

**Assessing seasonal relationships between chlorophyll a concentrations to phytoplankton composition, biomass, and abundance, emphasizing the bloom producing algae (HAB and others) within the James, Elizabeth, and Lafayette rivers in Virginia**

Primary Data Report  
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By

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**Introduction:**

Phytoplankton composition in Chesapeake Bay and its major tributaries has been reviewed by Marshall et al. (2005, 2009) who identified over 1,400 taxa, including 34 potentially harmful species, many of which have been classified as Harmful Algal Bloom (HAB) producers. Further reports regarding the status of HABs in the Bay and Virginia tributaries come from Marshall et al. (2008) and Marshall and Egerton (2009a, 2009b). On a broader scale of reference, there is a growing consensus that harmful algal blooms (HABs) are increasing in frequency and magnitude worldwide in response to increased eutrophication through greater nutrient loading (Anderson et al. 2002, Heisler et al. 2008). Within Virginia tidal waters, there have been an increased number of HABs in recent years including expanded development of toxin producing species (Marshall and Egerton 2009a). A common response world-wide involves increased efforts to develop nutrient criteria to reduce excess nitrogen and phosphorus from entering the water and causing blooms. To develop these criteria, it is necessary to understand how the phytoplankton community seasonally responds to varying (reduced/additional) nutrient levels. One of the most widely used methods for monitoring phytoplankton abundance is through chlorophyll *a* measurements. As different algal species and functional groups have differing physiologies and life histories (e.g. toxicity and bloom development) an important

component of this research identifies the relationship between chlorophyll and phytoplankton abundance, biomass, and composition.

Recent appraisals of HABs presence in Virginia rivers and the lower Chesapeake Bay have been discussed by Marshall et al. (2008), and Marshall and Egerton (2009a, 2009b, 2012). Several of these are dinoflagellates and residents of the James River, and other Virginia rivers. The dominant HAB species in the lower James River and its tributaries has been *Cochlodinium polykrikoides*, with a life cycle that includes cyst development. In recent decades it has expanded its geographic range and the duration of its blooms, with a seasonal presence that has been highly predictable, and the potential of degrading water quality (hypoxia) and impacting the life forms in these waters (Mullholland et al. 2009). Another HAB in these waters is *Prorocentrum minimum*. Even though it is a common bloomer within the tidal James River and lower Chesapeake Bay, and has the potential of producing hypoxic conditions, it has not been commonly associated locally with major fish kill events. The ichthyotoxic *Karlodinium veneficum*, is known for spring bloom development in the Potomac River and its associated inlets and streams (Li et al. 2000, Goshorn et al. 2004). It has also been noted more frequently in our monitoring of the James and York rivers in modest concentrations, but showing a potential for a broader range of bloom development in future years. What may be considered a recently (since 2007) established invasive species to these rivers and the Bay is the ichthyotoxic *Alexandrium monilatum*. It has produced modest blooms at the Chesapeake Bay entrance, plus being noted in the lower James and York rivers. It is also a cyst producer which would enhance its future distribution and establishment in this region. An HAB of concern in the tidal fresh water regions of the James River is the cyanobacterium *Microcystis aeruginosa*, which is discussed later for this region in reference to microcystin levels and chlorophyll standards.

Water quality parameters, chlorophyll concentrations and phytoplankton populations are routinely monitored in Virginia tidal waters by the Virginia Department of Environmental Quality and its Chesapeake Bay Monitoring Program partners including Old Dominion University (Marshall & Alden 1990, Marshall et al. 2003, Dauer et al. 2009). To better capture the spatial and temporal variability present in these dynamic ecosystems, a higher frequency monitoring strategy was employed in the tidal James River beginning in 2011 (Egerton et al. 2012). This report documents methods utilized by the Old Dominion University Phytoplankton

Analysis Laboratory (ODUPAL) in the James River chlorophyll a criteria study, and presents the results of the 2012 monitoring season. These data are also compared to James River monitoring results from 2011, as well as historical Chesapeake Bay Monitoring Program data.

