical fluorescence quenching" and recovery can involve the synthesis of new proteins to replaced damaged ones. Photoadaption includes mechanisms such as a change in the electron transport system leading to the electron acceptors NADP⁺ and ADP, a change in cellular chlorophyll concentration after repeated exposure to higher or lower light levels, or a reorganization of chlorophyll molecules in the chloroplasts. The amount of chlorophyll in a cell correlates with the total number of PSII reaction centers; high light adapted cells with fewer chlorophyll molecules have fewer centers whereas low light adapted cells with more chlorophyll molecules have more centers.

5.3.1. Photoinhibition patterns

The *in situ* results presented above show a light-driven daytime depression in chlorophyll fluorescence, and indicate a daily reduction in phytoplankton photosensitivity (Figure 5-1, 5-6). The depression occurs in surface waters of the deep station (Patuxent) and in shallow waters (Magothy, Severn), and apparently occurs year round. Departure from the maximal nighttime levels is largest when incident irradiance is highest.

At the Patuxent station monitored by Mikita (2002), comparisons between the significant daytime depressions in surface waters (Figure 5-4) and the persistent lack of a depression in bottom waters (Figure 5-5) attest to the influence of light intensity on photosensitivity. The number of fluorescing chlorophyll molecules measured at 0.5 m with an unshielded sensor begins to deviate significantly from the previous nighttime average when incident PAR reaches $\sim 100 \ \mu E \ m^{-2} \ s^{-1}$. Daytime values drop to $\sim 30\%$ of the nighttime average when incident irradiance exceeds $500 \ \mu E \ m^{-2} \ s^{-1}$. Chlorophyll fluorescence at 11 m showed no response to incident irradiance, indicating a gradient of photoinhibition occurs with the light gradient in the water column and extends down to some variable lower depth that depends on time of day. Figure 5-4 suggests the photoinhibition profile is strongest and deepest in the water column during early morning hours. The profile begins to shift upwards shortly after morning if irradiances are low (heavily overcast), and after solar noon if irradiances are high (clear). Surface and bottom sensors alone are not sufficient to test the progress of water column photoinhibition throughout the day, or to describe the photoinhibition profiles in detail.

In the shallow, well mixed Magothy and Severn systems, only a middle depth (1 m) was monitored and the results presumably reflect what is happening throughout the water column. Fluorescence minima tended to occur in late morning or at solar noon, regardless of weather conditions (Figure 5-9, 5-10). The % fluorescing chlorophyll dropped to a minimum of only ~ 60% of the nighttime average in both systems on bright days, as compared to 30% in the Patuxent surface waters. The shielding around the MDDNR sensor may be partially responsible for the higher midday photosensitive. The phytoplankton populations may also be high light adapted because they are confined to shallow waters and therefore regularly exposed to higher average daytime irradiances. Both factors are probably involved though not enough information is available to determine the relative importance of each with certainty. The issue of shielding *versus* photoadaptation could be resolved by doing side-by-side comparisons of shielded and unshielded sensor readings at several depths in deep and shallow systems, under a range of ambient light conditions.

5.3.2. Photoadaptation

Phytoplankton populations in the shallow Magothy and Severn systems are more likely to be adapted to higher light conditions than populations in the deeper Patuxent system. Median daytime PAR at 1 m in spring 2002 was estimated to be ~ 620 μ E m⁻² s⁻¹ in the Magothy and ~ 690 μ E m⁻² s⁻¹ in the Severn (assumes a Secchi depth of 0.95 m in the Magothy and 1.05 m in the Severn, with Kd = 1.43/Secchi). On May 9th, one of most overcast spring days in 2002, median daytime PAR at 1 m was estimated to be ~ 72 μ E m⁻² s⁻¹ in the Magothy and ~ 83 μ E m⁻² s⁻¹ in the Severn.

Populations at the deeper Patuxent station experienced average daytime light levels ranging from 3.4 - 46.6 μ E m⁻² s⁻¹ during spring 2001, and thus were exposed to average light levels ~1.5 orders of magnitude lower than phytoplankton populations in the two shallow systems. Exposure to near-surface irradiances represents a significant increase in the photon energy experienced by phytoplankton cells at the Patuxent station but not at the two shallow stations.

