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Data analysis of HAB species and phytoplankton community composition relationships to chlorophyll *a* of the James, Elizabeth and Lafayette Rivers.

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May, 2015

Executive Summary

Multiple phytoplankton metrics, including community characteristics and taxonomic group and individual species densities were compared to paired chlorophyll concentrations using several analytical techniques. The purpose of the analyses was to determine if there are relationships between chlorophyll *a* (Chl *a*) concentration and phytoplankton composition as it relates to the open water aquatic life designated use in the James River; and if so, if the current Chl *a* criteria are protective of these effects (i.e. do algal communities degrade at Chl *a* concentrations lower than are listed in the criteria).

Multi-metrics, including the Phytoplankton Index of Biotic Integrity (P-IBI) score and analyses using multiple variables such as community composition were especially variable. In general, the P-IBI score decreased with increasing Chl *a* concentrations, however, a significant percentage of samples with instantaneous Chl *a* values below the current criteria were classified as degraded based on the P-IBI score regardless of the season and salinity regime.

Additional community level analyses illustrated the differing relationships between Chl *a* and the phytoplankton composition that exist in the James River. In the upper James, increased Chl *a* is accompanied by an increased abundance of a diverse group of algal species, with little change in community structure at the major group level. In contrast, elevated Chl *a* in the lower James is strongly associated with a shift to a dinoflagellate dominated bloom, which in summer is almost always due to the harmful algal bloom species *Cochlodinium polykrikoides*. Paired Chl *a* concentrations at the current criteria (<10µg/L) are protective of *Cochlodinium* blooms, with 0% of these samples having cell concentrations considered fatal to fish/shellfish. In tidal fresh waters, the instantaneous Chl *a* concentration associated with the current summer criteria (<23µg/L) appears to be protective of toxic *Microcystis aeruginosa* blooms from a human health/recreation contact standpoint, with <2% of all samples meeting this criteria (1/78) associated with potentially toxic cell concentrations.

Multivariate analysis indicated that phytoplankton community composition as measured by (1) indicators of community health such as species diversity, species evenness, and abundance; (2) phylogenetic groups such as diatoms, dinoflagellates, cyanobacteria, etc. and; (3) taxonomic composition exhibited significant differences between groups of samples with concentrations above and below established water quality criteria for Chl *a*. In general, samples which exceeded established chlorophyll *a* criteria exhibited lower species evenness and higher community total abundance overall, higher abundances of cyanobacteria (Tidal Fresh), or dinoflagellates, and/or euglenophytes with salinity regime specific and season specific lists of individual taxa typically associated with high Chl *a* concentrations.

As a whole, the results varied considerably both seasonally and spatially (between river segments) with some data set combinations indicating negative relationships between Chl *a* and phytoplankton community characteristics, while other data sets indicated no apparent significant relationships. While these results focus entirely on the relationships between Chl *a* concentrations and various metrics of the phytoplankton community itself, they may provide additional evidence which may help to determine if the established Chl *a* criteria are protective of the aquatic habitat as a whole.

I. Introduction

Virginia Department of Environmental Quality (DEQ) requested that Old Dominion University (ODU) evaluate whether or not the current James River chlorophyll criteria are protective of aquatic life. The conditions described in Virginia's water quality standards for the Open water aquatic life designated use are "waters in the Chesapeake Bay and its tidal tributaries that protect the survival, growth and propagation of a balanced, indigenous population of aquatic life inhabiting open-water habitats." The objective of this analysis is to relate chlorophyll concentrations to biological metrics associated with "balanced, indigenous populations".

Chlorophyll *a* (Chl *a*) can serve as a proxy for phytoplankton biomass and act as a useful indicator and goal for water quality management if it can be related to biological/ecological responses and levels of impairment (Egerton et al., 2012, Harding et al. 2014). For Chl *a* to be useful as a management tool, it is necessary to understand how it relates to multiple lines of evidence that indicate conditions relative to the desired ecological conditions, including the abundance and composition of the phytoplankton community, both harmful algal bloom (HAB) and non-HAB species. These data needs were spelled out in Objectives 1 (Characterizing algal blooms) and 2 (Characterizing impairments associated with algal blooms) for the James River estuary by Bell et al. (2011). For these reasons our lab (Egerton/Marshall) has collected data on the phytoplankton composition of samples throughout the James River estuary paired with Chl *a* measurements during 2011-2013. In addition, long term phytoplankton and chlorophyll *a* data have been collected by Marshall and Egerton in the James River since 1985 as part of the Chesapeake Bay Monitoring Program. The data resulting from these efforts were used to address Subtask 1.1-Characterizing spatial and temporal patterns, and Subtask 1.2-Environmental factors favoring harmful algal blooms in both the Upper and Lower regions of the James River estuary.

While annual reports have summarized the 2011-2013 James River phytoplankton composition data and presented them to the scientific advisory panel (SAP), additional analyses are required to further assess the relationship between chlorophyll *a*, phytoplankton community composition, and concomitant and potentially adverse effects on natural resources as well as potential relations to water quality conditions. These analyses are addressed here in 3 main tasks as discussed by the Virginia Department of Environmental Quality and the James River data analysis working group:

- (1) Identifying relationships between Chl *a*, living resource conditions associated with a balanced and indigenous phytoplankton population, and overall water quality characteristics;
- (2) Relating HAB species abundances and the potential of exceeding HAB thresholds with Chl *a* concentrations, including concentrations listed in current Chl *a* criteria for the James River and;
- (3) Calculating the rate of threshold exceedance based on the central tendency of Chl *a*.

Task 3 was primarily handled by staff from Brown and Caldwell, with ODU assisting in the production of some of the interpolated data sets. Spatial interpolations were generated from DATAFLOW cruise data from 2005-2013 using the Chesapeake Bay and Tidal Tributary Interpolator. Using the interpolated maps, each season/segment combination was analyzed for percentage exceedance of 10µg/L Chl *a* bins ranging from 10-100 µg/L. Central tendency measurements were calculated as arithmetic and geometric means for each season/segment. These results were distributed electronically to all members of the SAP on 10/29/14.

Additional analysis and evaluation of the spatial interpolations included scatterplot and curve fitting, including LOWESS plots, along with the identification and corrections of initial miscalculations, with final corrected data resubmitted to Paul Bukaveckas in preparation of the November SAP meeting. Additional email correspondence, analysis and discussion between the data analysis group contributed to calculation of the probability analyses, including that of HABs included in the report (Empirical Relationships Linking Threats to Designated Uses with Algal Blooms in the James River Estuary) distributed by Bukaveckas on 11/14/14.

Additional data and analyses are included in this report or have been submitted separately in response to questions by DEQ and members of the SAP that have arisen during the period of this project. These include the relationship with Chl *a* and the possibility of human health concerns to *Microcystis aeruginosa*, and the calculation of long term phytoplankton diversity indices to support parallel analyses conducted by other investigators as well a short section (Appendix A) following up on a discussion with DEQ and SAP members on the relationship between the seasonal/segment means and the presence of bloom events.

II. Methods

A. Overview of Task 1 - Phytoplankton community composition/Chl *a* relationships

Relationships between Chl *a* and various estimates of the health of water quality and natural resources in the James River (e.g. the Phytoplankton Index of Biotic Integrity (P-IBI), diversity indices, etc.) were examined using multiple lines of evidence. At the request of DEQ and the SAP, particular interest was paid to the existing Chl *a* criteria (Table 1), to determine if they were protective of the Open-Water Aquatic life designated use. All analyses were conducted based on biological variables in comparison to the paired (instantaneous) Chl *a* concentrations present at the time of sampling. In general, this was accomplished by comparing multiple metrics between samples collected when chlorophyll *a* was above or below the threshold levels (the criteria listed in Table 1) for the standards for each salinity regime and season combination established for the James River watershed. Chl *a* criteria concentrations were considered protective for a given living resource metric if there was agreement between outcomes of comparisons to pre-established standards for both the Chl *a* criteria and living resource criteria (i.e. for the PIBI) or by using best professional judgment to whether or not phytoplankton community conditions were favorable or not. These metrics are best used collectively in a multiple line of evidence approach, and not meant to be viewed individually as representative of reference/degraded conditions.

B. Task1 - Univariate Comparisons

Univariate tests for significance differences between samples collected when Chl *a* was above and below the existing water quality thresholds were conducted for each salinity regime and season combination using a one-way analysis of variance for the P-IBI, species richness and species evenness. **Species richness** (S) was defined as number of taxa per sample while **evenness** (J) was that given by Pielou (1966) which is:

$$J = H'/\ln(S)$$

where H' is Shannon-Weiner diversity:

$$H' = - \sum_{i=1}^s \hat{p}_i \log \hat{p}_i$$

All univariate comparisons were conducted using SPSS software version 17.

C. Task 1 - Multivariate Comparisons

The overall objective of this study was to explore several broad questions concerning the relationships between Chl *a* and phytoplankton community composition. These questions included the following:

- (1) Are violations of Chl *a* of established water quality criteria associated with a concomitant response in phytoplankton community indicators (diversity, evenness, total abundance, etc.)?
- (2) Are violations of Chl *a* in established water quality criteria associated with a concomitant response in phytoplankton phylogenetic groups (e.g. diatoms, dinoflagellates, etc.) or individual taxa (species)?
- (3) Do any of the observed responses in phytoplankton communities change in response to changes in seasonality?

Given the number of combinations of variables, seasons and spatial groups (salinity regimes) involved in this study and the exploratory nature of the questions listed, multivariate statistical analyses provided a practical approach for addressing them. Therefore, two series of multivariate comparisons were made to assess general patterns in phytoplankton community characteristics in relation to Chl *a* concentrations. An initial series explored potential effects of and potential interactions between Chl *a* levels and seasonality. It was assumed that phytoplankton community composition would vary considerably in relation to seasonality. As such, any exploration of differences between sample groups associated with different Chl *a* levels would need to account for the effects of seasonality.

For this reason an initial series of two-way multivariate analyses was conducted to determine if seasons were an important source of variability in phytoplankton community composition. For this series three separate sets of multivariate comparisons of phytoplankton communities were conducted including: (1) indices of community health such as total abundance, number of species, species evenness, and average cell size; (2) abundances of major phylogenetic groups such as diatoms, dinoflagellates, cyanobacteria, chlorophytes, euglenophytes, and HAB species; and (3) abundances of all taxa collected. For context, in general within Chesapeake Bay, favorable phytoplankton assemblages include species that represent important food source for aquatic life, typically including diatoms, cryptophytes and chlorophytes (Marshall et al. 2009). Likewise, algal communities dominated by cyanobacteria and dinoflagellates, are seen as less favorable in part due to the presence of toxin producing species (Marshall et al. 2009). Variables were first \log_e -transformed both as a means of correcting for right-skewness and for weighting contributions of variables in the analysis. Community indices were also standardized to a mean of 0 and standard deviation of 1 prior to analysis, since each of these parameters were measured on different scales. If the results of the two-way analysis indicated that seasonality was an important source of variation, separate one-way multivariate comparisons were conducted for the same series. If no seasonal effects were detected, phytoplankton data collected during both seasons could be pooled to increase sample size for analysis.

Multivariate comparisons between groups of samples were made using one-way and two-way PERMANOVAs in combination with multivariate dispersion tests (PERMDISP) (Anderson, 2001a-b; Anderson et al., 2008). The PERMANOVA approach can best be described as the partitioning of multivariate model variation into mean squares based on symmetric distance measures such as Bray Curtis dissimilarity

(Anderson, 2001a-b; Anderson et al., 2008). Although no explicit assumptions about the distributions of the original variables are made (e.g. normality), sample independence and homogeneity of multivariate dispersions (in the space of the dispersion measure) are assumed. A distance-based pseudo-F statistic, analogous to Fisher's F-ratio, is calculated for each effect or term in the model and *P* values are obtained using permutation tests (Anderson, 2001a-b; Anderson et al., 2008). PERMANOVA is sensitive to differences in group variation (referred to as dispersion) between effects because the calculation of pseudo-F ratios uses pooled estimates of within-group variability. To test for potential violations of this assumption i.e. differences in variation between groups, the multivariate dispersion test or PERMDISP test was used which is a multivariate generalization of Levene's test applied to distance-based measures including but not limited to Euclidean distance (Anderson, 2006; Anderson et al., 2008). ANOSIM analysis (Clarke and Warwick, 2001) or analysis of similarities was used as a supplemental approach for testing for seasonal effects in the two-way series described above. This particular test identifies significant differences in rank similarities among and within the defined effects by using observed test statistic *R* for each effect defined as:

$$R = \frac{(\bar{r}_B - \bar{r}_W)}{\frac{1}{2}M}$$

Significance for each effect is based on the results of a permutation test (Clarke and Warwick, 2001). This test requires no underlying assumptions of the data whatsoever and provides a high degree of statistical power although it does not allow for testing for interaction effects as does the PERMANOVA test (Clarke and Warwick, 2001). It was felt that the application of both the PERMANOVA and ANOSIM analyses would insure the identification of any potential seasonal effects prior to additional interpretation or analysis.

Non-metric multidimensional scaling (nMDS) was used to create 2D and 3D ordinations of the rank similarities between samples (Clarke, 1993; Clarke and Warwick, 2001). Appendix B provides an explanation of how to interpret ordination plots produced in this study. Ordinations were distance based nMDS created using either simple Euclidean distance for the community indices:

$$d_{jk} = \sqrt{\sum_{i=1}^p (y_{ij} - y_{ik})^2}$$

or Bray-Curtis dissimilarity:

$$\delta_{jk} = 100 \frac{\sum_{i=1}^p |y_{ij} - y_{ik}|}{\sum_{i=1}^p (y_{ij} + y_{ik})}$$

for both abundances of the phylogenetic groups and the taxonomic abundance data (Clarke and Warwick, 2001). The resulting plots were used to assist in the verification of distinct sample groups and explain group differences in terms of the relative importance of group location (centroids) or dispersion (variability). Spearman rank correlations of the original variables to the nMDS axes were used to identify important parameters that contributed to pattern in rank dissimilarities between groups. All statistic procedures were

conducted using the PERMANOVA (ver. 1.02) and Primer (ver. 6.1.12) software packages, from PRIMER-E (www.primer-e.com).

D. Overview of Task 2: HAB/Chl *a* relationships

With respect to the designated open water use, a harmful algal bloom would be one example of an out of balance phytoplankton population, with the potential of negatively impacting other trophic levels as well. The purpose of this task was to quantify the relation(s) between Chl *a* and the potential for exceeding toxic thresholds of certain HAB cell densities in an attempt to determine the reliability of Chl *a* as a predictor of HAB-related toxic events. The results presented were generated from phytoplankton data and corresponding Chl *a* data collected during the long term Chesapeake Bay monitoring program (1985-2013) and the James River study (2011-2013). Threshold levels of HAB species abundances are based on the results of the James River bioassay study (Kim Reece) and literature review data (Anne Schlegel) for *Cochlodinium polykrikoides* at 1000 cells/ml (Bukaveckas 2014). The HAB *Prorocentrum minimum* was also included, with a threshold of 3000 cells/ml (Tango et al. 2004). *Microcystis aeruginosa* is considered a human health risk, with recreational warnings issued by Virginia Department of Health when densities of 20,000 cells/ml or greater are present (VDH, 2015 <http://www.vdh.virginia.gov/epidemiology/dee/habs/>). At concentrations greater than 20,000 cells/ml of toxic cyanobacteria, there are potential human health risks including skin irritations and gastrointestinal illness. These risks are greater at concentrations greater than 100,000 cells/ml and have the potential for long term illness as well (WHO 2003).

Univariate comparisons of HAB abundances between groups of samples with Chl *a* concentrations meeting (paired concentrations less than or equal to the values in Table 1) or exceeding (paired concentrations greater than the values in Table 1) existing criteria were made using one-way ANOVAs using SPSS software version 17. These analyses were conducted separately on the long term CBMP and 2011-2013 James River datasets. An additional analysis of *Cochlodinium* and *Microcystis* blooms was conducted by comparing the percentage of samples exceeding various cell density thresholds to the paired Chl *a* concentration (binned in 10µg/L groups).

III. Results and Discussion

A. Task 1 - Univariate analyses

For a larger perspective on the general relationship between Chl *a* and phytoplankton composition in Virginia, PIBI values were calculated for all long-term VA Chesapeake Bay Phytoplankton Monitoring Program collections. These results are summarized for the spring and summer seasons in Table 2 and illustrated in Figure 1. The PIBI score ranges from 1-5 and represents a gradient of the phytoplankton community as related to reference (good) or degraded (poor) conditions. Scores greater than 4 are considered good (shown in this report in blue), >3 to ≤ 4 are fair-good (green), >2.67 to ≤ 3 are fair (yellow), >2 to ≤ 2.67 are fair-poor (orange), and ≤ 2 are poor (red).

Within the VA CBP data set, there is a significant negative correlation in summer between average station Chl *a* and PIBI ($p=0.002$, $R=-0.758$), with no significant linear relationship in spring. Stations TF5.5 in the tidal fresh (lower) James River has the highest summer average Chl *a* concentration and lowest PIBI score in the state (Table 2), representative of poor/degraded conditions. The oligohaline RET5.2 and polyhaline LE5.5 also represent degraded conditions with poor or fair-poor summer conditions.

Looking at the James River specifically, there is considerable variability in the PIBI scores both spatially and seasonally, although on average a majority of the samples represent degraded (Fair-Poor or Poor) conditions (Figure 2). Spring is characterized generally as higher PIBI scores in the tidal fresh, and lowest values in the oligohaline and polyhaline, often associated with dinoflagellate (including *Prorocentrum minimum*) blooms. Summer includes poor PIBI scores in TF, OH and MH waters, and higher PIBI conditions in the polyhaline. The long term CBP data set is hampered by low sample size, with only 1 station per river segment in 3 of the 5 segments of the James River (lower TF, OH and PH). The more recent 2011-2013 data includes a larger number of stations for a better representation of the phytoplankton within each salinity zone. The data from these collections show relatively similar patterns as the long term data (i.e. the majority of degraded scores) with some notable exceptions, including a dominance of poor scores in the spring and summer MH due to the dinoflagellate blooms experienced in this region.

Focusing on the James River Chl *a* criteria, three metrics of phytoplankton community composition (PIBI, richness and evenness) were compared between samples that meet or exceed the current criteria using the long-term CBP data set (Table 3) and the more recent James River Chlorophyll Criteria Study data (Table 4). While methodological differences require these data sets to be analyzed separately, the trends in the data can be compared between the two groups (meeting/exceeding the Chl *a* criteria). Samples are assigned to salinity segments based on the field salinity measurement (TF, OH, MH, PH).

Beginning upstream in the **tidal-fresh** at station TF5.5, PIBI values were significantly lower in samples exceeding the current criteria in spring and summer (Table 3). Furthermore, PIBI values in samples that met the criteria were relatively high in spring, representing on average Fair-Good conditions. Less than 10% of spring samples that met the current criteria had Poor PIBI values opposed to 96% of samples that exceeded the criteria (Table 5). However in summer, while significantly higher than those that exceeded the criteria, >50% of the samples meeting the criteria were classified as poor (Table 5), with an average score of Fair-Poor (Table 3). When Chl *a* exceeded the current criteria, these values were significantly reduced representing Poor and Fair-Poor communities in 100% of the samples.

A similar pattern is observed in the 2011-2013 Tidal Fresh (lower) data, with significantly higher average PIBI values in samples meeting the criteria than those exceeding the criteria (Tables 4,6). In the upper Tidal Fresh segment, PIBI values are also significantly higher in samples meeting the criteria than exceeding it and generally good or fair-good regardless, with relatively low phytoplankton or Chl *a* throughout the year. In regards to phytoplankton diversity in the tidal fresh, there is in general a positive relationship with Chl *a* (Table 3-4). Species richness is higher in samples exceeding the Chl *a* criteria in both the long term and recent data. Evenness is reduced in tidal fresh samples exceeding the criteria, although significant reductions are only detected in the long term TF5.5 data (Table 3).

Taken together, these results indicate that within tidal fresh waters, samples meeting the current Chl *a* criteria contain a more evenly distributed phytoplankton community with fewer taxa that is on average more representative of reference conditions. In spring, the PIBI results suggest that samples meeting the current criteria contain phytoplankton communities that represent fair-good conditions, while the low (fair-poor) PIBI scores in summer samples meeting the criteria indicate that the current value may not be associated with the desired reference phytoplankton community.

Moving downstream to the **oligohaline** segment, at station RET5.2 there is a significantly higher PIBI value in samples meeting the spring criteria, with average values in the Fair category (Table 3), however 80% of

the samples are considered poor (Table 5). In summer at station RET5.2 there is no significant difference in the PIBI between those samples that meet or exceed the criteria, with both groups having an average poor score (Table 3). Similarly, there is no significant difference in species richness between criteria groups, with RET5.2 having the lowest richness in the river, characteristic of oligohaline salinities (Egerton 2012). It does not appear that at this segment, the current criteria relates to phytoplankton communities that would be characteristic of reference conditions in spring or summer, and provides evidence that a lower criteria might be required.