The objectives of this study were:

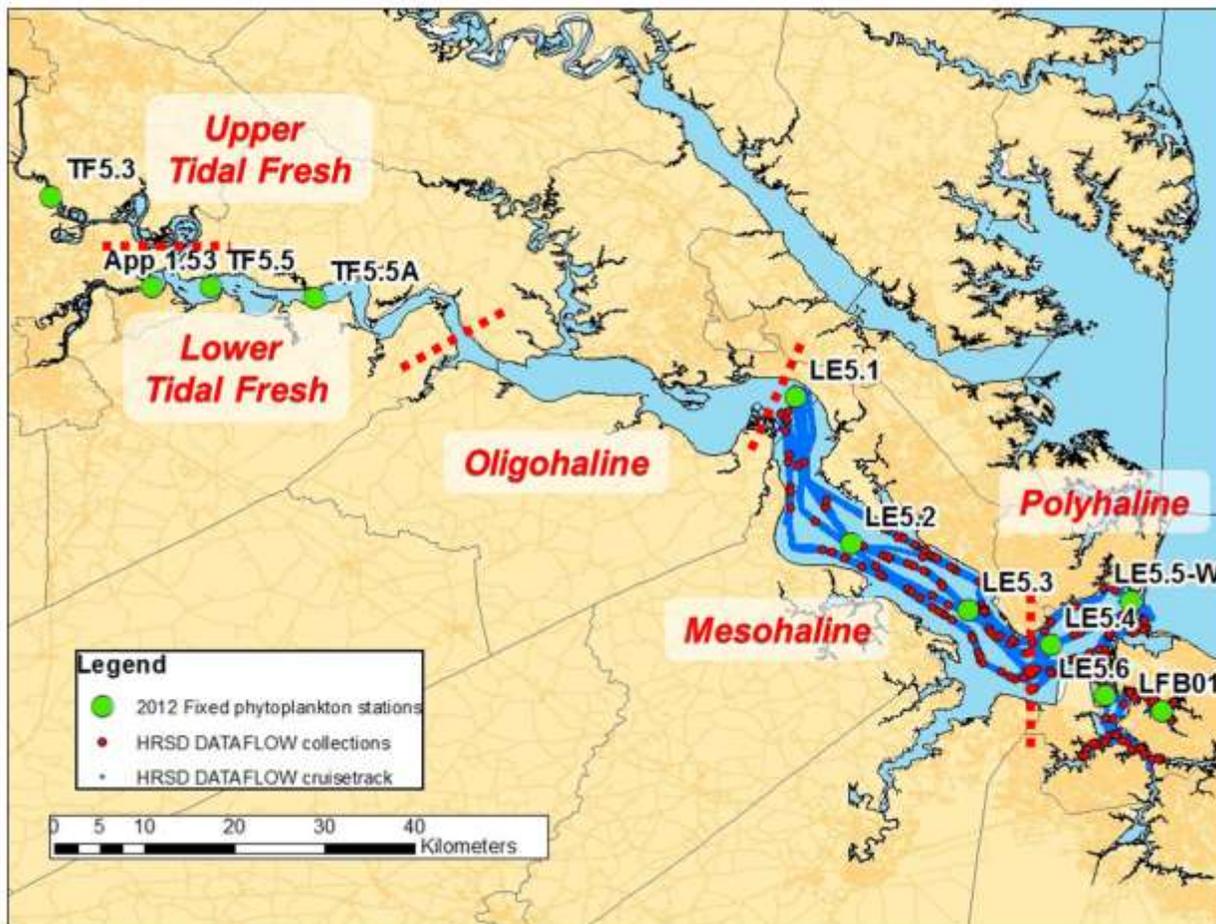
1. Identify weekly phytoplankton composition, abundance, and biomass in the James, Elizabeth and Lafayette rivers, including bloom producing species (HAB taxa and non-HAB bloom producers).
2. Relate phytoplankton composition, concentrations, and biomass to corresponding chlorophyll levels during the study period.
3. Follow the initiation, composition, location, progression, frequency, magnitude, and duration of blooms in these rivers.
4. Relate the significance of HAB producing species regarding their contribution to algal biomass and chlorophyll levels.
5. Additionally, after the study was conducted, the investigators were asked to make preliminary comparisons of 2012 bloom development to blooms occurring in 2011, and prior years including the period of 1991-2000, and 2007-2010 at these river sites.