The median Chl:Cl in the above-pycnocline layer at LE1.1, a CBP monitoring station near Mikita's Patuxent station, also indicates the phytoplankton were somewhat adapted to low light. Chl:C averaged 0.0125 (80.0 C:Chl) in spring 2001, which is higher than the ~ 0.008 Chl:C (12.5 C:Chl) found in the clearest waters at Chesapeake mesohaline and polyhaline stations (see Figure 3-3).

5.3.3. Photoinhibition, photoadapation, and P_{max}

Behrenfeld et al. (1998) found that P_{max} , or the light-saturated photosynthetic rate, does not necessarily decrease as photoinhibition reduces the proportion of light-sensitive PSII reaction centers, and the phytoplankton's photoadaptive state determines whether photoinhibition affects P_{max} . Falkowski and Raven (1997), Behrenfeld et al. (1998) and others relate P_{max} to the number of functional, light-sensitive PSII centers (*n*) and the steady state, light-saturated rate of electron turnover through functional PS II reaction centers and the electron transport system ($1/\tau_{PSII}$):

$$P_{max} = n * 1/\tau_{PSII}$$

As photoinhibition reduces *n*, $1/\tau_{PSII}$ can reciprocally increase up until a maximum electron turnover rate $(1/\tau_{PSII}^*)$. The electron transport system, composed of quinones, links the PSII centers in the chloroplast thylakoids with the Calvin cycle in the chloroplast stoma. Photosynthesis is limited by enzymatic rates in the Calvin cycle until $1/\tau_{PSII}^*$ is reached, after

which it is limited by the reduction of *n* caused by photodamage.

Behrenfeld et al. (1998) found that the P_{max} of low light adapted cells from cultures and natural phytoplankton populations in the south Pacific ocean were not decreased by photoinhibitory reductions of *n* up to 52%. Reciprocal increases in $1/\tau_{PSII}$ through the remaining *n* and the electronic transport system kept P_{max} at a high level as light intensity increased. When $1/\tau_{PSII}^*$ was reached, additional reductions in *n* could no longer be countered and P_{max} began to decrease. P_{max} therefore remained uncoupled to photoinhibition until $1/\tau_{PSII}^*$ was reached. P_{max} did not exhibit a comparable plateau after Ik in high light adapted (culture) populations, but rather decreased in proportion to the decrease in light-sensitive PSII centers. Apparently $1/\tau_{PSII}$ in low light adapted cells are initially low when irradiances reach Ik and can increase in response to further reductions in *n*; $1/\tau_{PSII}$ in high light adapted cells are close to $1/\tau_{PSII}^*$.

Based on Behrenfeld et al. (1998) findings, phytoplankton in deep, well mixed water columns in the Chesapeake system might be expected to exhibit the photosynthesis-irradiance (P-I) curves of low light adapted populations. According to this scenario, production in deep or subpycnocline waters of the Chesapeake will reach lk at modest irradiances and then plateau at P_{max} for an extended range of irradiances until a second threshold is reached. The second threshold, Ik_2 , will signal the irradiance at which additional photoinhibitory reductions in the number of light-sensitive PSII reaction centers are no longer compensated by reciprocal increases in electron transfer rates. P_{max} above Ik_2 will decline in proportion to further reductions in the number of reactive PSII centers. A decline in oxygen production above Ik_2 concomitant with the declining numbers of PSII reaction centers would be expected.

The P-I curve in shallow Chesapeake water columns can be expected to respond differently because the cells are adapted to higher daytime irradiances. The initial slope of the P-I curves (α) will be lower, production will reach Ik at higher irradiances, and Ik₂ will be closer to Ik, resulting in a shortened P_{max} plateau or peak before further photoinhibition causes P_{max} to decline. A decline in oxygen production above Ik₂ would again be expected. This scenario may also represent phytoplankton at the top of a deep water column, when laminar flow or a shallow pycnocline depth maintains populations near the surface.