The **mesohaline** and **polyhaline** segments both have the same Chl *a* criteria, and are subject to similar seasonal algal blooms. The long term CBP station LE5.5 is located within the polyhaline segment near the mouth of the James River, which sometimes experiences fresher mesohaline salinities. PIBI scores at this station are not significantly different between samples meeting or exceeding the Chl *a* criteria (Table 3). Data collected from 2011-2013 included a much greater spatial representation of the MH and PH segments, better capturing the spring (*Heterocapsa*) and summer (*Cochlodinium*) blooms than the long term monitoring program. PIBI values from this data set illustrate the poor/degraded composition representative of HABs (Table 4,6). These blooms result in very low evenness as they are dominated by single species, with significantly lower evenness in samples exceeding the Chl *a* criteria. No reduction in species richness during blooms was observed. Reduced richness could be considered an impact on the phytoplankton community and potentially on the system as a whole. Reductions in evenness as a result of a dominant bloom species without an impact on richness however are less clearly representative of impacted conditions. Even during very dense blooms (>90% of total biomass within single species), species richness has remained relatively unchanged (current data and Egerton et al. 2014). Whether or not a system with very low evenness affects other trophic levels cannot be determined based on the current data (2011-2013 James River). Analysis of CBP phytoplankton and zooplankton diversity data (1985-2001) identified no significant relationship between phytoplankton diversity (Shannon Diversity, which includes evenness) and zooplankton diversity (Shannon diversity) (Egerton 2013). Phytoplankton evenness seems to be a useful indication of bloom condition, but the current data does not on its own indicate that the system is impacted.

B. Alternative criteria

While the current criteria appear to somewhat differentiate between bloom and non-bloom conditions (based on evenness and previously reported composition data) the poorer PIBI values in samples meeting the criteria suggest that degraded conditions may still be present even at the poorer Chl *a* levels. As there were a significant percentage (72-100%) of poorer PIBI scores in samples meeting the existing criteria in the meso/polyhaline James River, alternative criteria were tested to see if scores differed significantly at lower Chl *a* concentrations. Concentrations of <5µg/L, ≥ 5 <10µg/L, ≥ 10 <15µg/L, ≥ 15 <20µg/L, and ≥ 20µg/L chl *a* were tested as alternative criteria.

PIBI scores within the mesohaline James were low across the ranges of Chl *a* tested (Table 7), with 100% of spring samples and 84% of summer samples in the Fair-Poor or worse range (≤ 2.67) even when Chl *a* is less than 5µg/L. Similarly, the majority of the polyhaline samples (63-69%) with Chl *a* less than 5µg/L also scored as Fair-Poor or worse. There is little differentiation between these Chl *a* bins at the 2.67 level. In comparison, at least within the polyhaline segment, the percentage of samples with PIBI ≤ 2 appears to be reduced at the lowest category, with only 22-25% of samples considered Poor having less than 5µg/L Chl *a*. This pattern however was not observed in the mesohaline segment, with similarly high proportions of poor samples (79-83%) at the lowest concentrations.

The phytoplankton community is influenced by multiple parameters, including light availability, nutrient concentrations, salinity, temperature and other factors. Composition changes, and thus PIBI scores are affected by more than just changes in total biomass (i.e. Chl *a*). This region includes a large percentage of dinoflagellates within the community, including those species (*Prorocentrum minimum*) whose dominance is included as a (negative) metric within the PIBI. These results suggest that at least in the lower James, the PIBI score might not provide useful diagnostic data to specifically relate to Chl *a*. For this reason, additional analyses were conducted on multiple phytoplankton community characteristics using multivariate statistics.

C. Task 1 - Multivariate Comparisons

1. Two-way analyses

For the lower tidal freshwater, there were significant differences in community indices, abundances of phylogenetic groups and phytoplankton taxonomic abundances between seasons but no significant differences between Chl *a* threshold groups (Tables 8-10). ANOSIM results obtained for the lower tidal freshwater indicated significant differences in dissimilarity between all seasons across all threshold groups and vice-versa for all parameter sets analyzed (Tables 8-10). Differences in dispersion (within-group variation) were indicated only for community indices (Table 8). Construction of 2D non-metric MDS (nMDS) ordination plots indicated little discernable patterns in any of the three data sets associated with Chl *a* thresholds although distinct groups of samples do appear to correspond to seasons with respect to both community indices and taxonomic composition (Figure 4A and 4C). Summer was characterized by higher total community abundance (excluding *Microcystis aeruginosa*), number of species, and *Microcystis aeruginosa* abundance while Spring samples had higher species evenness and average cell size (Figure 4A). With respect to taxonomic composition Spring samples were characterized by higher abundances of: (1) cyanobacteria including *Microcystis aeruginosa*, *Microcystis incerta*, *Merismopedia tenuissima*, *Pseudanabaena* sp. and several others; (2) chlorophytes of the genus *Pediastrum*, *Scenedesmus* and *Ankistrodesmus falcatus* and; (3) several diatom taxa including *Aulacoseira granulata*, and unidentified pennate (10-30µm length) and centric diatoms species (10-30 µm diameter) (Figure 4C). Summer samples were characterized by higher abundances of the diatoms *Skeletonema costatum* and *Navicula* sp. (Figure 4C). No additional analyses were performed using data collected within the lower tidal freshwater salinity regime.

The results for the oligohaline salinity regime were less clear. There were no significant differences in group centroids of community indices based on the results of the PERMANOVA however the ANOSIM analysis indicated a significant difference in group distance between seasons (Table 8). There were significant differences in phylogenetic groups and taxonomic composition between seasons as indicated by results of the PERMANOVA for both sets of parameters and for the ANOSIM analyses for phylogenetic groups (Table 9). Two-dimensional nMDS ordination plots indicated few if any discernable patterns in any of the three data sets associated with Chl *a* thresholds (Figures 5A-C). Examination of 3D nMDS plots (not presented here) revealed no additional usable information. No additional analyses were conducted using data collected in oligohaline salinity regime.

Two-way PERMANOVAs of community indices, abundances of phylogenetic groups, and phytoplankton taxonomic abundances indicated significant interactions between seasons and Chl *a* threshold groups as well as significant main effects for most salinity regimes except the lower tidal freshwater and oligohaline (Tables 8-10). Significant differences in dispersions were detected between combinations for all three sets of

phytoplankton community parameters in tidal freshwater, mesohaline and polyhaline areas (Tables 8-10). ANOSIM analyses indicated significant differences in all parameter groups analyzed for tidal freshwater, mesohaline and polyhaline salinity regimes (Tables 8-10). In the lower tidal freshwater, results indicated that there were significant differences in community indices, abundances of phylogenetic groups and phytoplankton taxonomic abundances between seasons but samples collected when Chl *a* was above and below the water threshold showed only differences in dispersions and nMDS plots suggested substantial overlap between samples. (Figures 4A-C; Tables 8-10). Overall the results indicated substantial differences in community indices, phylogenetic composition and taxonomic composition between seasons for tidal freshwater, mesohaline and polyhaline salinity regimes and that additional analyses would need to be conducted on a seasonal basis in order to adequately determine whether or not there were differences in phytoplankton communities between Chl *a* threshold groups and whether or not those differences were due mainly to differences in group location (centroids) or dispersion. Therefore, a second set of analyses comparing phytoplankton communities between threshold groups for these salinity regimes by seasons was conducted. These one-way comparisons were conducted on the same variable sets described for the two-way analysis.

2. One-way analyses

a) Community indices

Results of PERMANOVA and PERMDISP comparisons of community indices between samples collected when Chl *a* was above or below established water quality thresholds indicated significant differences between these two groups for all salinity regime and season combinations due in most cases to differences in group centroids in combination with group variability except for Mesohaline Summer and Polyhaline Spring for which group location was the primary source of differences (Table 11). ANOSIM analyses indicated there were significant differences in rank dissimilarity between threshold groups for all salinity regime and season combinations (Table 11). The nMDS ordination plots indicated that samples with Chl *a* above established thresholds for all salinity regime and season combinations tended to separate well along both nMDS axes although there was generally at least some overlap between threshold groups for each combination (Figures 6-8). Correlations of original variables to the nMDS axes indicate that, in general, species evenness and average cell size tended to be higher when Chl *a* was below the threshold while total abundance and bloom producing species abundances tended to be higher when Chl *a* was below the threshold, regardless of salinity regime or season (Figures 6-8; Table 12). In the Mesohaline and Polyhaline salinity regimes, total number of species tended to be higher in samples below the Chl *a* threshold during Spring and higher in samples above the Chl *a* threshold during Summer (Figures 7-8).

b) Phylogenetic groups

Results of PERMANOVA and PERMDISP comparisons of abundances of phylogenetic groups between samples collected when Chl *a* was above or below established water quality thresholds indicated significant differences between these two groups for all salinity regime and season combinations due in most cases to differences in group centroids in combination with group variability except for Polyhaline Spring and Summer for which group location was the source of differences as indicated by the lack of significance for the dispersion tests (Table 13). ANOSIM analyses indicated there were significant differences in rank dissimilarity between threshold groups for all salinity regime and season combinations except Polyhaline Spring (Table 13).

The 2D nMDS ordination plot for the Tidal Freshwater regime during Spring exhibited no distinct patterns associated with Chl *a* thresholds (Figures 9A) suggesting that despite the tests of significance, any inferences concerning differences in phylogenetic groups between threshold groups should be made with caution, if at all. The 2D ordination plot and correlations between the variables and nMDS axes for the Tidal Freshwater regime during Summer indicated: (1) that separation between samples collected above and below Chl *a* thresholds occurred primarily along nMDS axis 1 (Figure 9B); (2) nMDS axis 1 was highly correlated with abundances of *Microcystis aeruginosa*, other cyanobacteria, cryptophytes and diatoms (Figure 9B; Table 14); and (3) the direction of those correlations (negative) indicated that samples above the established Chl *a* threshold tended to have higher abundances of these groups (Figure 9B; Table 14).

The 2D nMDS ordination plots and correlations between variables and nMDS axes for the Mesohaline salinity regime for both Spring and Summer indicated that: (1) separation between samples collected above and below Chl *a* thresholds occurred primarily along nMDS axis 1 (Figure 10); (2) nMDS axis 1 was highly correlated with abundances of bloom producing species (*Heterocapsa triquerta* and *Prorocentrum minimum* in Spring; *Cochlodinium polykrikoides* in Summer), other dinoflagellates, euglenophytes (Summer only) and to a lesser degree chlorophytes (Figure 10; Table 14); (3) the direction of those correlations indicated that samples above the established Chl *a* threshold tended to have higher abundances for these taxa and phylogenetic groups. For both seasons, separation between samples also occurred along nMDS axis 2 in relation to cryptophyte abundance and in relation to euglenophyte abundance in Spring with both of these parameters tending to be higher in samples collected when Chl *a* was below the established water quality threshold (Figure 10A-B; Table 14).

The 2D nMDS ordination plot for the Polyhaline regime during Spring exhibited no distinct patterns in relation with Chl *a* thresholds (Figures 11A) suggesting that despite the tests of significance, few if any inferences concerning differences in phylogenetic groups between threshold groups should be made. The 2D nMDS ordination plot and correlations between the variables and nMDS axes for the Polyhaline regime during Summer indicated that separation between samples collected above and below Chl *a* thresholds occurred primarily along nMDS axis 1 which was highly correlated with abundances of *Cochlodinium polykrikoides*, and other dinoflagellates (Figure 11B; Table 14) and to lesser degree to nMDS axis 2 which was highly correlated with abundances of cryptophytes and euglenophytes (Figure 5B; Table 14). Direction of these correlations indicates that abundances of all of these groups were higher in samples collected when Chl *a* concentrations were above the established threshold (Figure 5B; Table 14).

c) Taxonomic composition

Results of PERMANOVA and PERMDISP comparisons of taxonomic composition between samples collected when Chl *a* was above or below established water quality thresholds indicated significant differences between these two groups for all salinity regime and season combinations due in most cases to differences in group centroids in combination with group variability (dispersion) except for Mesohaline Spring for which group location was the source of differences as indicated by the lack of significance for the dispersion tests (Table 15). ANOSIM analyses indicated there were significant differences in rank dissimilarity between threshold groups for all salinity regime and season combinations except for Polyhaline Spring (Table 15).

The 2D nMDS ordination plot and correlations between the variables and nMDS axes for the Tidal Freshwater regime during the Spring indicated that separation between samples collected above and below Chl *a* thresholds occurred primarily along nMDS axis 1 which was highly correlated with multiple

phytoplankton including *Skeletonema costatum*, *Ulothrix* sp., *Pleurosigma* sp., *Leptocylindrus danicus*, *Leptocylindrus danicus* and other taxa (Figure 12A; Table 16). The direction of these correlations indicates that the abundance of these taxa was higher in samples collected when Chl *a* concentrations were above the established threshold (Figure 12A; Table 16). Additional separation between threshold sample groups occurred along nMDS axis 2 although there was some overlap between samples along this axis (Figure 12A). The taxa *Aulacoseira granulata*, *Cyclotella* spp., and *Actinastrum hantzschii* were positively correlated with nMDS axis 2 indicating abundances of these taxa were higher when Chl *a* thresholds were exceeded (Figure 12A; Table 16). Unidentified pennate diatoms (length 31-60 μm), unidentified centric diatoms (dia. <10 μm), *Cryptomonas* sp. and *Aulacoseira granulata* var. *angus* were negatively correlated with this axis indicating these taxa were higher when Chl *a* concentrations were below the threshold.

The 2D nMDS ordination plot and correlations between the variables and nMDS axes for the Tidal Freshwater regime during the Summer indicated that separation between samples collected above and below Chl *a* thresholds occurred primarily along nMDS axis 1 and that a suite of nearly 30 taxa including cyanobacteria such as *Microcystis incerta*, *Microcystis aeruginosa*, *Pseudanabaena* sp., *Anabaena* sp., *Dactylococcopsis raphidioides*, *Anabaena circinalis*, *Chroococcus dispersus*, chlorophytes such as *Pediastrum duplex*, *Scenedesmus quadricauda*, *Ankistrodesmus falcatus*, *Merismopedia elegans*, and *Merismopedia tenuissima* and diatoms such as *Aulacoseira granulata*, *Cylindrotheca closterium*, unidentified pennate (<10 μm) and centric (dia. 10-30 μm) forms, had higher abundances when Chl *a* thresholds were exceeded (Figure 12B; Table 17).

The 2D nMDS ordination plot and correlations between the variables and nMDS axes for the Mesohaline regime during the Spring indicated that separation between samples collected above and below Chl *a* thresholds occurred primarily along nMDS axis 1 which was highly positively correlated with several phytoplankton taxa *Heterocapsa triquetra*, *Skeletonema costatum*, and *Prorocentrum minimum* indicating that abundances of these taxa were higher when Chl *a* exceeded established thresholds (Figure 13A; Table 18A). In contrast, *Katodinium rotundatum* was negatively correlated with this axis indicating abundances of this taxa were higher when Chl *a* were typically below the threshold (Figure 13A; Table 18A).

The stress value for the 2D ordination for the Mesohaline regime for Summer was too high (0.26) to make interpretation reliable (Figure 13B) therefore a 3D ordination was conducted. Results of the 3D ordination and correlations indicate that separation between samples collected above and below Chl *a* thresholds along nMDS axis 1 which was highly positively correlated with multiple dinoflagellate taxa including *Scrippsiella trochoidea*, *Cochlodinium polykrikoides*, *Gymnodinium* sp. (20 μm), and unidentified dinoflagellates along with the euglenophyte *Euglena* sp. indicating these taxa were more abundant when Chl *a* exceeded the established threshold (Figure 14; Table 18B). In contrast, the diatoms *Skeletonema costatum* and *Leptocylindrus minimus* were negatively correlated with nMDS axis 1 indicating abundances of these taxa were higher when Chl *a* concentrations were generally lower than the threshold (Figure 14; Table 18B).

The 2D nMDS ordination plot for the Polyhaline regime during Spring exhibited no distinct patterns associated with Chl *a* thresholds (Figure 15A; Table 19A) suggesting that despite the tests of significance, any inferences concerning differences in phylogenetic groups between threshold groups should be made with caution, if at all. A 3D ordination plot (not provided here) revealed no additional useful information. The stress value for the 2D ordination for the Polyhaline regime for Summer was too high (0.28) to make interpretation reliable (Figure 15B) therefore a 3D ordination was conducted. Results of the 3D ordination and correlations indicated that separation between samples collected above and below the Chl *a* thresholds

occurred along nMDS axis 1 (Figure 16). This axis was highly positively correlated with two diatom taxa (unidentified centric forms (10-3 μ m) and *Leptocylindrus minimus*) and highly negatively correlated with three dinoflagellate taxa (*Cochlodinium polykrikoides*, unidentified dinoflagellates and *Scrippsiella trochoidea*) indicating the diatoms were more abundant in samples with Chl *a* concentrations below the threshold and dinoflagellate taxa were more abundant in samples with Chl *a* concentrations exceeding the threshold (Figure 16; Table 19B).

3. Summary of results

In summary, two-way comparisons for lower tidal freshwater and oligohaline areas exhibited little if any variability for any of the parameter sets that could be associated with differences in Chl *a* levels although seasonal differences in community indices and taxonomic composition were observed in the lower tidal freshwater. In the lower tidal freshwater regime, Summer samples were characterized by higher total community abundance (excluding *Microcystis aeruginosa*), number of species, and *Microcystis aeruginosa* abundance while Spring samples had higher species evenness and average cell size. With respect to taxonomic composition, Spring samples were characterized by higher abundances of several cyanobacteria, chlorophyte and diatom taxa while Summer samples had higher abundances of several diatom taxa.

For most salinity regime and season combinations, species evenness and average cell size tended to be higher in samples collected when Chl *a* was below the threshold while total abundance and bloom producing species abundances tended to be higher when Chl *a* was above the threshold. In the Mesohaline and Polyhaline salinity regimes, total number of species tended to be higher in samples below the Chl *a* threshold during Spring and higher in samples above the Chl *a* threshold during Summer.

The analysis of the phylogenetic group data indicated that: (1) cyanobacteria, cryptophytes, diatoms and *Microcystis aeruginosa* in the Tidal Freshwater; (2) bloom producing and other dinoflagellates, euglenophytes (Summer only), cryptophytes and to a lesser degree chlorophytes in the Mesohaline; and (3) bloom producing and other dinoflagellates, cryptophytes, and euglenophytes in the Polyhaline tended to be higher in samples with Chl *a* concentrations above established water quality thresholds. Analysis of the taxonomic composition data, in general, reflected the observations of the results conducted on the phylogenetic groups while at the same time providing additional details as to the identity of individual taxa associated with the high Chl *a* concentration samples collected in the James River during each season.

D. Task 2: HAB/Chl *a* relationships

1) Dinoflagellate taxa

Seasonal dinoflagellate blooms are underrepresented in the long term CBP data set due to the low number of stations in the James River (3 only) and monthly sampling window. No records of *P. minimum* or *C. polykrikoides* exceeding the thresholds exist in the long term James River data (*P. minimum* max = 2500 cells/ml, *C. polykrikoides* max = 260 cells/ml). Within the long term data set, there are no significant differences in HAB densities between samples meeting or exceeding the current criteria (Table 20). The more intensive sampling approach used in the James from 2011-2013 provided a more accurate estimate of HAB densities and bloom duration by increasing sampling frequency and spatial coverage. Figure 17 illustrates the positive relationship between Chl *a* and *Cochlodinium*, showing increasing frequency of bloom densities with increasing Chl *a*. Within samples less than 10 μ g/L no samples contained *Cochlodinium* at 1000

cells/ml or greater. The percentage of summer samples above this threshold increased with a more or less linear relation to increasing Chl *a*, so that at concentrations of 60-70µg/L, over 90% of samples contained greater than 1000 cells/ml of *C. polykrikoides* (Table 21).

Utilizing the 2011-2013 James River study data set (Table 22), there are significant differences in HAB abundances in samples meeting and exceeding the existing criteria. *Prorocentrum minimum* densities are significantly greater in samples exceeding the threshold in the mesohaline segment both in Spring and Summer (Table 22). However, *Prorocentrum minimum* blooms are relatively scarce in the tributary. Out of the 1190 samples analyzed for this effort, there were 12 records of *P. minimum* densities exceeding 1000 cells/ml, including blooms in 2011 and 2013 with 3 samples exceeding 3000 cells/ml. Chl *a* in the samples exceeding 3000 cells/ml of *P. minimum* ranged from 19.1-60.6 µg/L.

If future assessments of the lower James River are to characterize the magnitude and duration of dinoflagellate HABs, increased sampling will be required. This could involve twice monthly collections during summer months. Most importantly a long term phytoplankton monitoring station should be established within the mesohaline segment. Further details and suggestions are included in Appendix A.

2) *Microcystis aeruginosa*

Within the Chesapeake Bay phytoplankton monitoring dataset (VA and MD 1984-2014) there are 808 records of *Microcystis aeruginosa* present at densities of 0.025-171,979 cell/ml. Of these there are 55 records of *M. aeruginosa* exceeding a 20,000 cells/ml threshold (VDH 2015, WHO 2003), with 29 (53%) of them within the James River at stations RET5.2 and TF5.5. Although the average *M. aeruginosa* density is greater in samples exceeding 23 µg/L Chl *a* threshold (in both the CBP longterm data and the 2011-2013 James data), there is considerable variability, and these differences are not significant (Table 20, 23). However, there is a general positive relationship with Chl *a* and *M. aeruginosa* in summer tidal fresh and oligohaline waters in both Virginia and Maryland, with a greater percentage of samples having the HAB species present, and at higher densities in samples with higher Chl *a* concentrations (Figure 18). In Virginia, 6 out of 68 Summer tidal freshwater (8.8%) samples exceeding the Chl *a* threshold (23µg/L) had *M. aeruginosa* densities of 20,000 cells/ml or greater, opposed to 1 out of 78 (1.3%) Summer VA tidal freshwater samples with Chl *a* less than 23µg/L. (Figure 19). A similar pattern was observed in Maryland Summer Tidal fresh waters, with 4.2% of samples (5/115) with Chl *a* >23µg/L having *M. aeruginosa* densities of at least 20,000 cells/ml, opposed to only 0.5% (1/213) of samples with <23µg/L Chl *a*. (Figure 19). In oligohaline waters, *M. aeruginosa* densities and exceedances were lower. Less than 1 % of Summer samples in both VA and MD that met the 23µg/L Chl *a* threshold had *M. aeruginosa* densities >20,000 cells/L, with no samples within collections >23µg/L having *M. aeruginosa* densities >20,000 cells/L. Within the 2011-2013 James River study, *M. aeruginosa* was present in 51 samples at densities of 40-11,440 cells/ml, with no exceedances of the 20,000 cell/ml threshold.