**Methods:**

Surface water samples were collected in the upper James River (Upper and Lower Tidal Fresh segments) by personnel from Virginia Commonwealth University, under the supervision of Dr. Paul Bukaveckas, and forwarded to the ODUPAL for analysis. These were tidal freshwater sites, although subject to periods of low saline intrusion. Samples were collected from May through October at 4 fixed DEQ stations TF5.3, TF5.5, TF5.5A, and App1.53. (Fig.1, Table 1). Chlorophyll measurements were taken by VCU and this data forwarded to the ODUPAL for correlations with the phytoplankton data. Preserved surface water samples from the Upper James (Bailey's Bay) were also provided monthly by the Hopewell Regional Waste Water Treatment Facility for analysis. In addition to the above collections, water samples provided to the Principle Investigator as part of his participation in the Chesapeake Bay Phytoplankton Monitoring Program in the James River were also included and provided additional historical data for this study.

Water samples from the lower James River were collected by personnel from the Hampton Roads Sanitation District (HRSD) under the supervision of Mr. Will Hunley. These were surface (<1m) samples (125ml) taken weekly from February through October, in the mesohaline and polyhaline James River, as well as the Elizabeth and Lafayette Rivers (Fig. 1, Table 1). This included weekly collections from 7 fixed DEQ stations (LE5.1, LE5.2, LE5.3, LE5.4, LE5.5, LE5.6, and LFB01) and additional collections based on in-situ DATAFLOW chlorophyll readings. The threshold value for bloom recognition and sample collection were chlorophyll readings  $>15 \mu\text{gL}^{-1}$  (Egerton et al. 2012). The samples were preserved immediately with Lugol's solution and delivered to the ODU Phytoplankton Analysis Laboratory (ODUPAL) for analysis (Marshall et al. 2005). Chlorophyll concentrations determined by HRSD were provided to the ODUPAL to determine chlorophyll relationships to phytoplankton composition, biomass, and abundance levels. Supplementary samples also came from members of the ODUPAL and citizen groups that provided additional information regarding extent of bloom developments beyond the standard station locations. When requested, non-preserved water samples were also provided for analysis.

Phytoplankton composition and concentration determinations followed standard protocols using light microscopy as followed in the Chesapeake Bay Phytoplankton Monitoring Program (Marshall et al. 2005). The ODUPAL contains extensive identification references, with a staff represented by H. Marshall and T. Egerton, plus 4 graduated research assistants. A scanning electron microscope facility, PCR laboratory, and cell culture room are located nearby and available for use. The methods and QA/QC standards followed are indicated in the 2012 "Work Quality Assurance Project Plan for Monitoring Phytoplankton and Picoplankton in the Lower Chesapeake Bay and Tributaries" (Marshall 2012). Biomass estimates were based on species specific biovolumes ( $\mu\text{m}^3$ ) and converted to carbon ( $\mu\text{gC/L}$ ) according to Smayda (1978). Pearson correlation analysis was used to compare chlorophyll ( $\mu\text{g/L}$ ) to algal biomass of total phytoplankton concentrations, major group totals, and major species.



**Figure 1:** 2012 James River phytoplankton collections. Fixed sites shown in green. HRSD DATAFLOW tract displayed in green with DATAFLOW bloom collections in red

**Table 1:** Fixed station coordinates.

River Segment	Station ID	Latitude	Longitude
James River Upper Tidal Fresh	TF5.3	37.4031	-77.3927
James River Lower Tidal Fresh	App1.53	37.3124	-77.2913
James River Lower Tidal Fresh	TF5.5	37.3127	-77.2328
James River Lower Tidal Fresh	TF5.5A	37.3017	-77.1284
James River Mesohaline	LE5.1	37.2030	-76.6483
James River Mesohaline	LE5.2	37.0560	-76.5931
James River Mesohaline	LE5.3	36.9904	-76.4754
James River Polyhaline	LE5.4	36.9549	-76.3928
James River Polyhaline	LE5.5W	36.9990	-76.3133
Elizabeth River	LE5.6	36.9046	-76.3384
Lafayette River	LFB01	36.8894	-76.2814