5.3.4. Ik and Ik₂

Onset of the daytime depression in chlorophyll fluorescence detected with an unshielded sensor may be a method of measuring Ik, the saturation intensity defined as the irradiance at which the initial slope of α intersects the value of P_{max}. We presume the chlorophyll fluorescence readings made at 0.5 m in the Patuxent with the unshielded sensor are measuring photoinhibition caused by the combined effects of short-term deactivation ("closing") of PSII centers and longer term photodamage. When incident PAR reaches ~100 µE m⁻² s⁻¹, % chlorophyll fluorescence begins to deviate noticeably from the nighttime average maximum (Figure 5-11). We interpret this to mean surface irradiances have exceeded Ik. An Ik of 100 µE m⁻² s⁻¹ is approximately 28% of the average incident daytime PAR measured 2-28 March 2001. Smith (1980) found Ik was ~30% of incident PAR in phytoplankton that had adjusted their chlorophyll cell content to ambient light conditions. The fluorescence-based Ik estimate approximates the Ik of 47.5 - 65 µE m⁻² s⁻¹ (white light) experimentally determined in the spring of 1982 for Chesapeake Bay mainstem populations (March 1982 cruise, stations I-IV, from Table 4 in Harding et al.1985). These populations were exposed to low light environments comparable to the Patuxent, with average light energies of 1.5 - 99 µE m⁻² s⁻¹ at the water column middepth.

We hypothesize that the onset of the daytime depression measured with shielded sensors in the shallow Magothy and Severn systems does not indicate Ik, but may indicate Ik₂ for high light adapted populations. The relationship Ik/Io $\approx 30\%$ presented in Smith (1980) would suggest that Ik occurs at $\sim 200 \ \mu E \ m^{-2} \ s^{-1}$ in these populations. Given the size of the covered PVC pipe shielding the sensors and the restricted water flow through the pipe, phytoplankton inside the pipe have likely recovered from photochemical inhibition and are experiencing only nonphotochemical inhibition. Chlorophyll fluorescence stimulated by a 470 nm fluorometer light beam inside the PVC pipe does not begin to deviate from the nighttime average until ambient incident PAR reaches ~900 $\mu E \ m^{-2} \ s^{-1}$, and then declines linearly as surface irradiance continues to increase. We interpret the inflection at ~900 $\mu E \ m^{-2} \ s^{-1}$ to indicate Ik₂ and the onset of photodamage to the PSII reactions centers that is not compensated by a reciprocal increase in electron transport rates. Similar productivity declines attributed to photodamage are found in lake systems beginning at ~ 200-1000 $\mu E \ m^{-2} \ s^{-1}$ (see Lampert and Sommer 1997).

The potential use of chlorophyll fluorescence to directly measure Ik and Ik_2 would need to be confirmed *in situ* or *in vitro* under different growth irradiances, with simultaneous measurements of ¹⁴C-uptake or oxygen production to generate P-I curves. We do not know if this has been accomplished yet with natural Chesapeake populations.

5.3.5. Models

The light threshold Ik is a key marker on the P-I curve. Cerco and Noel (2004) use Ik to divide the P-I curve into three regions in their Figure 10-4. For I<<Ik, or the initial slope of the P-I curve, production is strongly light limited (region A). For I < Ik, production is determined by the combined effects of temperature, nutrients and light (region B). For I>Ik, production is light saturated and nutrients and temperature are the primary controlling factors (region C). We propose the eventual use of Ik₂ as a second saturation threshold marker and the addition of a fourth region (D) for I>Ik₂, where production is increasingly controlled by light inhibition as well as nutrients and temperature. Given the frequency of phytoplankton fluorescence quenching in Chesapeake moored sonde data, photoinhibition is presently common in shallow embayments, nearshore regions, and near-surface waters of the Chesapeake Bay despite the bay's generally turbid condition. Nutrient and especially sediment reductions associated with bay restoration efforts are expected to improve water clarity and will serve to further increase the frequency and intensity of photoinhibition, as measured by chlorophyll fluorescence quenching. The expected shift in primary production from planktonic to benthic algae as water clarity improves will also be linked to phytoplankton photoinhibition. Understanding how photoinhibition affects phytoplankton P_{max} and how the relationship between the photoinhibition and production changes with light adaptation may increase the sensitivity of the Eutrophication Model's phytoplankton primary production component to future (improved) water quality conditions.