IV. Discussion

Both Baywide and in the James River, summer phytoplankton composition, as measured by the PIBI, generally declined (degraded) with increased Chl *a* concentrations. In the James River, the majority of samples within each salinity regime other than the upper tidal fresh had P-IBI values representative of degraded conditions (PIBI < 2.67). In the spring and summer lower tidal fresh (TF5.5), and spring oligohaline (RET5.2), PIBI scores were significantly higher (better) in samples meeting the current Chl *a* criteria than in

those exceeding it, with an average PIBI score that is representative of more favorable conditions (> 2.67), indicating that the current criteria can at least to some degree, differentiate between favorable and degraded phytoplankton conditions.

During summer in lower tidal freshwater areas (TF5.5), while the PIBI scores for samples meeting the criteria were significantly higher than those exceeding the criteria, the average PIBI score was still representative of degraded conditions (<2.67). In the summer oligohaline (RET5.2) and meso/polyhaline areas (LE5.5) there is no significant difference between samples meeting or exceeding the criteria, with lower (worse) PIBI scores. This suggests that either the current criteria are too high to differentiate the PIBI scores into degraded and reference conditions or: that in these communities, there may not be a recognizable linkage between Chl *a* and the PIBI metric.

While there may be significant differences in average PIBI score, a significant percentage of samples from each salinity/season outside of the upper tidal fresh (27.5-100%) that met the current criteria have Fair-Poor or Poor (< 2.67) PIBI scores. This suggests that either the current criteria are too high to completely protect from a degraded PIBI score, or that the PIBI metric might not be an achievable metric. The latter is supported by a large percentage (26-100%) of degraded PIBI (<2.67) scores in samples with very low Chl *a* (<5 µg/L), well below current criteria. It is not unreasonable to assume that the PIBI is functioning as it was designed i.e. a multimetric indicator of water quality conditions that responds to a suite of environmental characteristics that are not necessarily limited to the same predictive set as those that cause changes in chlorophyll *a* concentrations. Conversely, one component metric of the PIBI is chlorophyll *a* and therefore some similarity in response should be anticipated.

There was no negative relationship observed between Chl *a* and species richness at the ranges examined and where significant differences did occur, there were greater numbers of species in samples which exceeded the current criteria. Species evenness is generally reduced at increased Chl *a*, with significantly lower values in samples exceeding the current criteria, particularly within the recent data set in the meso/polyhaline segment. A reduction of species evenness, without a reduction of species richness, or a reduction of the actual densities of other co-occurring taxa was mostly probably associated with an increase in dominance of the bloom taxa. Currently, there are no data indicating that reduced evenness is in itself an indication of an impairment to the phytoplankton community. In addition, while this study has not looked at the potential effects of phytoplankton community composition or diversity on other trophic levels, the long term CBMP data does not indicate a significant relationship between Chesapeake Bay phytoplankton diversity and zooplankton diversity. It is recommended that a direct examination of the relationship between James River phytoplankton composition and diversity to zooplankton abundance and diversity be conducted.

HABs were generally more abundant at higher Chl *a* concentrations and no *Cochlodinium* (>1000 cells/ml) or *Prorocentrum* blooms (>3000 cells/ml) occurred in samples with less than 10µg/L Chl *a* (current meso/polyhaline criteria). Blooms of *Microcystis aeruginosa* in the James River have reached levels of potential human health concerns in the past, although densities observed in the most recent study (2011-2013) were lower than VDH/WHO threshold. Based on the long term CBMP data set, of the seven significant *Microcystis aeruginosa* blooms (>20,000 cells/ml) in Virginia summer tidal fresh waters, only 1 (1.3% of all summer TF samples with <23 µg Chl *a*/L), occurred in samples meeting current Chl *a* criteria. The other six blooms (8.8% of 68 samples) occurred when Chl *a* exceeded the criteria (>23 µg/L). No significant *Microcystis aeruginosa* blooms (>20,000 cells/ml) occurred in Tidal Fresh waters with less than

10µg/L Chl *a* in Virginia or Maryland, and only in 2.9% of Virginia Oligohaline samples with less than 10µg/L Chl *a*.

With respect to community indices, species evenness and average cell size tended to be higher in samples collected when Chl *a* was below the threshold while total abundance and bloom producing species abundances tended to be higher when Chl *a* was above the threshold For most salinity regime and season combinations. In the Mesohaline and Polyhaline salinity regimes, total number of species tended to be higher in samples below the Chl *a* threshold during Spring and higher in samples above the Chl *a* threshold during Summer.

The analysis of the phylogenetic group data indicated that: (1) cyanobacteria, cryptophytes, diatoms and *Microcystis aeruginosa* in the Tidal Freshwater; (2) bloom producing and other dinoflagellates, euglenophytes (Summer only), cryptophytes and to a lesser degree chlorophytes in the Mesohaline; and (3) bloom producing and other dinoflagellates, cryptophytes, and euglenophytes in the Polyhaline tended to be higher in samples with Chl *a* concentrations above established water quality thresholds.

Multivariate analysis of taxonomic composition data indicated that in the tidal fresh during spring abundances of *Aulacoseira granulata*, *Cyclotella* spp., and *Actinastrum hantzschii* were higher when Chl *a* thresholds were exceeded while during the Summer in these areas high Chl *a* concentrations were associated with a suite of nearly 30 taxa including cyanobacteria such as *Microcystis incerta*, *Microcystis aeruginosa*, *Pseudanabaena* sp., *Anabaena* sp., and many others. Oligohaline areas exhibited little few associations with respect to species and Chl *a* concentrations. In the mesohaline during the Spring, the phytoplankton taxa *Heterocapsa triquetra*, *Skeletonema costatum*, and *Prorocentrum minimum* had higher abundances when concentrations of Chl *a* exceeded water quality thresholds while during Summer abundances of *Scrippsiella trochoidea*, *Cochlodinium polykrikoides*, *Gymnodinium* sp., an unidentified dinoflagellate taxa and the euglenophyte *Euglena* sp. were higher when Chl *a* concentrations were above the threshold. In the polyhaline regime during Spring, no obvious patterns with respect to phytoplankton taxonomic composition in association with Chl *a* thresholds was observed. However, during Summer in the polyhaline regime three dinoflagellate taxa, *Cochlodinium polykrikoides*, *Scrippsiella trochoidea* and one unidentified taxa were found at higher densities in samples having Chl *a* concentrations above established thresholds.

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Tables

Table 1. Existing chlorophyll *a* criteria within segments of the James River ($\mu\text{g/L}$). Concentrations less than or equal to these values meet the criteria, while higher concentrations exceed the criteria. Note: current criteria are assessed as seasonal geometric means by DEQ. All comparisons in this study were made to the paired (instantaneous) Chl *a* concentrations in ($\mu\text{g/L}$).

	Spring (March-May)	Summer (July-September)
James Tidal Fresh (upper)	10	15
James Tidal Fresh (lower)	15	23
James Oligohaline	15	22
James Mesohaline	12	10
James Polyhaline	12	10

Table 2. Average chlorophyll *a* and PIBI scores for long term VA CBP phytoplankton monitoring stations (above pycnocline, 1985-2013).

Basin	Station	Mean Chl <i>a</i> ($\mu\text{g/L}$)		Mean PIBI	
		Spring	Summer	Spring	Summer
Chesapeake Bay	CB6.1	12.7	9.4	2.93	2.51
	CB6.4	12.1	8.1	3.10	2.73
	CB7.3E	8.5	6.4	3.11	2.89
	CB7.4	6.6	4.9	3.28	3.18
Rappahannock River	TF3.3	13.0	19.2	2.55	1.59
	RET3.1	14.5	15.6	2.57	1.93
	LE3.6	12.2	11.9	2.99	2.39
York River	TF4.2	4.3	5.7	2.71	3.34
	RET4.3	16.4	12.4	2.10	1.88
	WE4.2	10.8	13.9	2.49	2.46
James River	TF5.5	12.2	44.3	2.79	1.25
	RET5.2	13.4	10.3	2.50	1.98
	LE5.5	12.0	12.7	2.40	2.55
Elizabeth River	SBE5	6.8	8.2	1.90	2.06

Table 3. Phytoplankton community characteristics in relation to existing chlorophyll *a* standards. Values are PIBI values (1-5), Richness=number of species, Evenness= Pielou's evenness index (0-1). Data from 1985-2013 CBP stations. ANOVA results indicated by p and F values. Significant ANOVA ($p < 0.05$) in bold.

Parameter	Station	Season	Mean value of samples meeting existing standard	Mean value of samples exceeding existing standard	P	F
PIBI	TF5.5	Spring	3.2	1.8	0.000	61.68
		Summer	2.3	1.1	0.000	49.24
	RET5.2	Spring	2.7	2.0	0.004	9.41
		Summer	2.0	2.0	0.960	0.003
	LE5.5	Spring	2.3	2.5	0.242	1.16
		Summer	2.5	2.6	0.287	1.43
Richness	TF5.5	Spring	36.8	38.2	0.486	0.497
		Summer	45.6	51.4	0.016	6.25
	RET5.2	Spring	31.5	34.6	0.214	1.69
		Summer	32.9	35.3	0.612	0.261
	LE5.5	Spring	42.5	40.1	0.126	2.39
		Summer	41.3	40.6	0.702	0.147
Evenness	TF5.5	Spring	0.62	0.52	0.014	6.71
		Summer	0.65	0.56	0.045	4.25
	RET5.2	Spring	0.62	0.53	0.042	4.45
		Summer	0.59	0.53	0.286	1.16
	LE5.5	Spring	0.55	0.51	0.108	1.94
		Summer	0.48	0.55	0.001	11.57

Table 4. Phytoplankton community characteristics in relation to existing chlorophyll *a* standards. Values are PIBI values (1-5), Richness=number of species, Evenness= Pielou's evenness index (0-1). Data from 2011-2013 James River study. ANOVA results indicated by p and F values. Significant ANOVA (p<0.05) in bold.

Parameter	Segment	Season	Mean value of samples meeting existing standard	Mean value of samples exceeding existing standard	P value	F value
PIBI	JMSTFU	Spring	-	-	-	-
		Summer	4.1	3.5	0.083	3.23
	JMSTFL	Spring	-	-	-	-
		Summer	3.8	2.2	0.007	7.88
	JMSMH	Spring	1.7	1.5	0.037	4.41
		Summer	1.9	2.1	0.000	45.79
	JMSPH	Spring	2.4	2.0	0.038	4.49
		Summer	2.4	2.3	0.409	0.690
Richness	JMSTFU	Spring	5.9	12.0	0.029	7.56
		Summer	7.1	17.0	0.038	4.46
	JMSTFL	Spring	16.0	16.2	0.942	0.005
		Summer	19.7	26.2	0.019	5.96
	JMSMH	Spring	7.5	8.2	0.268	1.23
		Summer	5.7	9.6	0.000	78.40
	JMSPH	Spring	10.8	10.5	0.797	0.007
		Summer	8.1	8.8	0.347	0.896
Evenness	JMSTFU	Spring	0.79	0.46	0.142	2.74
		Summer	0.72	0.84	0.553	0.360
	JMSTFL	Spring	0.70	0.59	0.154	2.289
		Summer	0.62	0.56	0.543	0.375
	JMSMH	Spring	0.74	0.26	0.000	242.56
		Summer	0.65	0.52	0.001	10.74
	JMSPH	Spring	0.76	0.47	0.000	35.12
		Summer	0.81	0.35	0.000	106.82

Table 5. Percentage of samples from James River CBP dataset (1985-2013) below or exceeding existing chlorophyll *a* standards with Fair-Poor or Poor (≤ 2.67) and Poor (≤ 2.0) PIBI score for each season/salinity regime.

Season	Salinity Regime	% Samples Fair-Poor or Poor		% Samples Poor	
		Meets current Chl <i>a</i> standard	Exceeds current Chl <i>a</i> standard	Meets current Chl <i>a</i> standard	Exceeds current Chl <i>a</i> standard
Spring	TFL	27.5	96.0	9.8	96.0
	OH	80.0	50.0	80.0	0.0
	MH	47.8	48.3	26.1	10.3
	PH	82.9	100.0	60.0	66.7
Summer	TFL	69.2	100.0	53.8	97.5
	OH	77.8	100.0	59.3	100.0
	MH	100.0	90.9	56.3	45.5
	PH	64.6	45.8	27.1	16.7

Table 6. Percentage of samples from James River chlorophyll *a* study (2011-2013) below or exceeding existing chlorophyll *a* standards with Fair-Poor or Poor (≤ 2.67) and Poor (≤ 2.0) PIBI score for each season/salinity regime.

Season	Salinity Regime	% Samples Fair-Poor or Poor		% Samples Poor	
		Meets current Chl <i>a</i> standard	Exceeds current Chl <i>a</i> standard	Meets current Chl <i>a</i> standard	Exceeds current Chl <i>a</i> standard
Spring	TFU				
	TFL				
	OH				
	MH	100.0	95.8	94.7	77.1
	PH	72.2	69.4	66.7	34.7
Summer	TFU	0.0	0.0	0.0	0.0
	TFL	64.4	0.0	40.0	0.0
	OH	100.0	100.0	100.0	100.0
	MH	81.0	85.2	63.1	77.8
	PH	86.1	71.1	27.8	37.8

Table 7. Percentage of samples from lower James River chlorophyll *a* study (2011-2013) at lower chlorophyll *a* thresholds with Fair-Poor or Poor (≤ 2.67) and Poor (≤ 2.0) PIBI score for each season/salinity regime.

Season	Salinity Regime	% Samples Fair-Poor or Poor (PIBI (≤ 2.67))				
		<5 $\mu\text{g/L}$	≥ 5 <10 $\mu\text{g/L}$	≥ 10 <15 $\mu\text{g/L}$	≥ 15 <20 $\mu\text{g/L}$	$\geq 20\mu\text{g/L}$
Spring	MH	100.0	95.0	88.9	100.0	100.0
	PH	68.8	66.7	72.7	60.0	100.0
Summer	MH	84.2	87.5	50.0	77.8	89.8
	PH	63.0	83.3	71.4	66.7	92.3
<hr/>						
Season	Salinity Regime	% Samples Poor (PIBI (≤ 2.0))				
		<5 $\mu\text{g/L}$	≥ 5 <10 $\mu\text{g/L}$	≥ 10 <15 $\mu\text{g/L}$	≥ 15 <20 $\mu\text{g/L}$	$\geq 20\mu\text{g/L}$
Spring	MH	83.3	75.0	66.7	90.9	96.2
	PH	25.0	46.7	72.7	60.0	75.0
Summer	MH	78.9	75.0	25.0	77.8	71.2
	PH	22.2	61.1	57.1	33.3	19.2

Table 8.

Summary of two-way multivariate comparisons of community indices for the effects of season and chlorophyll *a* threshold for all salinity regimes. Provided are multivariate permutation tests of significance for differences between centroids (PERMANOVAs), multivariate dispersion tests, and two-way analyses of similarities (ANOSIM).

Tidal Freshwater								Dispersion Test	ANOSIM
PERMANOVA									
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.			
Season	1	70.52	70.52	21.20	<0.01	9937	F=9.05;P<0.01	R=0.34;P<0.01	
Chl <i>a</i> Threshold	1	88.23	88.23	26.53	<0.01	9952			
Interaction	1	11.48	11.48	3.45	0.03	9950			
Residual	121	402.39	3.33						
Total	124	620							
Lower Tidal Freshwater								Dispersion Test	ANOSIM
PERMANOVA									
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.			
Season	1	56.72	56.72	17.21	<0.01	9946	F= 4.18;P=0.01	R=0.54;P<0.01	
Chl <i>a</i> Threshold	1	8.53	8.53	2.59	0.07	9946			
Interaction	1	2.65	2.65	0.80	0.43	9939			
Residual	59	194.47	3.30						
Total	62	310							
Oligohaline								Dispersion Test	ANOSIM
PERMANOVA									
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.			
Season	1	13.13	13.13	3.06	0.07	9942	F=6.23;P<0.01	R=0.28;P<0.01	
Chl <i>a</i> Threshold	1	6.18	6.18	1.44	0.15	9939			
Interaction	1	9.75	9.75	2.27	0.08	9935			
Residual	49	209.99	4.29						
Total	52	260							
Mesohaline								Dispersion Test	ANOSIM
PERMANOVA									
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.			
Season	1	186.01	186.01	27.22	<0.01	9951	F=11.40;P<0.01	R=0.21;P<0.01	
Chl <i>a</i> Threshold	1	635.21	635.21	92.95	<0.01	9932			
Interaction	1	161.38	161.38	23.62	<0.01	9949			
Residual	462	3157.1	6.83						
Total	465	4185							
Polyhaline								Dispersion Test	ANOSIM
PERMANOVA									
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.			
Season	1	31.62	31.62	10.62	<0.01	9952	F=8.87;P=0.01	R=0.39;P<0.01	
Chl <i>a</i> Threshold	1	69.69	69.69	23.40	<0.01	9946			
Interaction	1	34.48	34.48	11.58	<0.01	9954			
Residual	288	857.62	2.98						
Total	291	1164							

Table 9.

Summary of two-way multivariate comparisons of measures of phytoplankton phylogenetic groups for the effects of season and chlorophyll *a* threshold for all salinity regimes. Provided are multivariate permutation tests of significance for differences between centroids (PERMANOVAs), multivariate dispersion tests, and analyses of similarities (ANOSIM).

Tidal Freshwater								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(permutation)	# Perm.		
Season	1	6278	6278	11.36	<0.01	9967	F=32.59;P<0.01	R=0.34;P<0.01 R=0.30;P<0.01
Chl <i>a</i> Threshold	1	14107	14107	25.52	<0.01	9961		
Interaction	1	1520	1520	2.75	0.06	9964		
Residual	123	67985	553					
Total	126	97296						
Lower Tidal Freshwater								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(permutation)	# Perm.		
Season	1	1400	1400	7.62	<0.01	9961	F=2.03;P=0.26	R=0.37;P<0.01 R=0.32;P<0.01
Chl <i>a</i> Threshold	1	58	58	0.32	0.77	9959		
Interaction	1	270	270	1.47	0.22	9968		
Residual	123	11022	184					
Total	126	13930						
Oligohaline								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(permutation)	# Perm.		
Season	1	5252	5252	4.95	0.02	9943	F=2.00;P=0.47	R=0.26;P<0.01 R=0.10;P=0.10
Chl <i>a</i> Threshold	1	500.47	500	0.47	0.75	9948		
Interaction	1	1429	1429	1.35	0.18	9942		
Residual	50	53109	1062					
Total	53	68990						
Mesohaline								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(permutation)	# Perm.		
Season	1	34117	34117	44.89	<0.01	9962	F=8.94;P<0.01	R=0.23;P<0.01 R=0.24;P<0.01
Chl <i>a</i> Threshold	1	52329	52329	68.85	<0.01	9951		
Interaction	1	14446	14446	19.01	<0.01	9959		
Residual	462	351160	760					
Total	465	450490						
Polyhaline								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(permutation)	# Perm.		
Season	1	8657	8657	16.24	<0.01	9956	F=2.10;P=0.13	R=0.35;P<0.01 R=0.38;P<0.01
Chl <i>a</i> Threshold	1	7930	7930	14.87	<0.01	9961		
Interaction	1	3001	3001	5.63	<0.01	9951		
Residual	288	153560	533					
Total	291	203530						

Table 10. Summary of two-way multivariate comparisons of measures of phytoplankton taxonomic composition for the effects of season and chlorophyll *a* threshold for all salinity regimes. Provided are multivariate permutation tests of significance for differences between centroids (PERMANOVAs), multivariate dispersion tests, and analyses of similarities (ANOSIM).