**Results:****Phytoplankton composition, including HABs, and non-harmful bloom producers within the James River, plus the Elizabeth and Lafayette rivers.**1: Overview: Upper James River:

In contrast to the Lower James, the floral composition upstream was composed predominantly of freshwater chlorophytes, diatoms (both pelagic and benthic), and cyanobacteria, with background taxa typically composed of cryptomonads, and to a much lesser degree dinoflagellates. Common dominant diatoms included *Aulacoseira granulata*, *Aulacoseira granulata v. angustissima*, *Aulacoseira distans*, *Cyclotella meneghiniana*, and *Skeletonema potamos*. Common chlorophytes were *Ankistrodesmus falcatus*, *Chlorella* spp., *Pediastrum duplex*, and representatives from the *Desmodesmus/Scenedesmus* complex. Present among the cyanobacteria were *Aphanocapsa incerta*, *Microcystis aeruginosa*, *Merismopedia tenuissima*, and *Synechococcus* spp., among others. The collections provided by the Hopewell Regional Water Treatment Facility mainly expanded the cyanobacteria representation which included a variety of filamentous taxa (e.g. *Limnothrix redekei*, *Pseudanabaena limnetica*, *Aphanizomenon* sp., *Anabaena viguier*, and *Jaaginema neglecta*). These are all common bloom producers.

2: HABs present in the Upper James:

The only HAB species present in the VCU station samples was *Microcystis aeruginosa*. From the Bailey's Bay collections, the filamentous cyanobacterium *Limnothrix redekei*, a potential toxin bloom producer, was present, but neither of these taxa produced major blooms during the collection period.

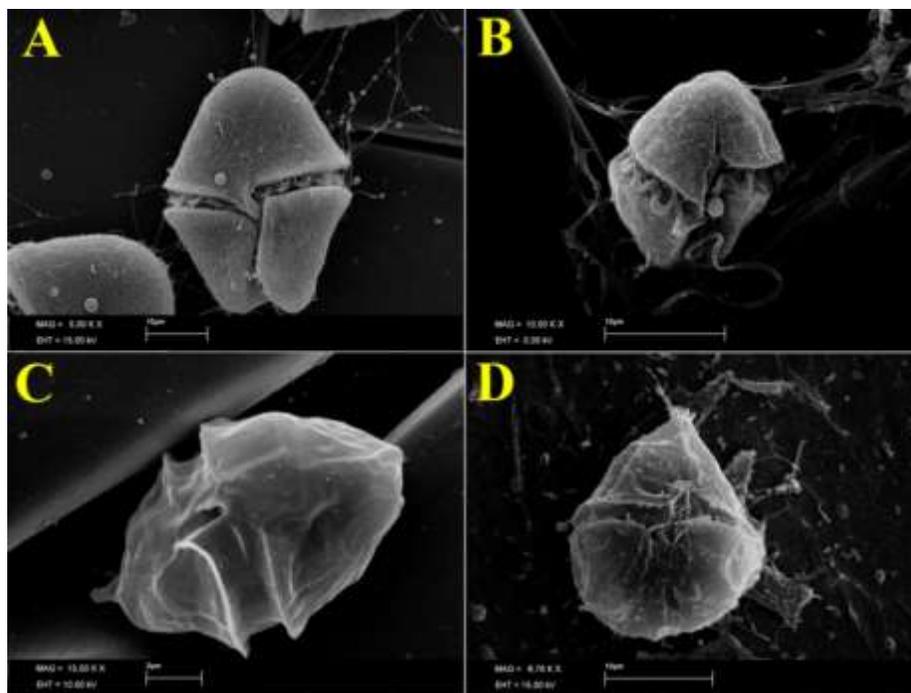
3: Overview: lower James River, and the Elizabeth and Lafayette rivers:

Algal composition in these rivers was dominated by a diverse assemblage of benthic and pelagic diatoms. Commonly co-occurring with these taxa is a variety of dinoflagellates and cryptomonads, with other taxonomic groups present mainly as background species. These would include chlorophytes, cyanobacteria, raphidophytes, and others. Increased diatom concentrations characterize the late winter/early spring months, with the dominant species including