The sensitivity of future eutrophication models might be improved if Ik is made a function of the average local light conditions experienced by sampled phytoplankton population. In the current model, Cerco and Noel (2004) derive Ik from seasonal constants for P^Bm (chlorophyll *a*-specific, light-saturated photosynthesis rate at T_{opt} , under nutrient saturation) and α (chlorophyll *a*-specific initial slope of the P-I curve) as follows:

$$Ik = \frac{P^{B}m}{\alpha}$$

They then uses Ik in conjunction with $P^{B}m(N,T)$ to construct for each model cell at each time step an individual P-I curve. $P^{B}m(N,T)$ is $P^{B}m$ adjusted by temperature and limiting-nutrient coefficients. The spring algal group is assigned values of $P^{B}m = 300 \text{ g C g Ch}^{-1} \text{ d}^{-1}$ and $\alpha = 8 \text{ g}$ C g Chl⁻¹ (E m⁻²)⁻¹; the summer algal group is assigned values of P^Bm = 350 g C g Chl⁻¹ d⁻¹ and α = 8 g C g Chl⁻¹ (E m⁻²)⁻¹ (Table 10-1, Cerco and Noel 2004). The lk derived from these P^Bm and α values are 434 µE m⁻² s⁻¹ (spring algal group) and 506 µE m⁻² s⁻¹ (summer algal group). They are noticeably higher than Ik observed in the Chesapeake mainstem (Harding et al. 1985) or inferred in the Patuxent chlorophyll fluorescence data (Mikita 2002) for low light adapted populations. The adjustments to P_{max} recommended in Chapter 4 will tend to further increase model Ik values in some season-salinity zones if the equation above continues to be used as is. The elevated model Ik values may also be caused by the low value of 8 C g Chl⁻¹ (E m⁻²)⁻¹ assigned to α . The α derived from spring 1982 productivity measurements by Harding et al. (1985) produce values between 8.8 and 51.3 g C g Chl⁻¹ (E m⁻²)⁻¹ in the bay mainstem, with the lowest value occurring in clearer waters of the lower bay and the highest value occurring in the turbidity maximum. (The α values reported in Table 10-1 of Harding et al. (1985) were converted from $\mu g C \mu g Chl^{-1} h^{-1} (uE m^{-2} s^{-1})^{-1})$.

If Ik can in fact be accurately measured with unshielded chlorophyll fluorescence, it's relationship to the average ambient irradiances in phytoplankton environments can be quantified in a series of curves for Ik *versus* average daily irradiance representing Chesapeake Bay populations across a range of photoadaptation states. The constant Ik in eutrophication models would become a variable responsive to light environment and thus capable of imitating actual Ik changes in natural populations. By default, α could no longer be a model constant but would vary reciprocally with Ik, as it does in natural populations as a result of cellular Chl:C adjustments.

5.4. Summary

- Chlorophyll fluorescence monitoring results in the Chesapeake point to a vertical gradient of photoinhibition driven by the light gradient in the water column, and extending down to some variable lower limit that depends on time of day.
- The results raises questions about Chesapeake phytoplankton photoinhibition and its potential effects on P_{max} and oxygen production.
- The unshielded chlorophyll fluorescence sensor appears to measure Ik, the irradiance at which the initial slope of the production versus irradiance (P-I) relationship intersects the value of P_{max} . It may be the better tool for measuring photosensitivity of *in situ* populations.
- The shielded chlorophyll fluorescence sensor may be measuring Ik_2 , or the onset of a decline in productivity due to photodamage to the PSII reactions centers that is not compensated by a reciprocal increase in electron transport rates. The shielded sensor is probably the better tool for estimating chlorophyll *a* concentrations from fluorometric measurements.
- These results suggest that several changes in standard water quality model assumptions

may better simulate phytoplankton production/light response curves and allow improved predictions of dissolved oxygen concentrations. However, photosensitivity of phytoplankton with depth, under different light conditions, has not been adequately characterized with chlorophyll fluorescence to our knowledge.

Figure 5-1. Time series of fluorometric-based chlorophyll estimates (green line) and surface photosynthetically active radiation, or PAR (purple line) in the lower Patuxent River for 2-28 March, 2001 (Data collected by Kim Mikita).



Figure 5-2. Comparison of extractive and *in situ* fluorometric chlorophyll *a* measurements collected with an unshielded sensor, Mikita data. Line, 1:1 relationship; \blacktriangle , the average of the previous night's high frequency (15 min) fluorometric measurements compared to the following day's extractive measurement; \Box , extractive measurement compared to the time-matched, daytime fluorometric measurement. Samples for extractive chlorophyll measurements were collected in the morning once a week between 3/2/2001 and 5/31/2001, and immediately filtered and frozen. The close correspondence between nighttime fluorometric measurements and daytime extractive measurements ($r^2 = 0.985$, p<0.001) indicates the former is a good surrogate for the latter.