Tidal Freshwater								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Season	1	20000	20000	10.21	<0.01	9922	F=51.23;P<0.01	R=0.41;P<0.01 R=0.39;P<0.01
Chl <i>a</i> Threshold	1	22926	22926	11.70	<0.01	9911		
Interaction	1	5992.3	5992.3	3.06	<0.01	9912		
Residual	123	240970	1959.1					
Total	126	303550						
Lower Tidal Freshwater								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Season	1	10204	10204	7.86	<0.01	9937	F=3.09;P=0.1817	R=0.74;P<0.01 R=0.35;P<0.01
Chl <i>a</i> Threshold	1	1836.1	1836.1	1.41	0.15	9918		
Interaction	1	937.1	937.1	0.72	0.72	9932		
Residual	59	76642	1299					
Total	62	96949						
Oligohaline								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	#		
Season	1	5349.6	5349.6	2.64	0.02	9922	F=7.05;P=0.03	R=0.01;P=0.43 R=0.10;P=0.17
Chl <i>a</i> Threshold	1	3307.2	3307.2	1.63	0.12	9915		
Interaction	1	4336.2	4336.2	2.14	0.04	9935		
Residual	49	99254	2025.6					
Total	52	115970						
Mesohaline								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Season	1	82312	82312	39.17	<0.01	9935	F=4.51;P=0.01	R=0.30;P<0.01 R=0.20;P<0.01
Chl <i>a</i> Threshold	1	53286	53286	25.36	<0.01	9930		
Interaction	1	39003	39003	18.56	<0.01	9923		
Residual	462	970890	2102					
Total	465	115130						
Polyhaline								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Season	1	24253	24253	12.31	<0.01	9935	F=19.44;P<0.01	R=0.41;P<0.01 R=0.44;P<0.01
Chl <i>a</i> Threshold	1	11964	11964	6.07	<0.01	9949		
Interaction	1	9735	9735	4.94	<0.01	9933		
Residual	288	567580	1971					
Total	291	665220						

Table 11. Summary of one-way multivariate comparisons of measures of phytoplankton community indices for all salinity regime and season combinations between samples collected when chlorophyll *a* was above or below the threshold level. Provided are multivariate permutation tests of significance for differences between centroids (PERMANOVAs), multivariate dispersion tests, and one-way analyses of similarities.

Tidal Freshwater - Spring								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Chl <i>a</i> Threshold	1	24.12	24.12	11.85	<0.01	9949	F=15.42;P<0.01	R=0.26;P<0.01
Residual	33	67.176	2.04					
Total	34	91.296						
Tidal Freshwater - Summer								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Chl <i>a</i> Threshold	1	115.56	115.56	30.34	<0.01	9946	F=4.82;P=0.03	R=0.35;P<0.01
Residual	88	335.22	3.81					
Total	89	450.78						
Mesohaline - Spring								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Chl <i>a</i> Threshold	1	287.46	287.46	65.87	<0.01	9954	F=12.53;P<0.01	R=0.27;P<0.01
Residual	278	1213	4.36					
Total	279	1501						
Mesohaline - Summer								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Chl <i>a</i> Threshold	1	201.19	201.19	60.01	<0.01	9966	F=0.11;P=0.76	R=0.37;P<0.01
Residual	184	616.93	3.35					
Total	185	818.12						
Polyhaline - Spring								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Chl <i>a</i> Threshold	1	15.05	15.05	7.17	<0.01	9944	F=0.16;P=0.78	R=0.32;P<0.01
Residual	29	60.84	2.10					
Total	30	75.89						
Polyhaline - Summer								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perm.		
Chl <i>a</i> Threshold	1	167.61	167.61	37.26	<0.01	9962	F=35.25;P<0.01	R=0.06;P=0.03
Residual	259	1165.20	4.50					
Total	260	1332.8						

Table 12. Summary of Spearman rank correlation coefficients between 2D nMDS axes and the original log-transformed standardized community indices.

Tidal Freshwater						
Spring	Total Abundance	Average Cell Size	Number of Species	Species Evenness	<i>Microcystis aeruginosa</i>	
nMDS1	0.85	0.06	0.74	-0.93	0.00	
nMDS2	0.04	-0.96	-0.13	-0.30	0.00	
Summer	Total Abundance	Average Cell Size	Number of Species	Species Evenness	<i>Microcystis aeruginosa</i>	
nMDS1	-0.96	0.64	-0.78	0.74	-0.82	
nMDS2	0.10	-0.62	-0.26	-0.61	0.03	
Mesohaline						
Spring	Total Abundance	Average Cell Size	Number of Species	Species Evenness	Dinoflagellate to Diatom Ratio	
nMDS1	-0.93	-0.12	-0.35	0.81	-0.78	
nMDS2	-0.11	-0.65	-0.83	-0.48	0.15	
Summer	Total Abundance	Average Cell Size	Number of Species	Species Evenness	Dinoflagellate to Diatom Ratio	
nMDS1	-0.84	-0.65	-0.57	0.50	-0.81	
nMDS2	0.45	-0.64	0.23	-0.43	-0.06	
Polyhaline						
Spring	Total Abundance	Average Cell Size	Number of Species	Species Evenness	Dinoflagellate to Diatom Ratio	
nMDS1	0.94	-0.47	0.78	-0.79	0.36	
nMDS2	-0.15	0.69	0.57	0.41	-0.33	
Summer	Total Abundance	Average Cell Size	Number of Species	Species Evenness	Dinoflagellate to Diatom Ratio	
nMDS1	0.80	0.84	0.02	-0.90	0.72	
nMDS2	-0.26	-0.16	-0.87	-0.07	-0.03	

Table 13. Summary of one-way multivariate comparisons of phytoplankton phylogenetic groups for salinity regime and Season combinations between samples collected when chlorophyll *a* was above or below the threshold level. Provided are multivariate permutation tests of significance for differences between centroids (PERMANOVAs), multivariate dispersion tests, and analyses of similarities.

Tidal Freshwater - Spring									
PERMANOVA							Dispersion Test	ANOSIM	
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.			
Chl <i>a</i> Threshold	1	3761	3761	6.66	<0.01	9959	F=21.16;P<0.01	R=0.16;P<0.01	
Res	33	18630	565						
Total	34	22391							
Tidal Freshwater - Summer									
PERMANOVA							Dispersion Test	ANOSIM	
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.			
Chl <i>a</i> Threshold	1	18429	18429	33.61	<0.01	9954	F=77.79;P<0.01	R=0.37;P<0.01	
Res	90	49355	548						
Total	91	67784							
Mesohaline -Spring									
PERMANOVA							Dispersion Test	ANOSIM	
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.			
Chl <i>a</i> Threshold	1	24184	24184	31.92	<0.01	9958	F=10.68;P<0.01	R=0.16;P<0.01	
Res	278	210610	758						
Total	279	234800							
Mesohaline -Summer									
PERMANOVA							Dispersion Test	ANOSIM	
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.			
Chl <i>a</i> Threshold	1	39371	39371	51.54	<0.01	9955	F=17.46;P<0.01	R=0.38;P<0.01	
Res	184	140550	764						
Total	185	179920							
Polyhaline -Spring									
PERMANOVA							Dispersion Test	ANOSIM	
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.			
Chl <i>a</i> Threshold	1	1705	1705	3.82	<0.01	9964	F=3.42;P=0.11	R=0.07;P=0.19	
Res	29	12928	446						
Total	30	14633							
Polyhaline -Summer									
PERMANOVA							Dispersion Test	ANOSIM	
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.			
Chl <i>a</i> Threshold	1	35622	35622	66.48	<0.01	9955	F=4.00;P=0.06	R=0.38;P<0.01	
Res	259	138770	536						
Total	260	174400							

Table 14. Summary of Spearman rank correlation coefficients between 2D nMDS axes and the original log-transformed phylogenetic group variables.

Tidal Freshwater							
Spring							
	Diatoms	Dinoflagellates	Cyanobacteria	Chlorophytes	Cryptophytes	Euglenophytes	<i>Microcystis</i> abundance
MDS1	0.55	-0.10	0.84	0.46	0.66	0.29	0.00
MDS2	0.02	-0.13	-0.49	0.23	0.22	0.07	0.00
Summer							
	Diatoms	Dinoflagellates	Cyanobacteria	Chlorophytes	Cryptophytes	Euglenophytes	<i>Microcystis</i> abundance
MDS1	0.83	0.48	0.89	0.81	0.22	0.33	0.82
MDS2	0.17	-0.50	0.21	0.35	-0.27	-0.43	0.40

Mesohaline										
Spring										
	Diatoms	Dinoflagellates	Cyanobacteria	Chlorophytes	Cryptophytes	Euglenophytes	Abundance	<i>Cochlodinium</i> Abundance	<i>Heterocapsa</i> Abundance	<i>Prorocentrum</i> Abundance
MDS1	0.07	-0.71	-0.07	-0.33	0.00	-0.21	0.01	-0.69	-0.73	-0.73
MDS2	-0.34	0.16	-0.02	-0.04	-0.81	-0.45	0.02	0.28	-0.12	-0.12
Summer										
	Diatoms	Dinoflagellates	Cyanobacteria	Chlorophytes	Cryptophytes	Euglenophytes	Abundance	<i>Cochlodinium</i> Abundance	<i>Heterocapsa</i> Abundance	<i>Prorocentrum</i> Abundance
MDS1	-0.27	0.83	-0.17	-0.36	-0.23	0.58	0.66	0.02	0.23	0.23
MDS2	-0.04	-0.01	-0.04	-0.24	-0.81	-0.37	0.20	-0.09	-0.26	-0.26

Polyhaline									
Spring									
	Diatoms	Dinoflagellates	Chlorophytes	Cryptophytes	Euglenophytes	Prasinophytes	Abundance	<i>Heterocapsa</i> Abundance	<i>Prorocentrum</i> Abundance
MDS1	-0.13	0.44	-0.07	0.56	0.66	0.65	0.65	-0.39	-0.32
MDS2	-0.14	0.04	0.07	-0.14	0.15	0.52	0.52	-0.44	-0.75
Summer									
	Diatoms	Dinoflagellates	Cryptophytes	Euglenophytes	Abundance	<i>Cochlodinium</i> Abundance	<i>Heterocapsa</i> Abundance	<i>Prorocentrum</i> Abundance	
MDS1	-0.23	0.84	-0.48	0.25	0.81	0.00	0.16	0.16	
MDS2	-0.18	-0.18	-0.70	-0.62	-0.15	0.08	0.00	0.00	

Table 15. Summary of one-way multivariate comparisons of phytoplankton taxonomic composition for all salinity regime and season combinations between samples collected when chlorophyll *a* was above or below the threshold level. Provided are multivariate permutation tests of significance for differences between centroids (PERMANOVAs), multivariate dispersion tests, and analyses of similarities.

Tidal Freshwater - Spring								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.		
Chl <i>a</i> Threshold	1	6701	6701	3.37	<0.01	9928	F=35.33;P<0.01	R=-0.17;P<0.01
Residual	33	65524	1985.					
Total	34	72225						
Tidal Freshwater - Summer								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.		
Chl <i>a</i> Threshold	1	34130	34130	18.03	<0.01	9925	F=124.41;P<0.01	R=0.429;P<0.01
Residual	89	168510	1893					
Total	90	202640						
Mesohaline - Spring								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.		
Chl <i>a</i> Threshold	1	31142	31142	14.52	<0.01	9956	F=2.32;P=0.17	R=0.12;P<0.01
Residual	278	596220	2145					
Total	279	627360						
Mesohaline Summer								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.		
Chl <i>a</i> Threshold	1	55764	55764	27.77	<0.01	9942	F= 9.86;P<0.01	R=0.38;P<0.01
Residual	183	367540	2008					
Total	184	423310						
Polyhaline Spring								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.		
Chl <i>a</i> Threshold	1	4214	4214	2.69	<0.01	9932	F=8.09;P=0.02	R=0.01;P=0.42
Residual	28	43829	1565					
Total	29	48043						
Polyhaline Summer								
PERMANOVA							Dispersion Test	ANOSIM
Source	DF	SS	MS	Pseudo-F	P(perm)	# Perms.		
Chl <i>a</i> Threshold	1	60390	60390	30.11	<0.01	9939	F=45.93;P<0.01	R=0.45;P<0.01
Residual	259	519430	2006					
Total	260	579820						

Table 16. Summary of Spearman rank correlation coefficients between 2D nMDS axes and the original log-transformed phylogenetic group variables for the Tidal Freshwater regime during Spring. Only those taxa with Spearman rank correlations > 0.40 are provided.

Spring		
Taxon	MDS1	MDS2
<i>Skeletonema costatum</i>	-0.69	
<i>Ulothrix</i> sp.	-0.64	
<i>Pleurosigma</i> sp.	-0.63	
<i>Leptocylindrus danicus</i>	-0.63	
<i>Leptocylindrus minimus</i>	-0.54	
<i>Asterionella formosa</i>	-0.50	
<i>Coscinodiscus</i> sp.	-0.49	
Unid. pennate diatoms (10-30µm)	-0.49	
<i>Nitzschia</i> sp.	-0.46	
<i>Cryptomonas erosa</i>	-0.46	
Unid. centric diatoms(10-30µm)	-0.44	0.47
<i>Thalassionema nitzschioides</i>	-0.42	
<i>Chaetoceros</i> sp.	-0.40	
<i>Surirella</i> sp.	-0.40	
<i>Aulacoseira granulata</i>		0.59
<i>Cyclotella</i> spp.		0.57
<i>Actinastrum hantzschii</i>		0.46
<i>Navicula</i> sp.		0.43
<i>Aulacoseira granulata</i> var. <i>angus</i>		-0.36
<i>Cryptomonas</i> sp.		-0.37
Centric diatom (<10µm)		-0.41
Unid. pennate diatoms(31-60µm)		-0.42

Table 17.

Summary of Spearman rank correlation coefficients between 2D nMDS axes and the original log-transformed taxonomic abundances for the Tidal Freshwater regime during Summer. Only those taxa with Spearman rank correlations > 0.40 are provided.

Summer		
Taxon	MDS1	MDS2
Unid. Centric Diatoms(10-30 μm)	-0.84	
<i>Pediastrum duplex</i>	-0.80	
<i>Microcystis incerta</i>	-0.80	
<i>Aulacoseira granulata</i>	-0.79	
<i>Scenedesmus quadricauda</i>	-0.77	
Unid. Pennate Diatoms(<10 μm)	-0.73	
<i>Microcystis aeruginosa</i>	-0.70	
<i>Cylindrotheca closterium</i>	-0.69	
<i>Ankistrodesmus falcatus</i>	-0.69	
<i>Pseudanabaena</i> sp.	-0.66	-0.47
<i>Anabaena</i> sp.	-0.65	
<i>Merismopedia elegans</i>	-0.64	
<i>Dactylococcopsis raphidioides</i>	-0.64	
<i>Merismopedia tenuissima</i>	-0.63	
<i>Anabaena circinalis</i>	-0.61	
<i>Chroococcus dispersus</i>	-0.60	
<i>Cyclotella</i> spp .	-0.58	
<i>Scenedesmus acuminatus</i>	-0.54	
<i>Pediastrum simplex</i>	-0.53	
<i>Pediastrum biradiatum</i>	-0.49	
<i>Leptocylindrus minimus</i>	-0.49	
<i>Coscinodiscus</i> sp .	-0.48	
<i>Crucigenia tetrapedia</i>	-0.47	
<i>Cylindropermopsis philippinensi</i>	-0.47	
<i>Closteriopsis longissima</i>	-0.43	
<i>Scenedesmus dimorphus</i>	-0.43	
<i>Cosmarium</i> sp .	-0.41	
<i>Actinastrum hantzschii</i>	-0.39	
<i>Scenedesmus bernardii</i>	-0.35	
<i>Skeletonema costatum</i>		0.48
<i>Achnanthes</i> sp.		0.44
<i>Aulacoseira granulata</i> var. <i>angus</i>		0.37
<i>Skeletonema potamus</i>		-0.36

Table 18. Summary of Spearman rank correlation coefficients between A) 2-Dimensional nMDS axes for Spring and Summer and B) 3-Dimensional nMDS axes for Summer and the original log-transformed phylogenetic group variables for the Mesohaline regime. Only those taxa with Spearman rank correlations > 0.40 are provided.

A) 2-Dimensional nMDS

Spring		
Taxon	MDS1	MDS2
<i>Heterocapsa triquetra</i>	0.77	
<i>Skeletonema costatum</i>	0.44	-0.45
<i>Prorocentrum minimum</i>	0.39	0.35
<i>Katodinium rotundatum</i>	-0.48	
Unid. Pennate Diatoms (< 10 µm)		-0.43
Summer		
Taxon	MDS1	MDS2
<i>Scrippsiella trochoidea</i>	-0.70	
<i>Cochlodinium polykrikoides</i>	-0.63	
<i>Gymnodinium</i> sp. (20 µm)	-0.50	0.42
Unid. Dinoflagellates	-0.44	
<i>Cryptomonas</i> sp.		0.55
<i>Akashiwo sanguinea</i>		0.39
<i>Katodinium rotundatum</i>		0.37
<i>Skeletonema costatum</i>		-0.49

B) 3-Dimensional nMDS

Summer			
Taxon	MDS1	MDS2	MDS3
<i>Scrippsiella trochoidea</i>	0.72		
<i>Cochlodinium polykrikoides</i>	0.64		
<i>Gymnodinium</i> sp.(20µm)	0.53		0.41
Unid. Dinoflagellates	0.43		
<i>Euglena</i> sp.	0.38		
<i>Skeletonema costatum</i>	-0.39	0.40	-0.48
<i>Leptocylindrus minimus</i>	-0.39		
<i>Cylindrotheca closterium</i>		0.40	
<i>Pleurosigma</i> sp.		0.38	
<i>Prorocentrum micans</i>		-0.47	
<i>Cryptomonas</i> sp.			0.62
<i>Katodinium rotundatum</i>			0.45
<i>Akashiwo sanguinea</i>			0.43

Table 19. Summary of Spearman rank correlation coefficients between A) 2-Dimensional nMDS axes for Spring and Summer and B) 3-Dimensional nMDS axes for Summer and the original log-transformed phylogenetic group variables for the Polyhaline regime. Only those taxa with Spearman rank correlations > 0.40 are provided.

A) 2-Dimensional nMDS

Spring		
Taxon	MDS1	MDS2
<i>Skeletonema costatum</i>	0.69	
<i>Pseudo nitzschia pungens</i>	0.63	
Unid. pennate diatoms(<10 µm)	0.61	
<i>Asterionellopsis glacialis</i>	0.60	
<i>Heterocapsa triquetra</i>	0.58	
<i>Prorocentrum minimum</i>	0.58	
<i>Cerataulina pelagica</i>	0.50	
<i>Rhizosolenia setigera</i>	0.43	
<i>Katodinium rotundatum</i>	-0.72	
Unid. centric diatoms(10-30 µm)	-0.64	
<i>Amphidinium</i> sp.	-0.50	
<i>Pyramimonas</i> sp.	-0.48	
<i>Euglena</i> sp.	-0.48	
<i>Cryptomonas</i> sp.	-0.39	
<i>Chaetoceros</i> sp.		0.52
<i>Scrippsiella trochoidea</i>		0.45
<i>Coscinodiscus</i> sp.		-0.69
<i>Thalassionema nitzschioides</i>		-0.54
<i>Cylindrotheca closterium</i>		-0.41
Summer		
Taxon	MDS1	MDS2
Unid. centric diatoms(10-30 µm)	0.45	
<i>Leptocylindrus minimus</i>	0.44	-0.43
<i>Skeletonema costatum</i>	0.33	-0.61
Unid. pennate diatoms(<10 µm)	0.32	0.33
<i>Cryptomonas</i> sp.	0.30	
<i>Cochlodinium polykrikoides</i>	-0.64	
Unid. dinoflagellates	-0.44	
<i>Scrippsiella trochoidea</i>	-0.37	
<i>Gyrodinium</i> sp .	-0.32	
<i>Gymnodinium</i> sp.(20 µm)	-0.30	
<i>Prorocentrum micans</i>		0.29

B) 3-Dimensional results

Taxon	MDS1	MDS2	MDS3
Unid. centric diatoms(10-30 µm)	0.43		
<i>Leptocylindrus minimus</i>	0.42	-0.37	
<i>Cochlodinium polykrikoides</i>	-0.71		
Unid. dinoflagellates	-0.47		
<i>Scrippsiella trochoidea</i>	-0.42		-0.48
Unid. pennate diatoms(10-30µm)		0.38	-0.37
<i>Skeletonema costatum</i>		-0.66	
<i>Prorocentrum micans</i>			0.35
<i>Prorocentrum minimum</i>			-0.37

Table 20. Harmful algal bloom species in relation to existing chlorophyll *a* standards. Values are cells/ml. Data from 1985-2013 CBP stations.

Parameter	Station	Season	Mean value of samples meeting existing standard	Mean value of samples exceeding existing standard	p	F (df)
<i>Microcystis aeruginosa</i>	TF5.5	Spring	2.3	0.0	0.197	1.73 (1,35)
		Summer	0.6	5,007.7	0.391	0.75 (1,44)
	RET5.2	Spring	884.6	949.4	0.963	0.002 (1,38)
		Summer	536.2		-	-
	LE5.5	Spring	14.4	0.0	0.421	0.65 (1,84)
		Summer	10.2	10.3	0.991	0.00 (1,102)
<i>Prorocentrum minimum</i>	TF5.5	Spring	0.1	0.5	0.306	1.08 (1,35)
		Summer	0.0	0.1	0.623	0.245 (1,44)
	RET5.2	Spring	0.0	0.1	0.321	1.01 (1,38)
		Summer	29.8		-	-
	LE5.5	Spring	96.3	199.8	0.236	1.42 (1,84)
		Summer	25.3	9.4	0.334	0.941 (1,102)
<i>Cochlodinium polykrikoides</i>	TF5.5	Spring	0	0	-	-
		Summer	0	0	-	-
	RET5.2	Spring	0	0	-	-
		Summer	0		-	-
	LE5.5	Spring	0.0	0.0	-	-
		Summer	3.3	7.8	0.476	0.51 (1,102)

Table 21. Data plotted in Figure 17 along with sample size of each chlorophyll *a* bin. Values are % of samples exceeding thresholds within each chlorophyll *a* bin.