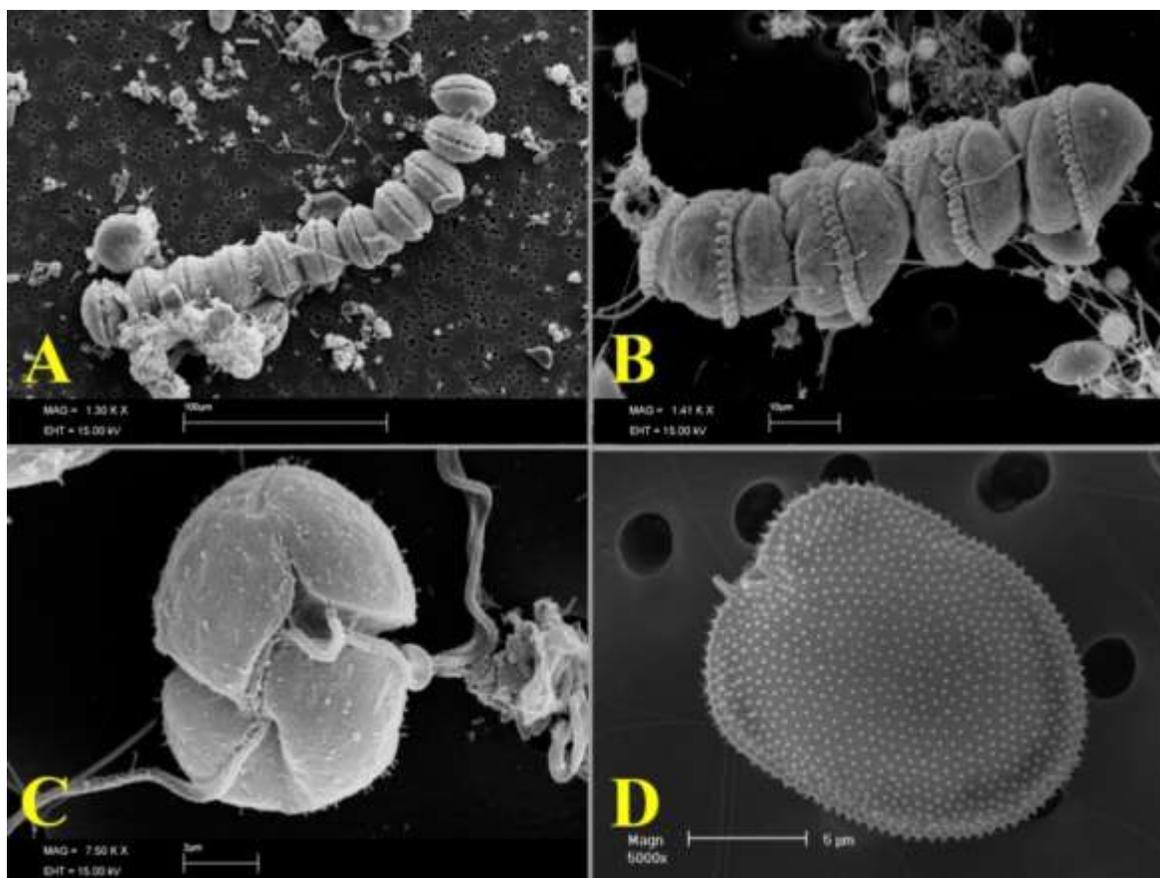
*Skeletonema costatum*, *Cerataulina pelagica*, *Leptocylindrus minimus*, plus several benthic taxa. This period is followed by a sequence of bloom producing dinoflagellates, with peak representation and abundance typically occurring in summer and early autumn. Typical non-HAB species of dinoflagellate bloomers during this period include *Akashiwo sanguinea*, *Gymnodinium* spp., *Heterocapsa triquetra*, and *Scrippsiella trochoidea* (Fig. 2).