Figure 5-3a. Daytime fluorometric chlorophyll *a* in the surface layer expressed as a percent (%) of the average nighttime fluorometric chlorophyll *a* concentration on three heavily overcast days. The incident PAR time series for each day is presented in the box.



Heavy Overcast

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Figure 5-3b. Daytime fluorometric chlorophyll *a* in the surface layer expressed as a percent (%) of the average nighttime fluorometric chlorophyll *a* concentration on three partly cloudy days. The incident PAR time series for each day is presented in the box.



Partly Cloudy

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Figure 5-3c. Daytime fluorometric chlorophyll *a* in the surface layer expressed as a percent (%) of the average nighttime fluorometric chlorophyll *a* concentration on three clear or slightly hazy days. The incident PAR time series for each day is presented in the box.



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Figure 5-4. Chlorophyll *a* photosensitivity measured in Patuxent River surface waters (0.5 m) with an unshielded sensor, under different incident PAR irradiances between dawn and dusk. Chlorophyll *a* photosensitivity is the amount of fluorescing chlorophyll *a* expressed as a percent (%) of that date's average nighttime fluorescing chlorophyll *a*. Incident PAR is grouped as follows: 50, 0 - 100 μ E m⁻² sec⁻¹; 150, 100 - 200 μ E m⁻² sec⁻¹; etc.; time is grouped by hours after dawn as follows: 0.5, 5:00-6:00; 1.5, 6:00-7:00, etc. Each point represents the average of 5 or more data for each PAR-hour combination. Approximate solar noon is indicated by ∇ on the x-axis. Each point is the average value of %fluorescing chlorophyll *a* in each PAR-time class. On heavily overcast days, when irradiances remained low, the % fluorescing chlorophyll recovers almost linearly during the day from post-dawn minimums. On clear days, when incident irradiances are maximal, % fluorescing chlorophyll remains at minimum levels of 30% - 40% until after solar noon, then increases sharply as ambient irradiance begin to decrease rapidly.



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Figure 5-5. Chlorophyll *a* photosensitivity measured in Patuxent River bottom waters (\sim 11 m) with an unshielded sensor, under different incident PAR irradiances between dawn and dusk. See figure 5-4 heading for details.



Figure 5-6. Time series of fluorometric chlorophyll estimates (green line) measured at 1 m in surface waters of the Magothy and Severn rivers with a shielded sensor, and incident photosynthetically active radiation, or PAR (purple line).



Figure 5-7. Comparison of extractive and *in situ* fluorometric chlorophyll *a* measurements collected with shielded sensors, MDDNR 2002 data for the Stonington site on the Magothy River (left panel) and the Sherwood site on the Severn River (right panel). Fluorometric sensors at both sites are positioned at 1 m depth. Line, 1:1 relationship; \blacktriangle , the average of the previous night's high frequency (15 min) fluorometric measurements compared to the day's extractive measurement; \Box , extractive measurement compared to the time-matched, daytime fluorometric measurement.



Figure 5-8. Chlorophyll *a* photosensitivity measured in Magothy and Severn river surface waters (1 m) with a shielded sensor, under different incident PAR irradiances between dawn and dusk. Each point represents the average of 5 or more data for each PAR-hour combination. Spring, March-May; summer, June-September; autumn, October-November. See figure 5-4 heading and text for further detail.



Figure 5-9. Magothy River near Stonington, for 1 - 18 April, 2002. Nighttime chlorophyll fluorescence was less variable on these dates (see Figure 5-6).







Figure 5-11. Morning (5:30 - 11:30 am) chlorophyll fluorescence at 0.5 m depth, expressed as a percent of the average nighttime fluorescence, *versus* incident PAR at Mikita's Patuxent station, 2-28 March 2001. Lines and symbols: dashed line, 100%; solid line, 20-period moving average; x, 5:30 - 9:30 am; o, 9:30 - 11:30 am. The break in the moving average at ~100 μ E m⁻² s⁻¹ is thought to indicate Ik in this low light adapted population.



Figure 5-12. Morning (4:00 am - noon) chlorophyll fluorescence at 1 m depth, expressed as a percent of the average nighttime fluorescence, *versus* incident PAR for MDDNR Magothy (1-18 April 2002) and Severn (23 May - 2 June 2002) river data. Lines and symbols: dashed line, 100%; solid line, 20-period moving average; x, Severn; o, Magothy. The break in the moving average at ~900 μ E m⁻² s⁻¹ is thought to indicate Ik₂ in these high light adapted populations.



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