Chl <i>a</i> bin	present (>10/ml)	>1000 cells/ml	>3000 cells/ml	>6000 cells/ml	>12,000 cells/ml	n
0-10	7.9	0.0	0.0	0.0	0.0	151
10-20	53.1	14.1	0.0	0.0	0.0	64
20-30	52.0	20.0	4.0	2.0	2.0	50
30-40	73.0	40.5	5.4	2.7	0.0	37
40-50	83.3	50.0	29.2	8.3	0.0	24
50-60	87.5	62.5	31.3	12.5	0.0	16
60-70	91.7	91.7	58.3	16.7	0.0	12
>70	87.0	64.1	52.2	40.2	30.4	92

Table 22. Harmful algal bloom species in relation to existing chlorophyll *a* standards. Values are cells/ml. Data from 2011-2013 James River study.

HAB	Segment	Season	Mean value of samples meeting existing standard	Mean value of samples exceeding existing standard	P	F (df)
<i>Microcystis aeruginosa</i>	JMSTFU	Spring	0	0	-	-
		Summer	0	0	-	-
	JMSTFL	Spring	0	0	-	-
		Summer	146.7	1,276.2	0.28	1.20 (1,46)
	JMSMH	Spring	0	0	-	-
		Summer	0	0	-	-
JMSPH	Spring	0	0	-	-	
	Summer	0	0	-	-	
<i>Prorocentrum minimum</i>	JMSTFU	Spring	0	0	-	-
		Summer	0	0	-	-
	JMSTFL	Spring	0	0	-	-
		Summer	0.0	0.2	0.799	0.065 (1,46)
	JMSMH	Spring	20.9	211.7	0.042	4.21 (1,189)
		Summer	3.1	14.8	0.002	9.94 (1,169)
JMSPH	Spring	123.5	225.0	0.411	0.685 (1,65)	
	Summer	5.8	3.3	0.987	0.755 (1,79)	
<i>Cochlodinium polykrikoides</i>	JMSTFU	Spring	0	0	-	-
		Summer	0	0	-	-
	JMSTFL	Spring	0	0	-	-
		Summer	0	0	-	-
	JMSMH	Spring	0	0	-	-
		Summer	0.4	5,044.0	0.000	14.13 (1,169)
JMSPH	Spring	0	0	-	-	
	Summer	16.3	8,174.7	0.000	27.93 (1,79)	

Table 23. Data plotted in Figure 18 along with sample size of each chlorophyll *a* bin. Values are % of samples exceeding thresholds of *Microcystis aeruginosa* within each chlorophyll *a* bin. Data are summer CBMP samples 1984-2013.

State	Salinity regime	Chl <i>a</i> bin	Present	>5000 cells/ml	>10,000 cells/ml	>20,000 cells/ml	n
Virginia	Tidal Fresh	<5	18.8	6.3	3.1	0.0	32
		>5<10	14.3	0.0	0.0	0.0	21
		>10<15	33.3	11.1	0.0	0.0	9
		>15<20	45.5	0.0	0.0	0.0	11
		>20<25	55.6	33.3	11.1	11.1	9
		>25	48.4	29.7	15.6	9.4	64
	Oligohaline	<5	11.1	0.0	0.0	0.0	9
		>5<10	40.0	8.6	5.7	2.9	35
		>10<15	43.3	3.3	0.0	0.0	30
		>15<20	30.8	0.0	0.0	0.0	26
		>20<25	26.7	6.7	6.7	0.0	15
>25	36.4	27.3	0.0	0.0	11		
Maryland	Tidal Fresh	<5	2.9	0.0	0.0	0.0	34
		>5<10	8.6	1.4	0.0	0.0	70
		>10<15	19.2	5.8	0.0	0.0	52
		>15<20	27.6	6.9	3.4	3.4	29
		>20<25	25.0	6.8	2.3	2.3	44
		>25	37.8	18.1	9.4	3.9	127
	Oligohaline	<5	3.9	0.0	0.0	0.0	77
		>5<10	11.5	0.0	0.0	0.0	78
		>10<15	11.4	2.9	2.9	2.9	35
		>15<20	7.7	0.0	0.0	0.0	26
		>20<25	13.6	4.5	0.0	0.0	22
>25	10.2	3.7	0.0	0.0	108		

Figures

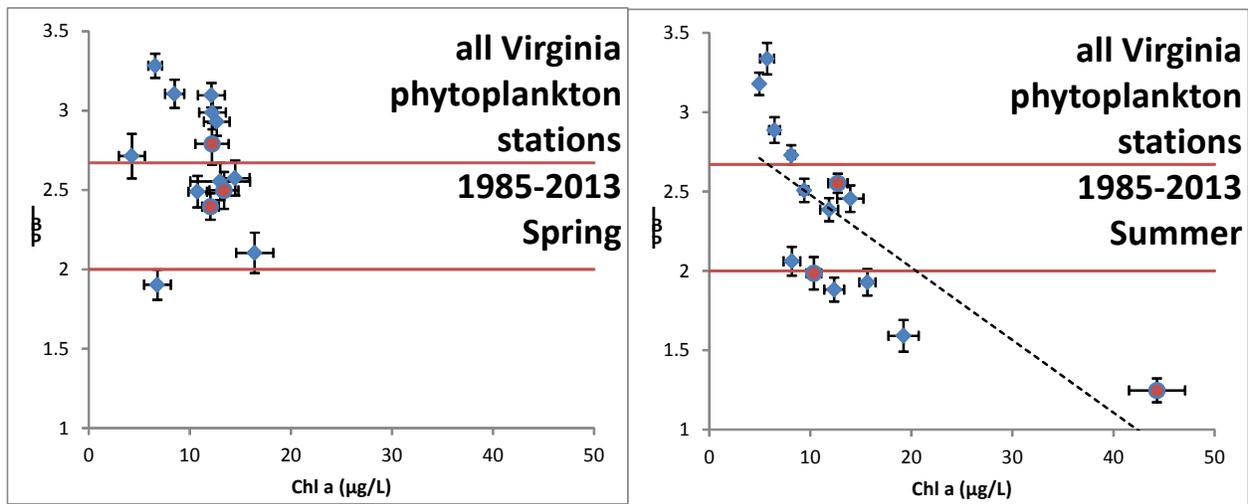


Figure 1. Relationship between chlorophyll *a* and PIBI scores for long term VA CBP phytoplankton monitoring stations (above pycnocline, 1985-2013), significant negative relationship during summer. James River stations in red, other stations in blue. Tidal freshwater James R. station TF5.5 is shown on far right of summer plot with lowest PIBI and highest chlorophyll *a*.

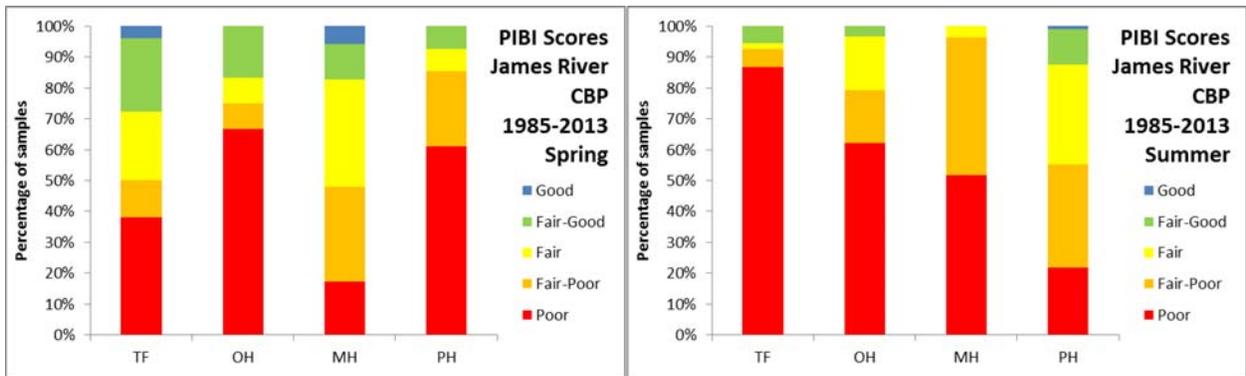


Figure 2. Average PIBI distribution of each season/salinity regime from CBP long term data set.

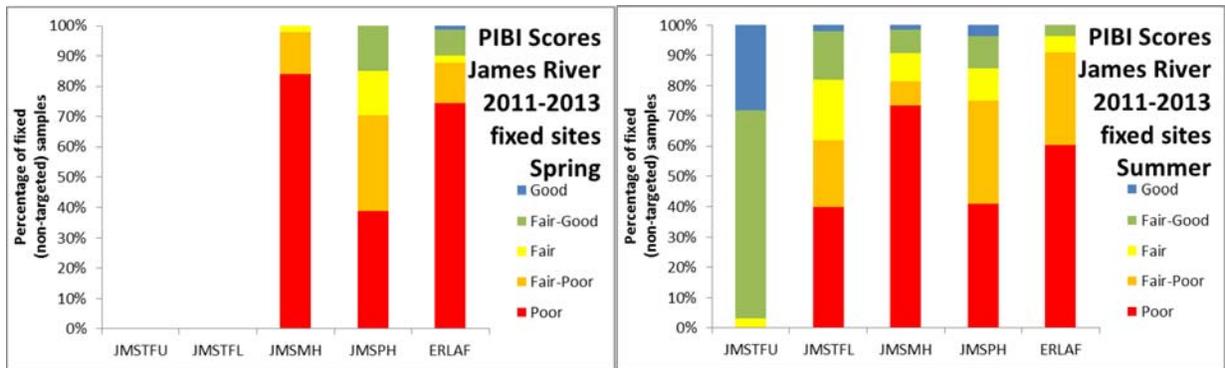


Figure 3. PIBI distribution of each season/salinity regime from 2011-2013 James River study using only fixed (non-bloom targeted sites).

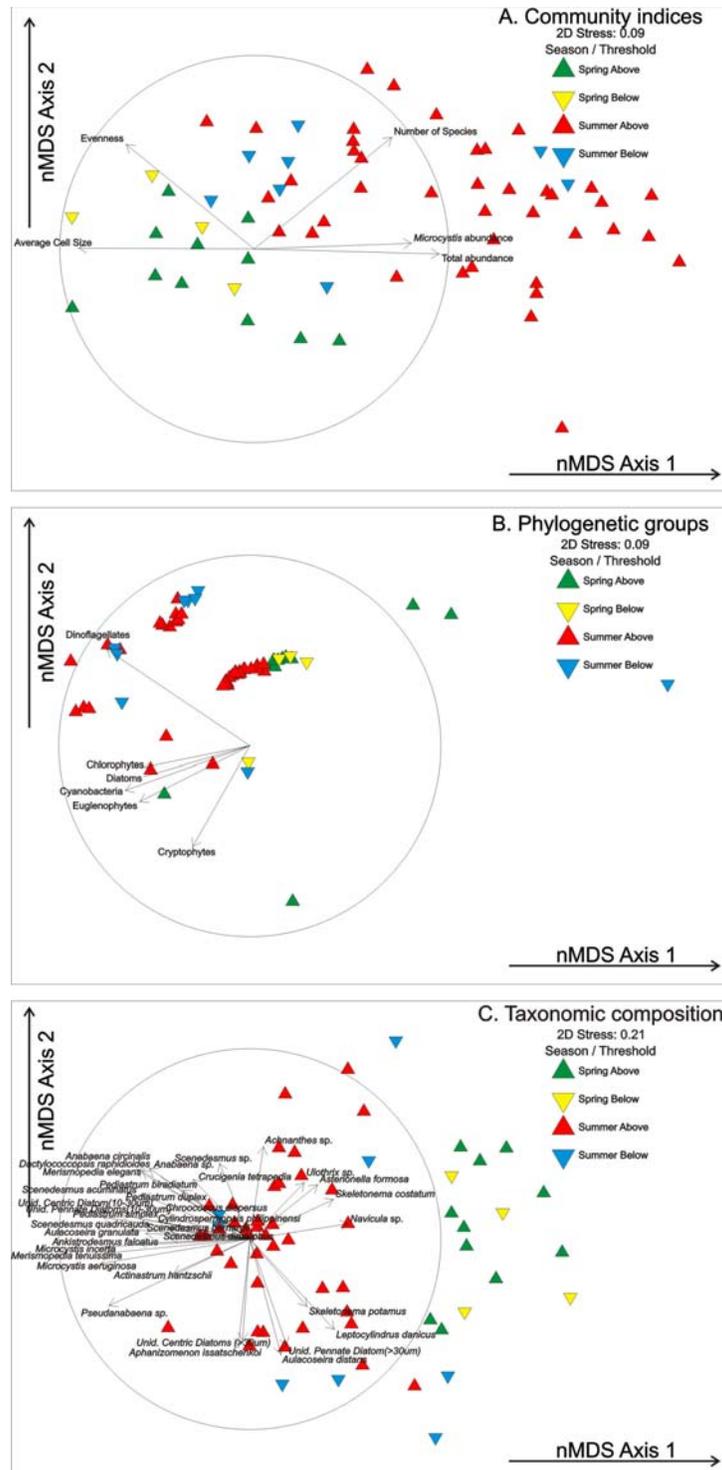


Figure 4. Two-dimensional nMDS ordination of A. Community indices, B. Phylogenetic groups and C. Taxonomic composition comparing samples collected when chlorophyll *a* was Above or Below established water quality thresholds for the lower Tidal Freshwater areas of the James River during the Spring and Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

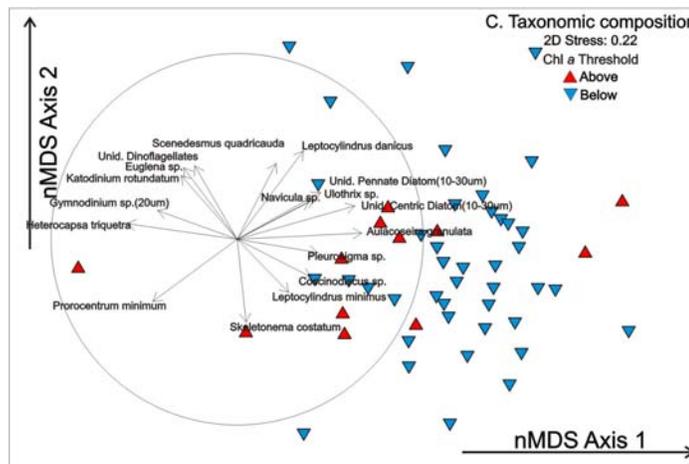
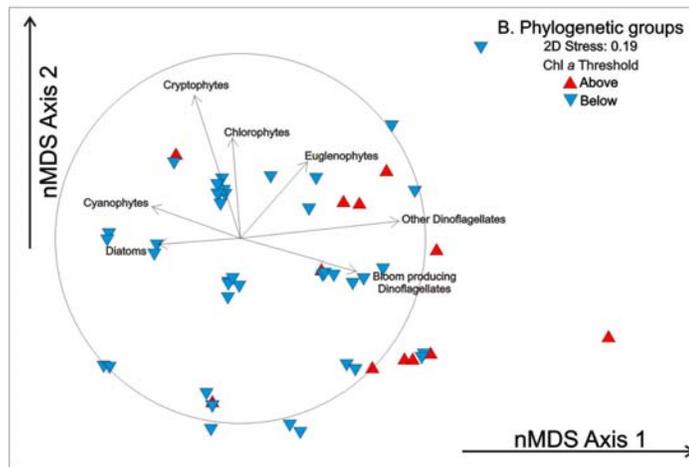
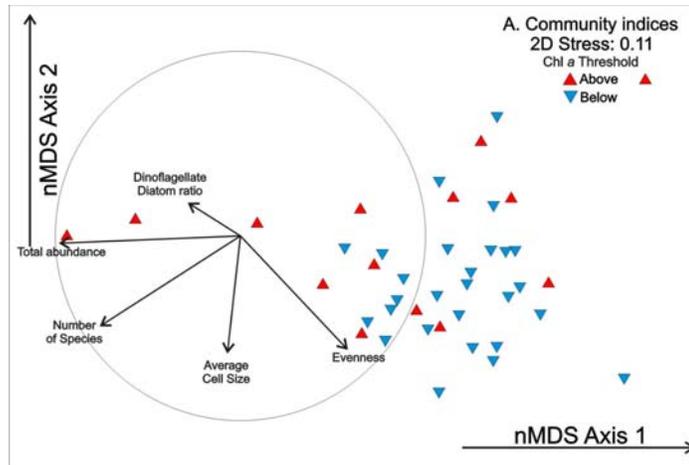


Figure 5. Two-dimensional nMDS ordination of A. Community indices, B. Phylogenetic groups and C. Taxonomic composition comparing samples collected when chlorophyll *a* was Above or Below established water quality thresholds for Oligohaline areas of the James River during the Spring and Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

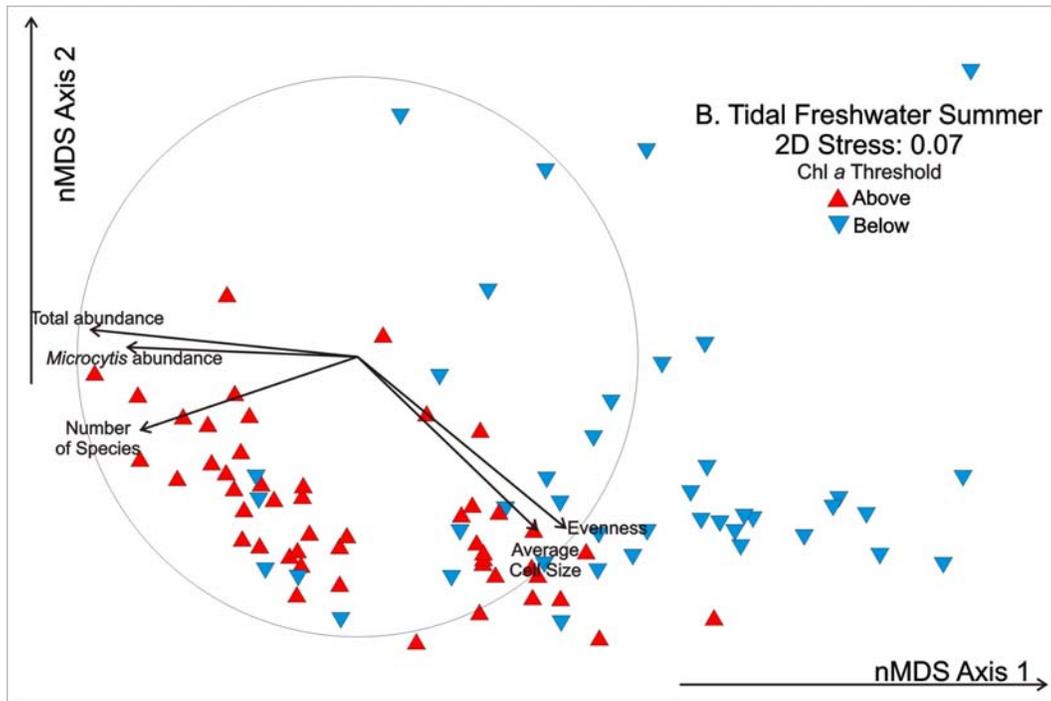
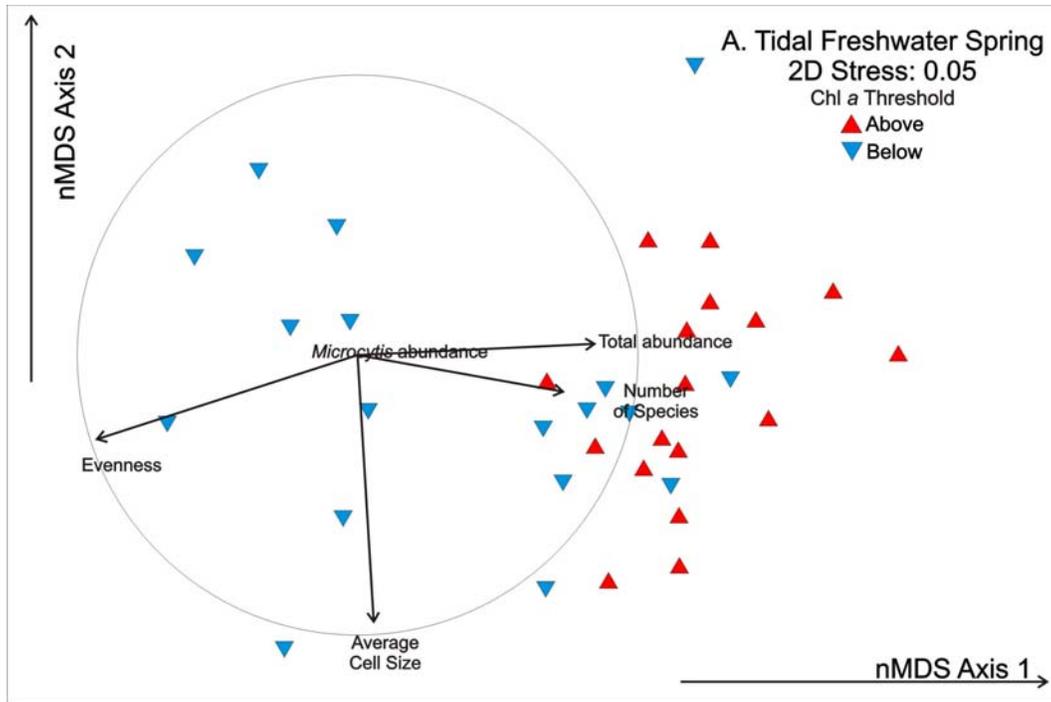


Figure 6. Two-dimensional nMDS ordination of phytoplankton community indices for comparing samples collected when chlorophyll *a* was Above or Below established water quality thresholds for Tidal Freshwater areas of the James River during the A. Spring and B. Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

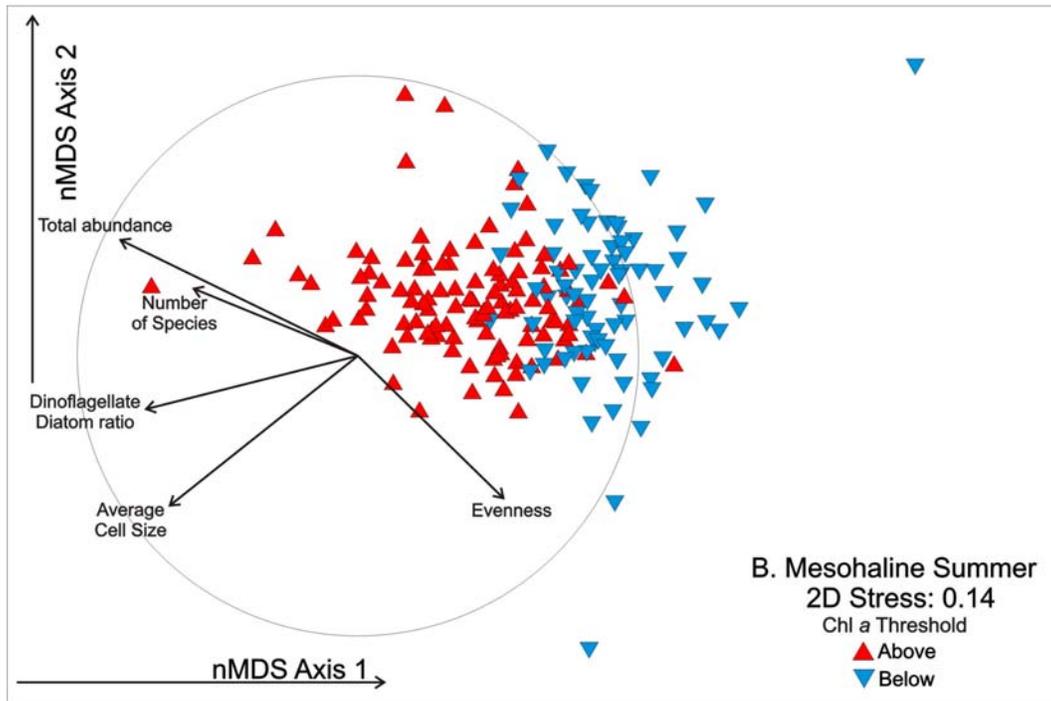
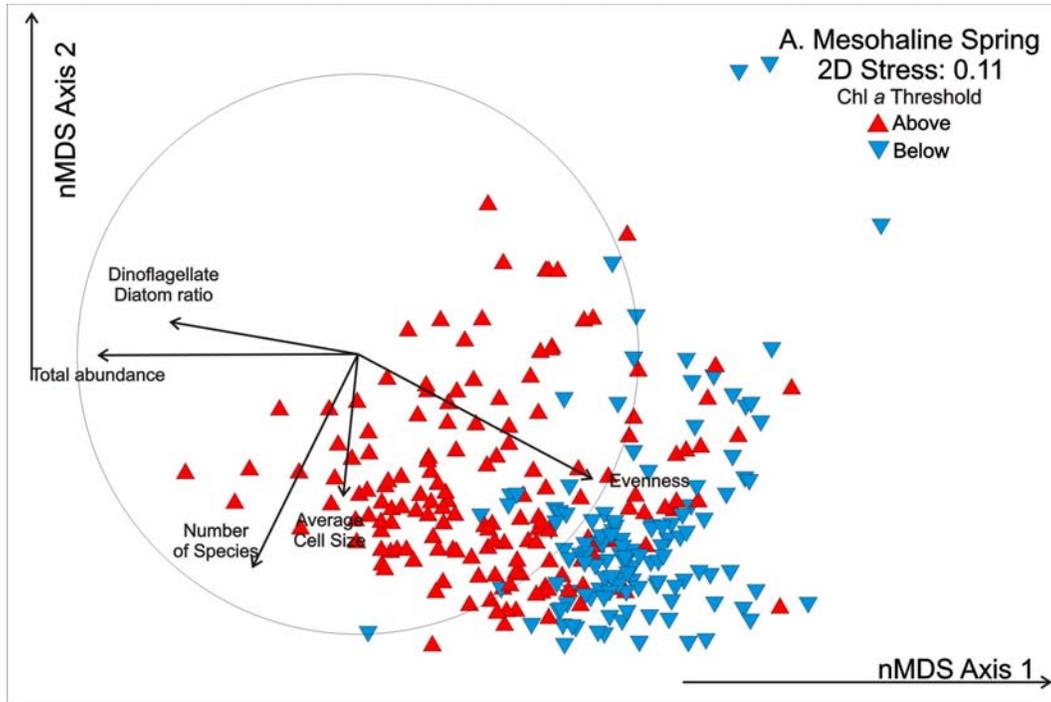


Figure 7. Two-dimensional nMDS ordination of phytoplankton community indices for comparing samples collected when chlorophyll *a* was Above or Below established water quality thresholds for Mesohaline areas of the James River during the A. Spring and B. Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

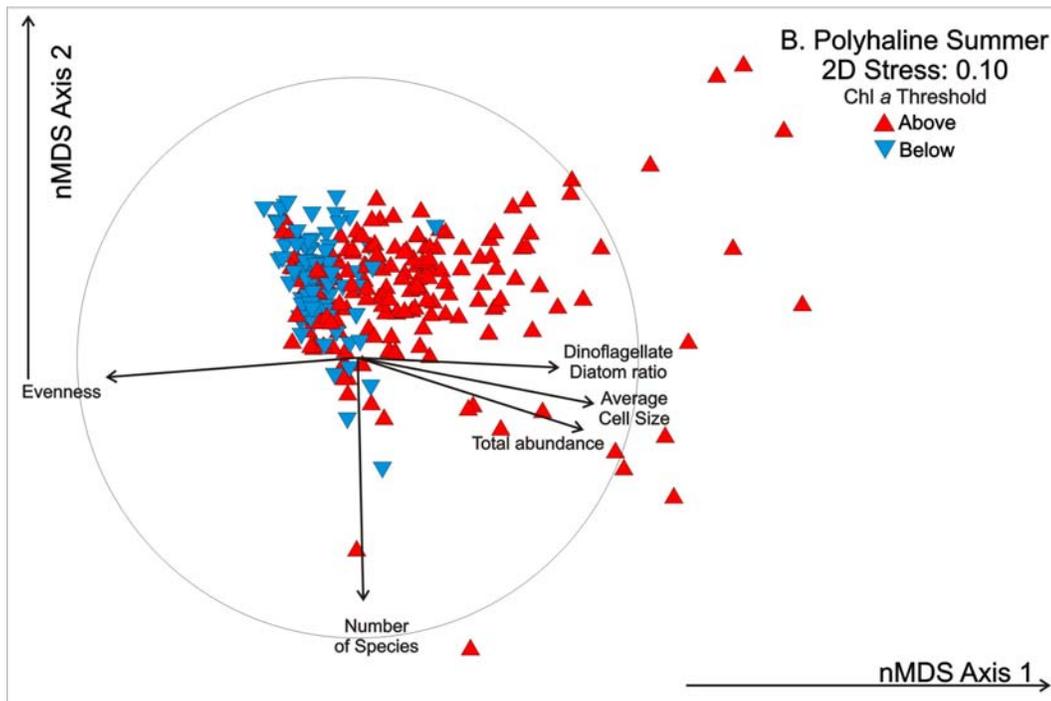
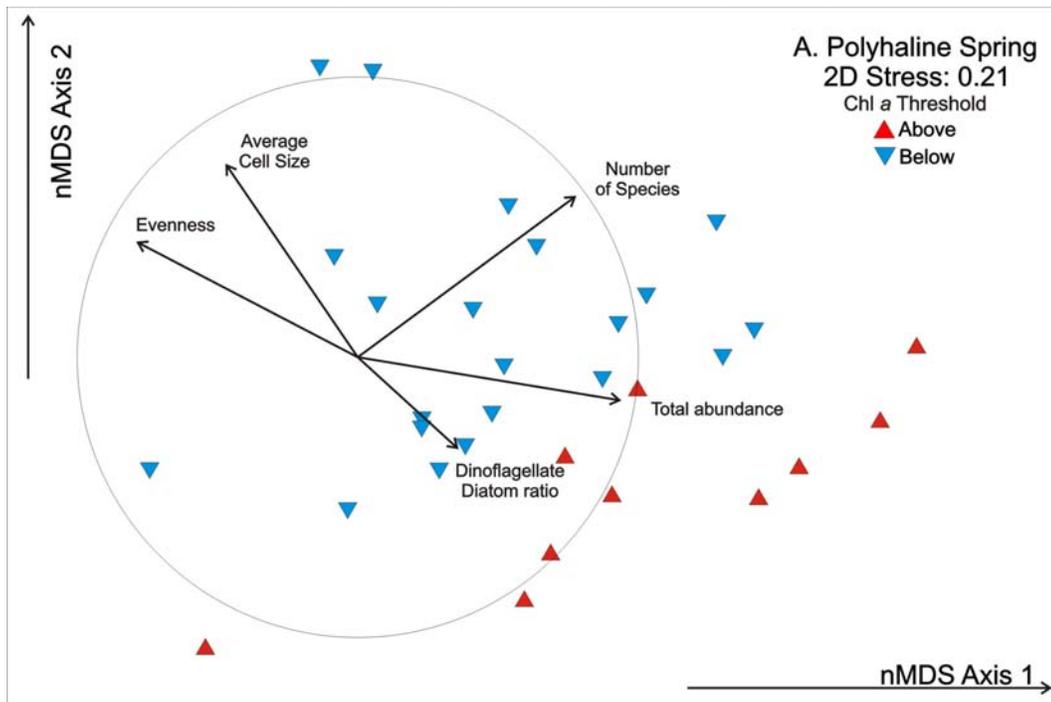


Figure 8. Two-dimensional nMDS ordination of phytoplankton community indices for comparing samples collected when chlorophyll *a* was Above or Below established water quality thresholds for Polyhaline areas of the James River during the A. Spring and B. Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

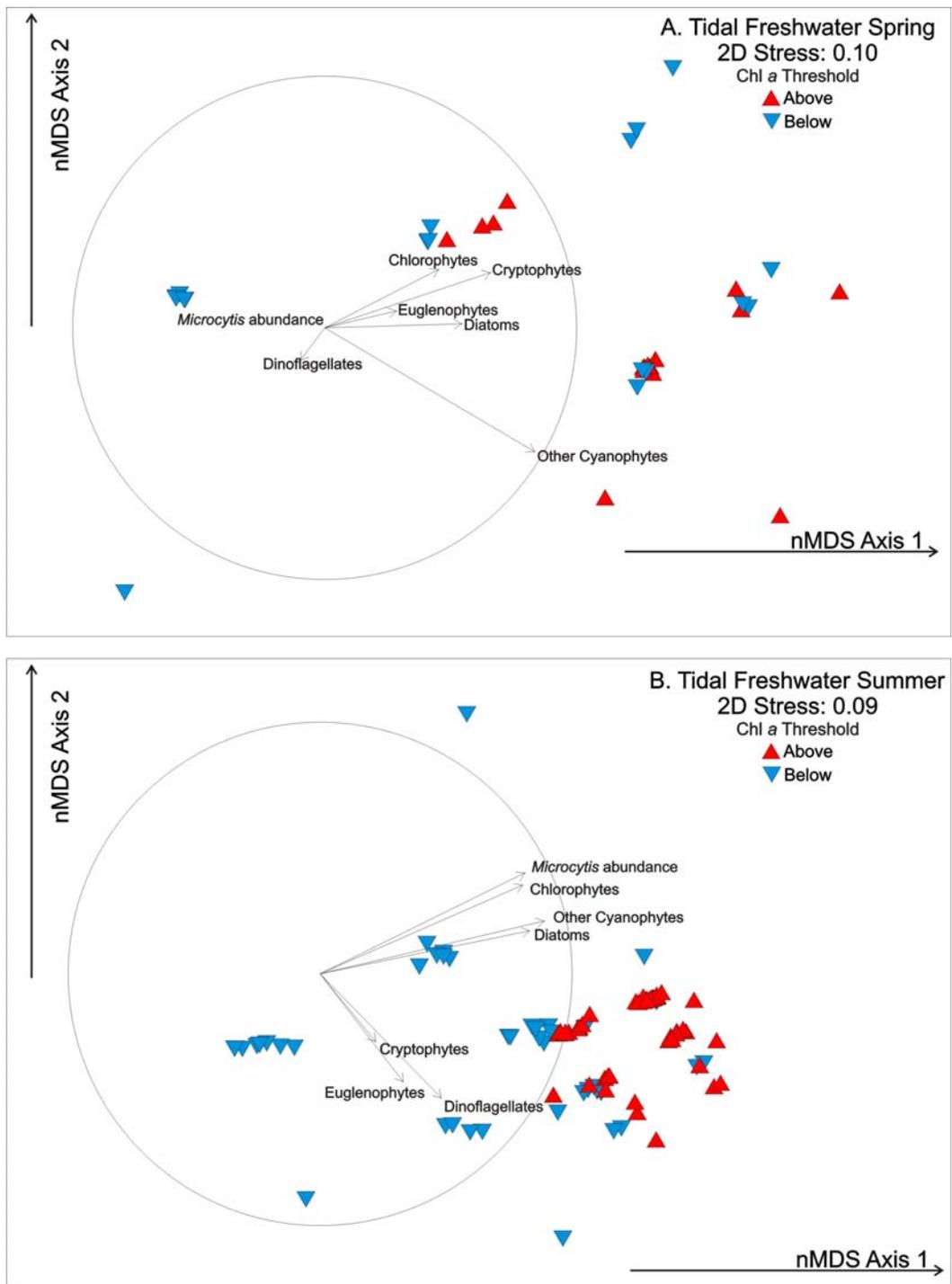


Figure 9. Two-dimensional nMDS ordination of abundances of phytoplankton phylogenetic groups comparing samples collected when chlorophyll a was above or below established water quality thresholds for Tidal Freshwater areas of the James River during A. Spring and B. Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

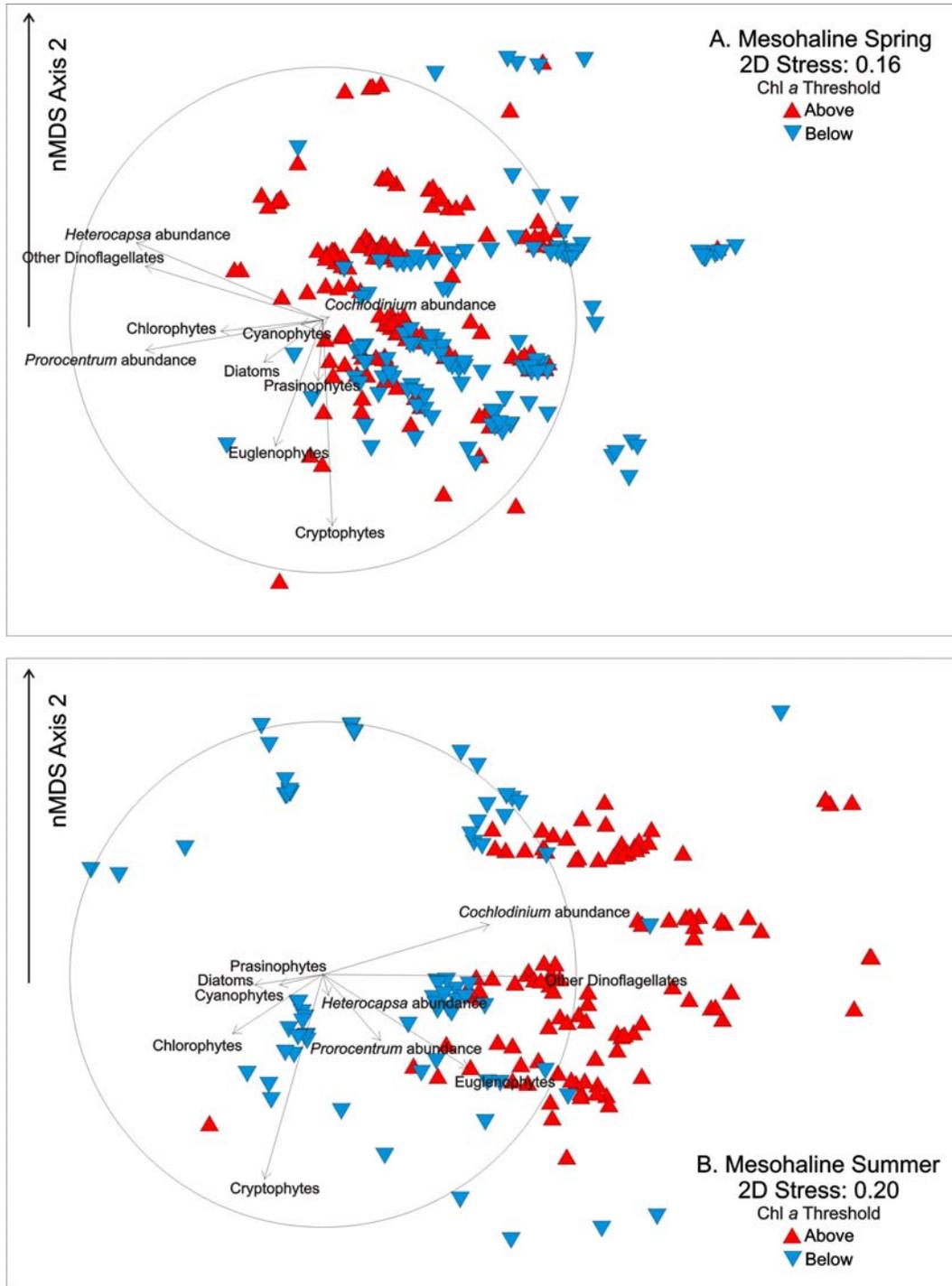


Figure 10. Two-dimensional nMDS ordination of abundances of phytoplankton phylogenetic groups comparing samples collected when chlorophyll a was above or below established water quality thresholds for Mesohaline areas of the James River during A. Spring and B. Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

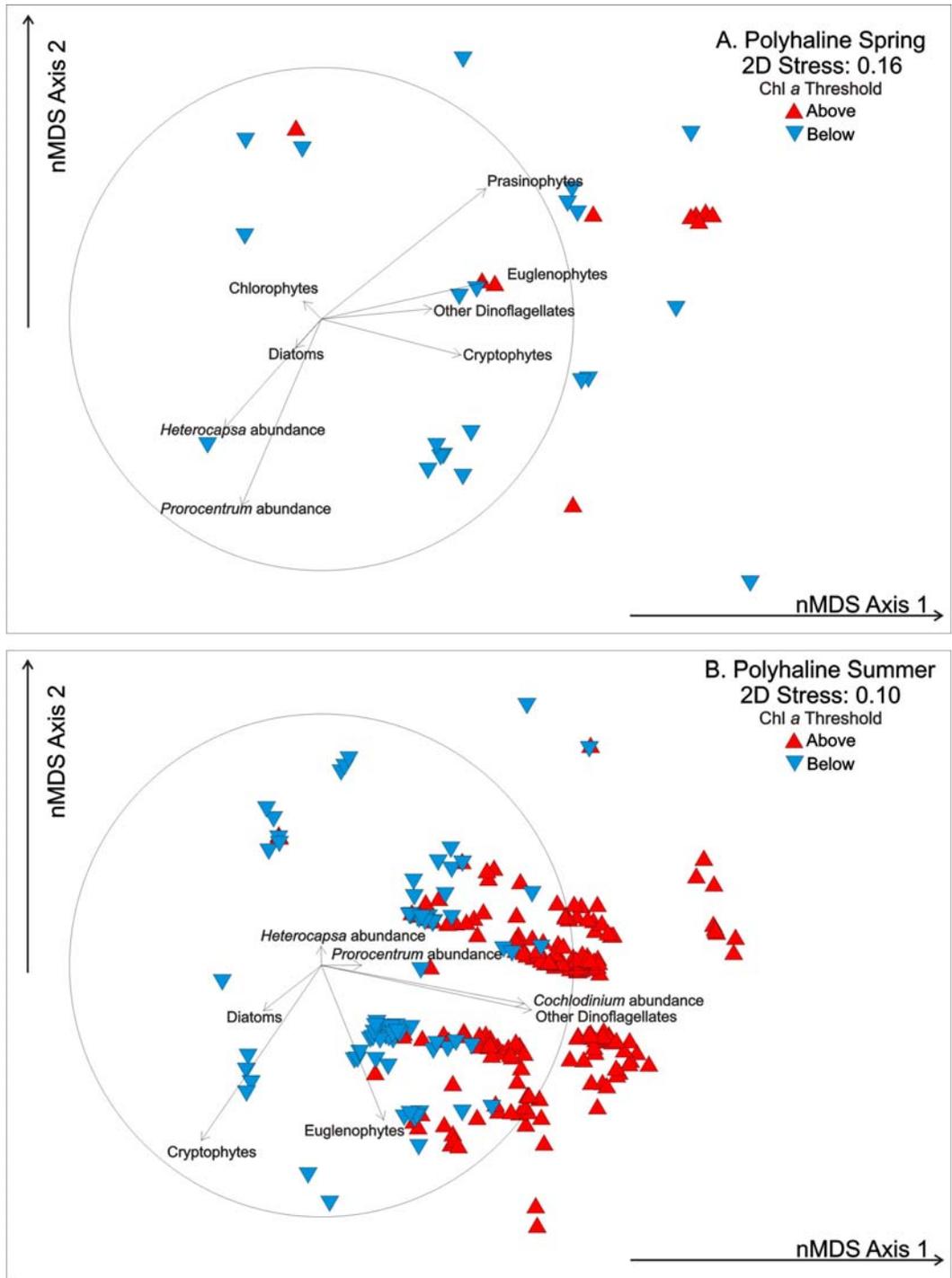


Figure 11. Two-dimensional nMDS ordination of abundances of phytoplankton phylogenetic groups comparing samples collected when chlorophyll a was above or below established water quality thresholds for Polyhaline areas of the James River during A. Spring and B. Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

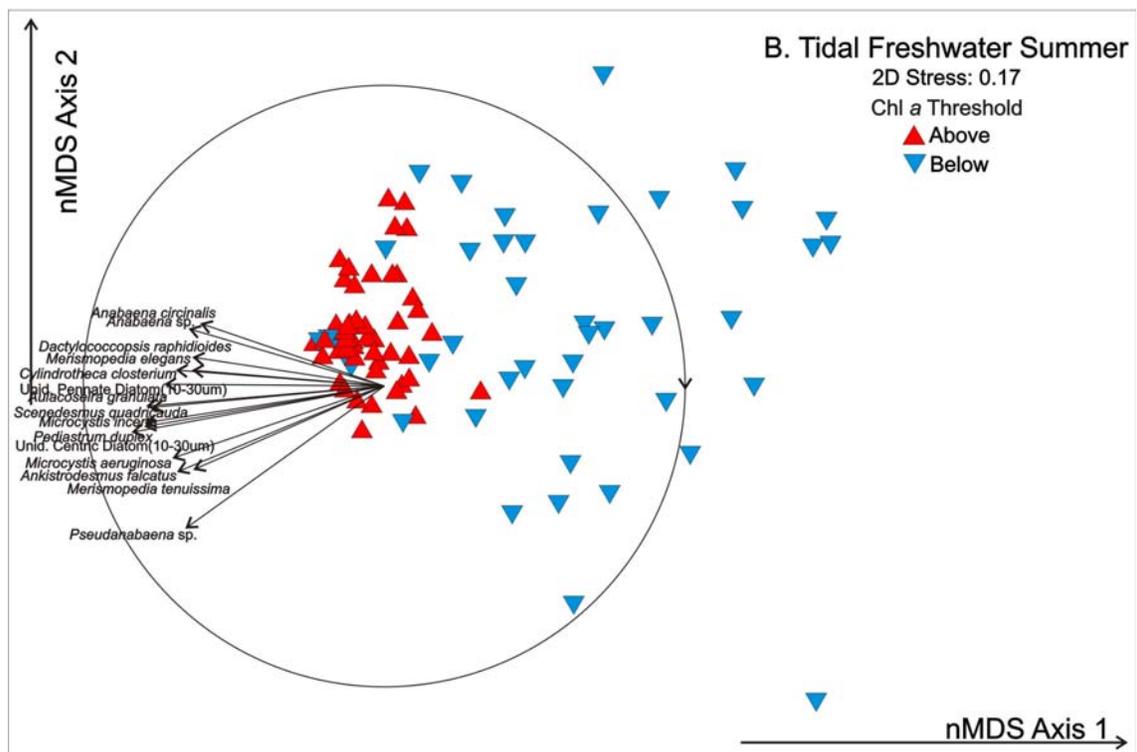
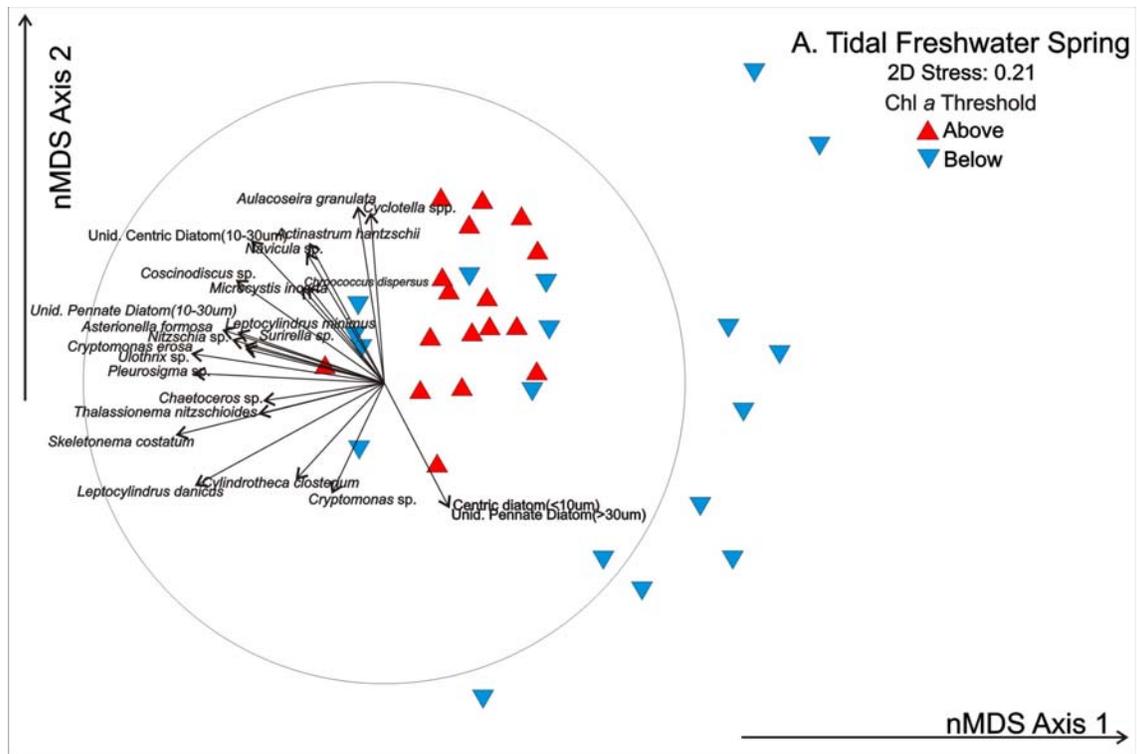


Figure 12. Two-dimensional nMDS ordination of phytoplankton taxonomic composition comparing samples collected when chlorophyll a was above or below established water quality thresholds for Tidal Freshwater areas of the James River during A. Spring and B. Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

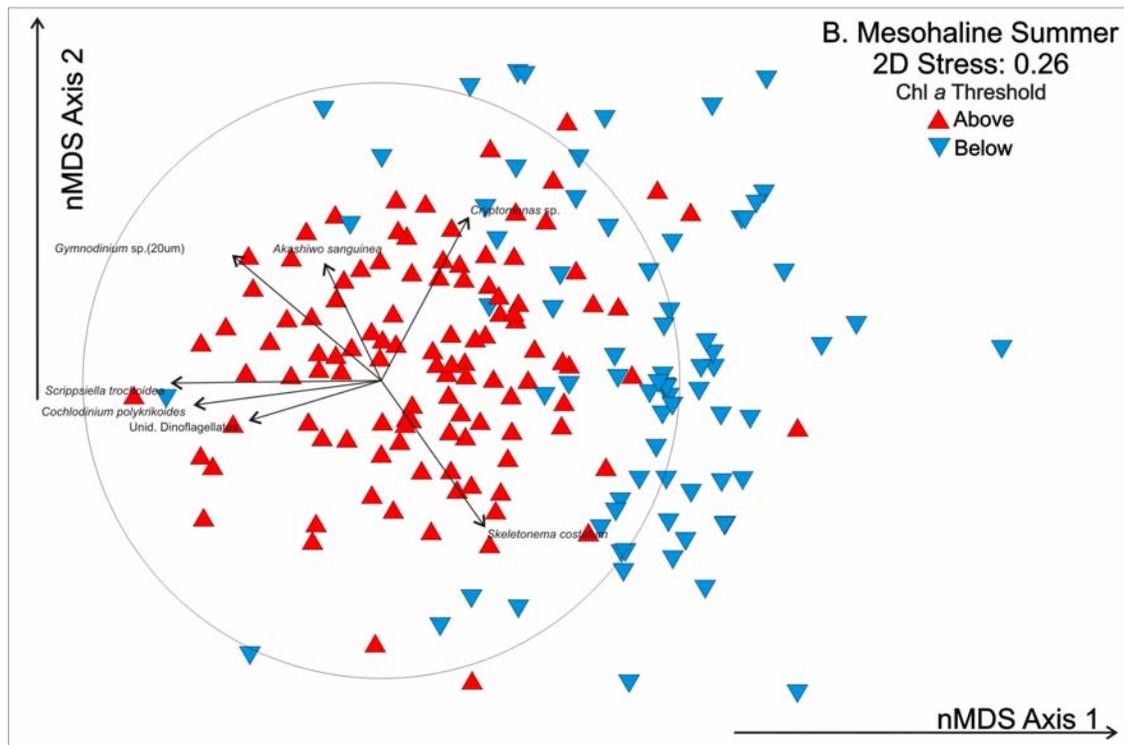
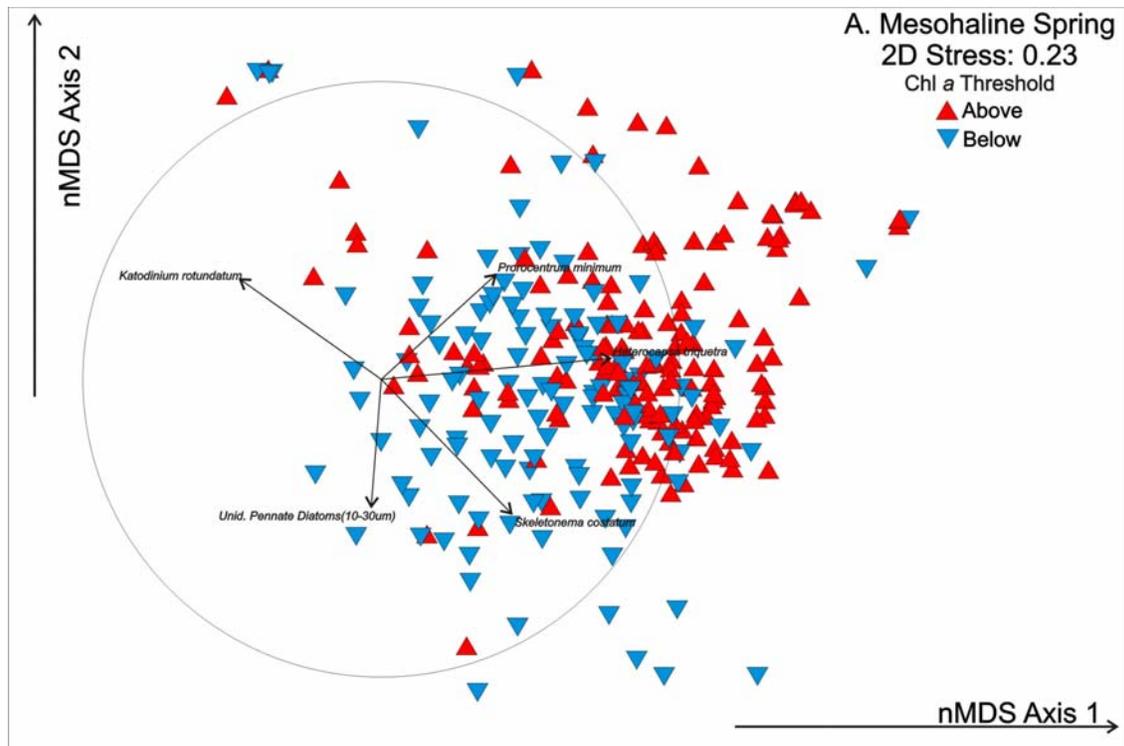


Figure 13. Two-dimensional nMDS ordination of phytoplankton taxonomic composition comparing samples collected when chlorophyll a was above or below established water quality thresholds for Mesohaline areas of the James River during A. Spring and B. Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

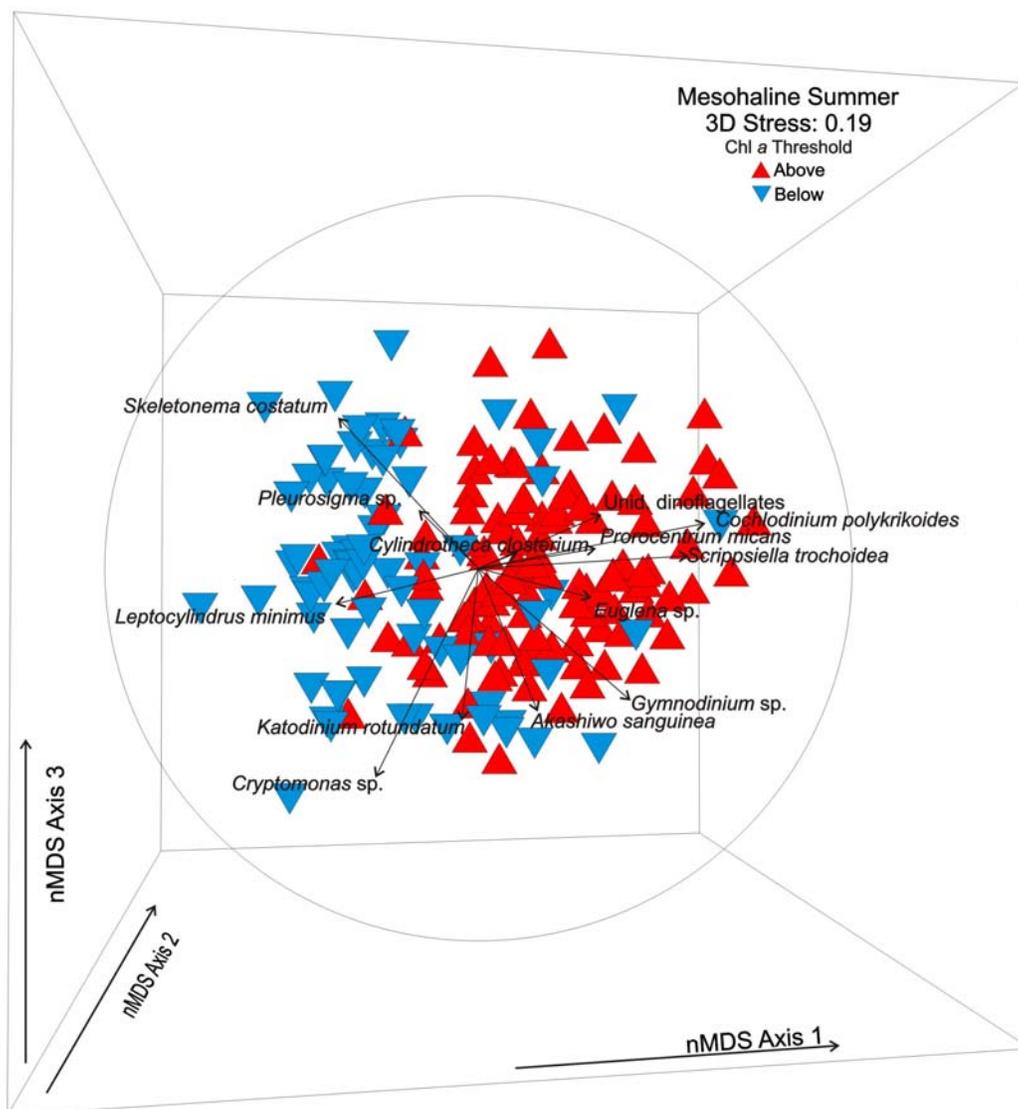


Figure 14. Three-dimensional nMDS ordination of phytoplankton taxonomic composition comparing samples collected when chlorophyll a was above or below established water quality thresholds for Mesohaline areas of the James River during Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

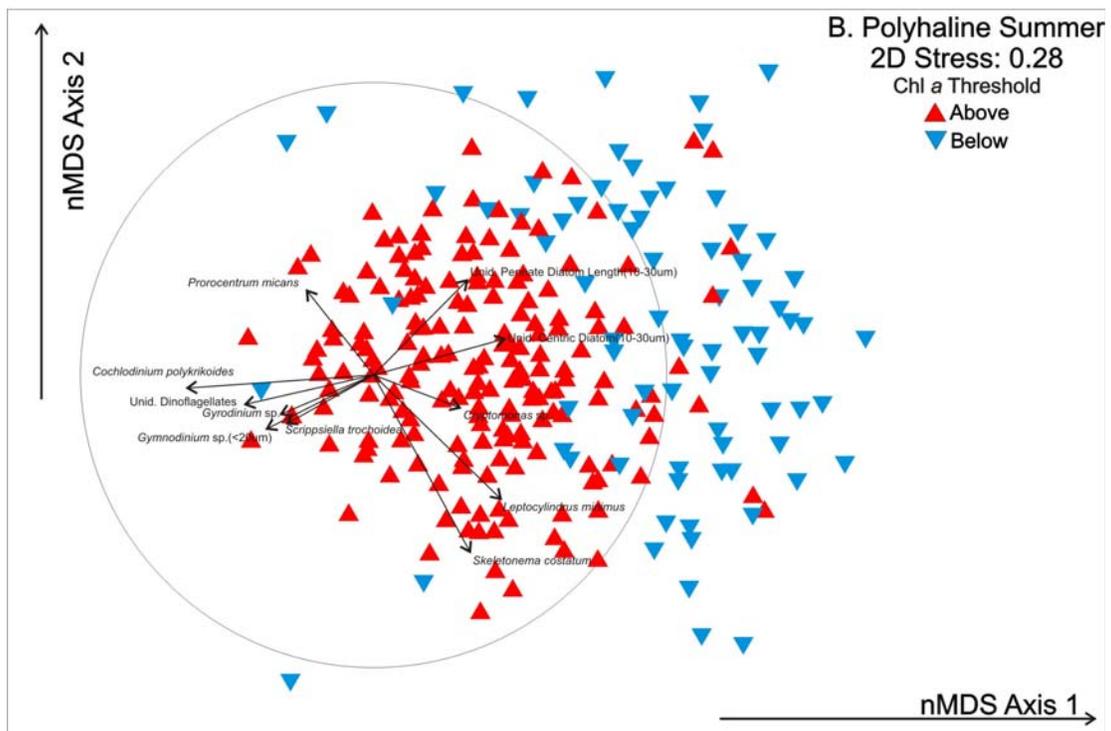
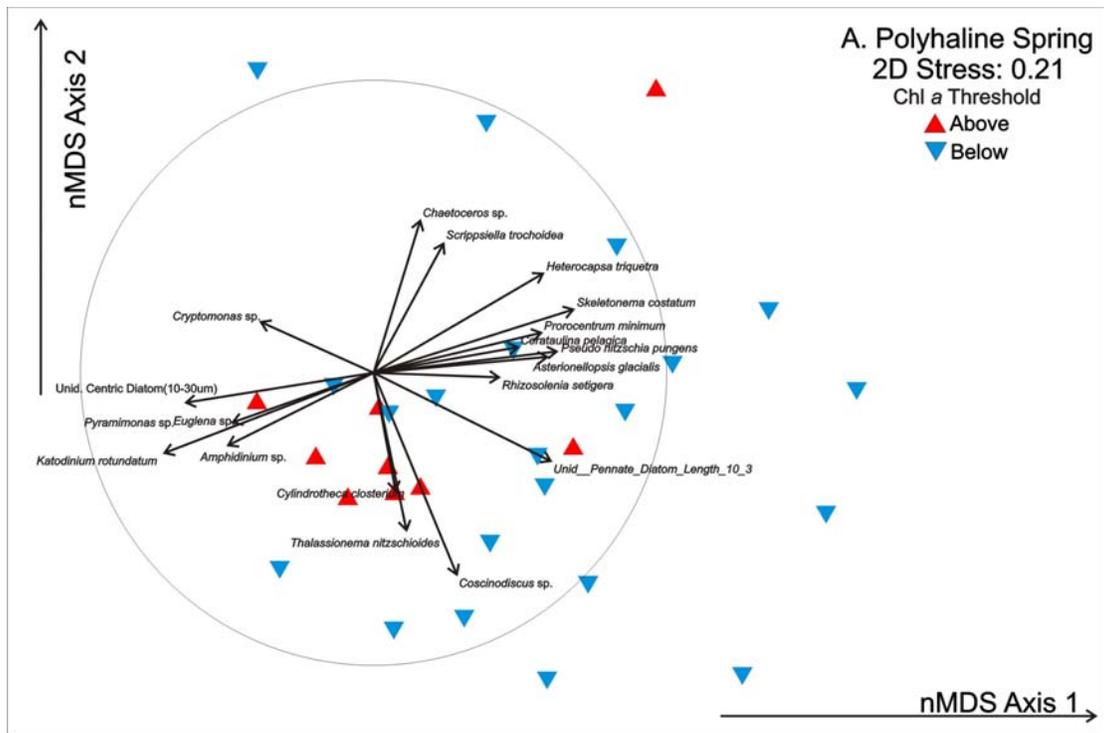


Figure 15. Two-dimensional nMDS ordination of phytoplankton taxonomic composition comparing samples collected when chlorophyll a was above or below established water quality thresholds for Tidal Freshwater areas of the James River during A. Spring and B. Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

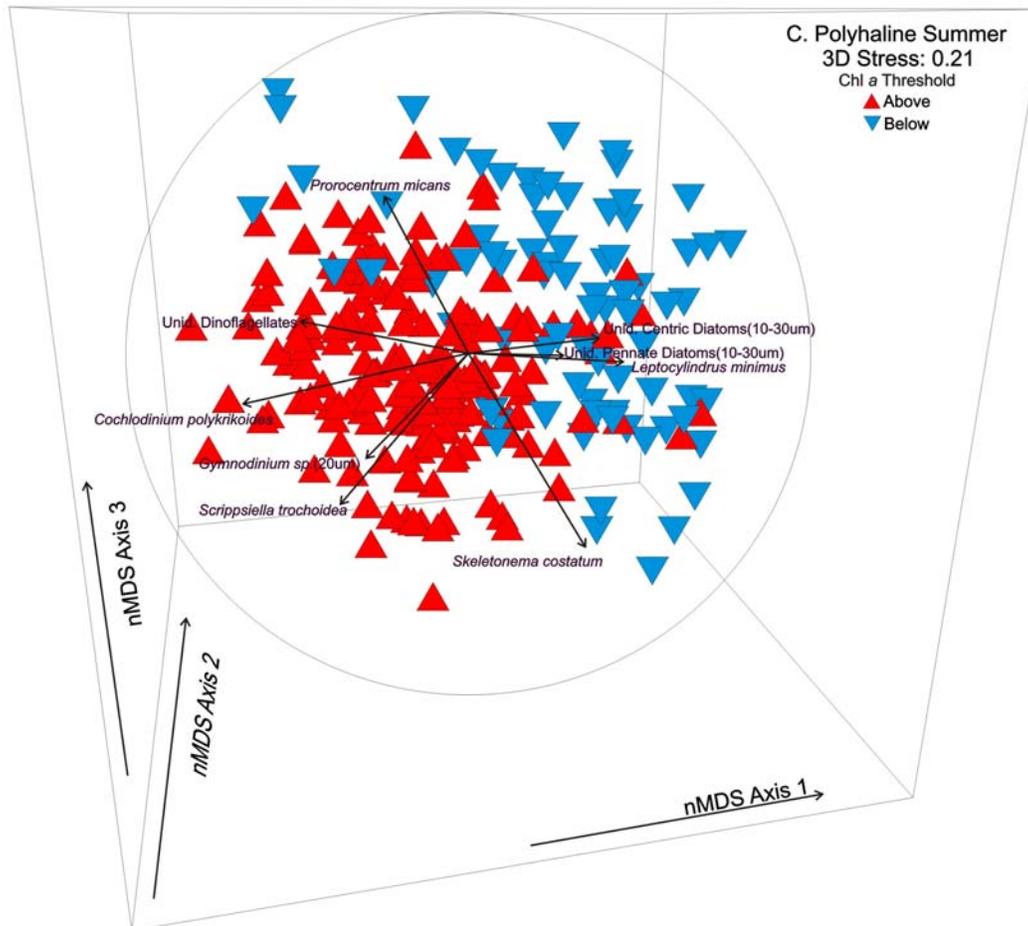


Figure 16. Two-dimensional nMDS ordination of phytoplankton taxonomic composition comparing samples collected when chlorophyll a was above or below established water quality thresholds for Polyhaline areas of the James River during Summer of 2011, 2012, and 2013. Arrows indicate direction and magnitude of correlations between the log transformed and standardized versions of the variables listed and the nMDS axes.

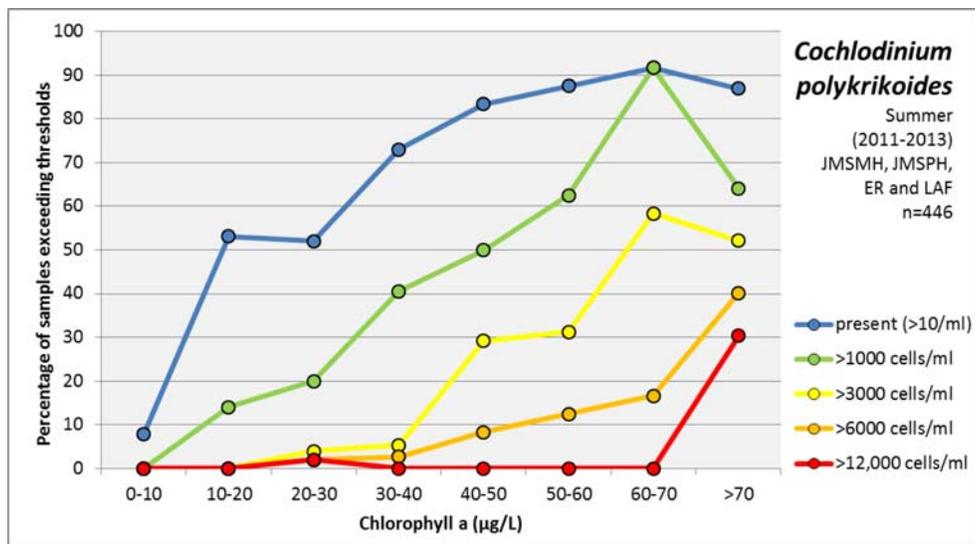


Figure 17. Plot of percentage of Summer samples from Lower James River (inclusive of Elizabeth and Lafayette Rivers) with *Cochlostinium polykrikoides* densities exceeding multiple thresholds within each chlorophyll *a* bin.

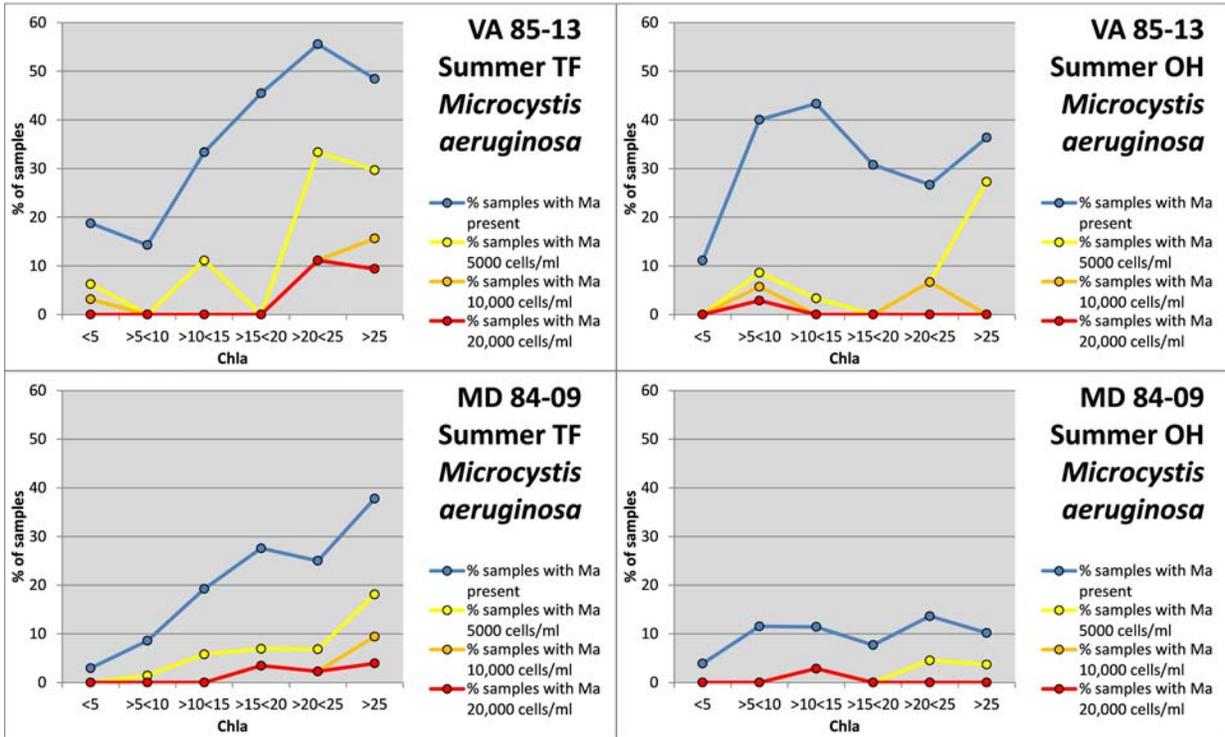


Figure 18. Plot of percentage of Summer samples from Virginia and Maryland TF and OH (CBP long term monitoring data) with *Microcystis aeruginosa* densities exceeding multiple thresholds within each chlorophyll *a* bin.

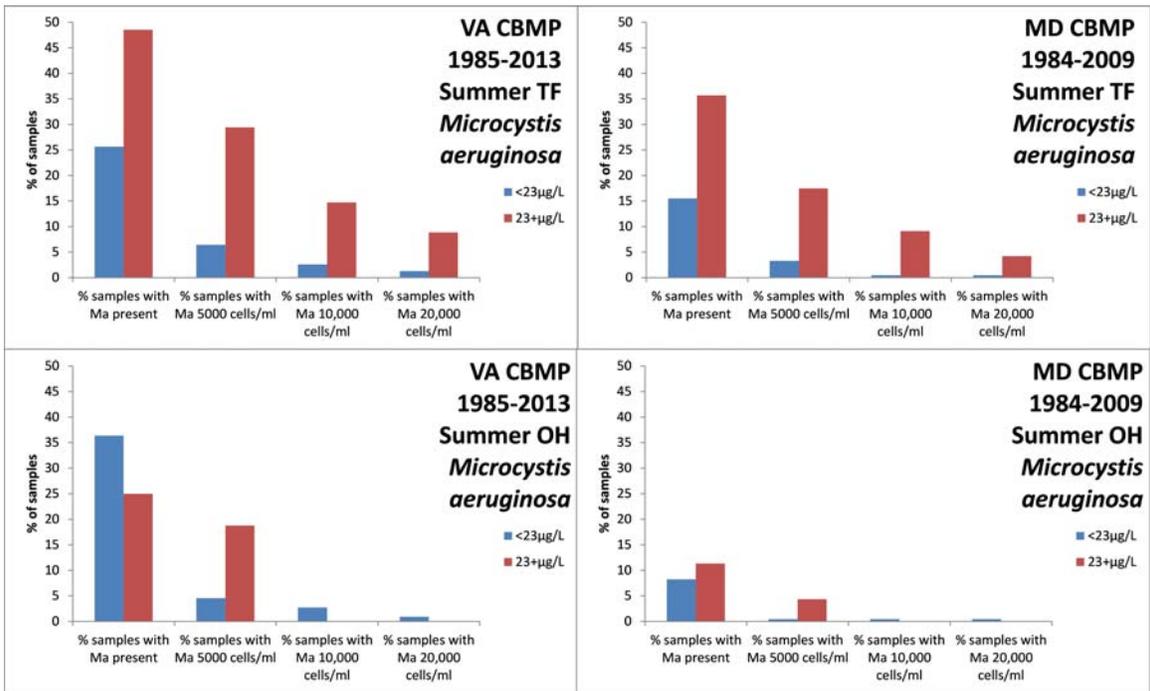


Figure 19. Percentage of samples with Chl *a* below or above 23µg/L that contain *Microcystis aeruginosa* at different densities (>0, ≥5000, ≥10,000, ≥20,000 cells/ml).

Appendix A Chl *a* criteria assessment results in comparison to bloom events in lower James River.

Todd Egerton, Old Dominion University

Following the data analysis meeting on Jan 20, 2014 with DEQ and members of the SAP, there was an extended discussion regarding the chlorophyll *a* assessment results and an apparent disconnect with bloom conditions in the lower James River. At the request of DEQ, aspects of this discussion are included here.

The major concern that was voiced was the way in which the chlorophyll *a* criteria are currently assessed, specifically that they are seasonal and spatial means calculated over both long periods of time and large areas. This appears to be especially relevant to the James River Mesohaline segment which spans a length of over 35 km. Using the current approach, by calculating the likelihood of occurrence in both time and space over the entire segment/season, even though they can be large and widespread, blooms end up being quantified as being relatively rare. In the most recent summary report, the annual probabilities of a *Cochlodinium* bloom (>1000 cells/ml) were calculated as 1.7-4.4% of time/space, and this included years when massive blooms were known to have occurred. For example in 2011, the mean Chl *a* in both the polyhaline and mesohaline was <10µg/L (meeting the current criteria), however *Cochlodinium* was present within the system at densities >1000 cells/ml for 6 weeks. Based on the bioassay experiments conducted at VIMS, *Cochlodinium* can cause mortality to zooplankton and fish and shellfish larvae over a period of hours to days. The presence of these high density, extended duration blooms suggest that the current criteria assessment may not be protective of the aquatic life designated use. All of the analyses and recommendations for criteria protectiveness in the accompanying report are based on paired/instantaneous chlorophyll concentrations, not geographic seasonal means.

Alternative approaches that were suggested included smaller, more representative spatial segments. One suggestion was that the Mesohaline segment could possibly be split into upper mesohaline (5-10 ppt) and lower mesohaline (10-18 ppt) segments. This division would be based on the conditions (salinity) present at the time of sampling, and would not necessarily be geographically constant. This would result in the size of the segment changing with every tidal cycle, and likely lead to complications with implementation and regulation. Alternatively, the segment could be divided geographically into fixed size segments based on average salinity calculations or other criteria.

In terms of temporal variability, there were multiple suggestions as well. This could include simply a smaller window of time that would be averaged (ie. monthly instead of seasonal). Alternatively, as suggested, the use of temporal means could be dropped altogether and instantaneous values of chlorophyll *a* could be used to determine whether an individual area (such as an interpolation cell) meets or exceeds a threshold for each date available.

A large part of the problem with developing a chlorophyll *a* criteria, is that unlike dissolved oxygen or other criteria, that have a direct relationship between the variable measured/managed for and the impairment, elevated i.e. chlorophyll *a* itself is not necessarily an impairment. The most significant and obvious indicator appears to be the presence and abundance of harmful algal bloom species, an alternative criteria could explicitly include cell densities of HAB species. This could possibly be implemented as a dual criteria, that would include routine chlorophyll *a* measurements and cell counts of HAB species when elevated Chl *a* was detected. The use of *in situ* fluorescence measurements in the field could allow for real time decisions to be made by the sampling crew to collect samples for HAB enumeration. This is similar to the approach used by HRSD to target bloom sampling. If samples contained HAB abundances in excess of a designated threshold, than they would violate the criteria. Based on the current study, the most obvious HAB candidate would be *Cochlodinium polykrikoides*. If the bloom species is not associated with toxicity (based on bioassay

and literature reviews), it would be more difficult to decide impairment is indicated (i.e. Spring blooms of *Heterocapsa triquetra*). However, extremely high concentrations of any phytoplankton, including non-toxic species can negatively impact aquatic life by shading and/or reduced oxygen. These impairments might already be protected against by separate turbidity/TSS and DO criteria, and would be unnecessary to include in a Chl *a*/HAB criteria.

Appendix B Interpreting nMDS ordinations

An ordination can be defined as a two or three dimensional graphical display of sample points such that the distances between points in the graph are representative of the actual distances between samples in whatever variables and at whatever scales the samples were originally measured. In this study the distances measured are Euclidean or Bray-Curtis dissimilarities calculated from multiple suites of either community indices, phylogenetic groups or individual taxa as described in the methods. In the ordinations provided by the Primer-E software, the representation of the original dissimilarities between samples are expressed as rank order distances between samples so that distances are expressed on relative scale (e.g. sample A is larger than sample B etc.) rather than an absolute scale (Clarke and Warwick, 2001). This is the reason why the ordination plots provided by Primer-E software have no x or y axis scales (e.g. Figure B1).

For the purposes of this study, predetermined groups had already been identified with sample groups being assigned on the basis of instantaneous sample chlorophyll *a* concentrations (i.e. above or below established water quality thresholds). As a result, for this study, nMDS analysis primarily served as a tool to confirm patterns identified by the multivariate statistical comparisons and to identify variables that might explain differences between the predetermined groups. Figure B1 presents an example of the ordination graphs produced from one of the nMD analyses performed for this study. In this case, the example represents a comparison of phytoplankton phylogenetic groups between samples collected when chlorophyll *a* was above or below established water quality thresholds for Mesohaline areas of the James River during Summer of 2011, 2012, and 2013.

Results of the multivariate analyses for this group indicated significant differences in group centroids (i.e. multivariate means) using a PERMANOVA test as well as a significant difference in group dispersion (variability) using the PERMDISP test (see Table 13) . An initial evaluation of the nMDS ordination would seem to confirm these results.

The ordination shown in Figure B1 has a stress value of 0.2 suggesting that the plot is useful for drawing some general conclusions about the data but not necessarily for describing detailed relationships between individual samples. Note that as a general rule, two dimensional ordinations with stress values less than or equal 0.1 can be interpreted in detail, while those with stress values between 0.1 and 0.2 can be interpreted with some degree of skepticism regarding the details to (e.g. relationships between individual samples) (Clarke and Warwick, 2001). Stress values above 0.3 indicate random placement of the samples in the ordination (Clarke and Warwick, 2001). Typically, for two dimensional MDS ordinations with stress values higher than 0.2 three dimensional MDS ordinations are attempted to further reduce and improve visualizations.

The groups appear to be reasonably well separated from one another primarily along the first nMDS axis (Figure B1). Note if you observe from left to right along the horizontal axis of Figure B1, the blue (below threshold) and red (above threshold) triangles form two groups in roughly the left and right halves of the plot respectively although there is some overlap between groups. Note that the red triangles are enclosed by a red ellipse (Note: added manually not software generated) (Figure B1). Additionally it should be evident that the spread of the samples of the two different groups is different with the below threshold group having a somewhat wider dispersion (variability) than the above threshold group. There is some additional separation along the second nMDS axis as well.

The arrows on the plot indicate direction and magnitude of correlations between the log transformed and standardized versions of the original variables labeled and the nMDS axes. The circle with the dotted line is simply an indicator against which the length of the arrows representing the correlations can be estimated

since it represents a maximum correlation value of 1. The longer the arrow, the higher the magnitude of the correlation while the direction indicates whether the variable is positively or negatively correlated with a given nMDS axis. For example, *Cochlidinium* abundance, indicated by the red arrow, is highly correlated with the horizontal nMDS axis (Spearman's rank correlation of 0.66) in a positive direction toward samples with chlorophyll a concentrations above the threshold suggesting that this dinoflagellate might be responsible in part for the high chlorophyll a concentrations observed. A similar statement could be made for Other dinoflagellates. Conversely, cryptophyte abundance, indicated by the blue arrow, was highly negatively correlated with the second nMDS axis (Spearman's rank correlation=-0.81). The general direction of the arrow is away from samples above and towards samples below the chlorophyll a threshold. Note that the dotted black circle indicates the maximum possible value for the correlation coefficient and is used to visually assess the importance of individual variables.

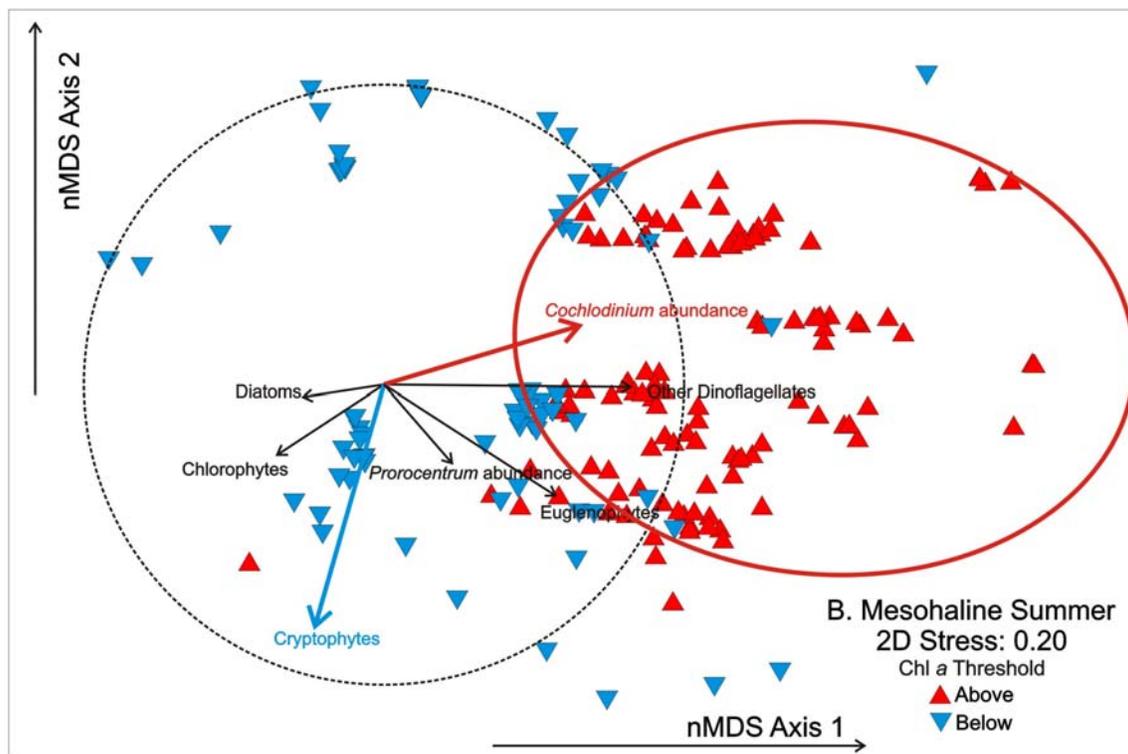


Figure B1 Example nMDS ordination plot. Arrows indicate magnitude and direction of Spearman rank correlations to nMDS axes. The circle with the dotted line indicates the maximum possible value for the correlation value i.e. 1. The red ellipse demarcates the "Above" Threshold samples from "Below" Threshold samples and was manually added to the graph.