#### 4: HAB species from the Lower James River, and Elizabeth and Lafayette rivers:

It is recognized that any algal species producing a major, extensive, or long duration bloom may produce degrading water quality conditions (e.g. hypoxia) that may be harmful to biota present. Reference to HAB here emphasizes those taxa typically characterized as toxin producers, and /or have been associated with hypoxia related faunal deaths or illness. HABs identified in these rivers during this time period include the dinoflagellates *Alexandrium monilatum*, *Cochlodinium polykrikoides*, *Karlodinium veneficum*, and *Prorocentrum minimum* (Fig. 3). Although not noted in this year's collections, other HABs have been recorded in this river complex previously and other Virginia rivers including the raphidophyte *Chattonella subsalsa* (Marshall and Egerton 2009a).



**Figure 2:** Representative non-harmful bloom forming dinoflagellates within the lower James River estuary. A: *Akashiwo sanguinea*, B: *Gymnodinium aureolum*, C: *Heterocapsa triquetra*, D: *Scrippsiella trochoidea*.

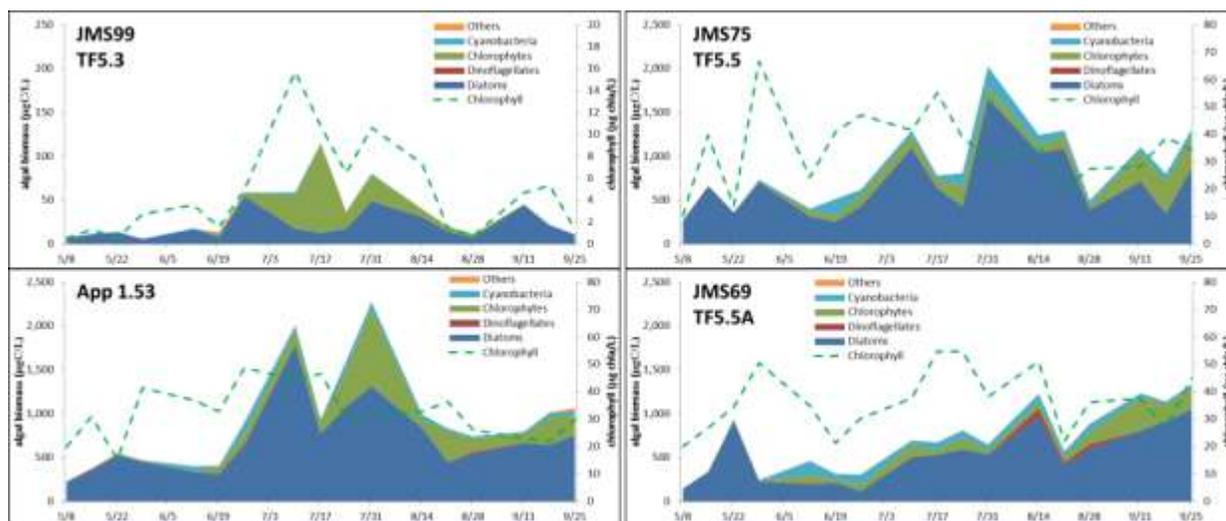


**Figure 3:** Harmful bloom forming dinoflagellates present within the lower James River estuary. A: *Alexandrium monilatum*, B: *Cochlodinium polykrikoides*, C: *Karlodinium veneficum*, and D: *Prorocentrum minimum*.

### Characterization of cell abundance and biomass in Upper James during 2012

In contrast to the lower section of the James River, the Upper James River stations had a general increase in a summer abundance pulse, but had no distinct mono-specific algal blooms during 2012. Instead the community was composed of a diverse group of taxa which in general did not have clear periods of bloom development. Algal biomass was lowest at the Upper tidal fresh water station (TF5.3) during spring when diatoms (e.g. *Aulacoseira granulata*) represented in excess of 90% the total biomass present (Figs. 4-5, Tables 2-3). This changed in summer, with increased biomass coming from the chlorophytes. There was an increase in total biomass between these seasons from 8 to 115  $\mu\text{gC/L}$  (Table 2). In contrast, downstream tidal freshwater stations (TF5.5, TF5.5A, and App1.53) had much greater biomass values (200 to  $>2000$   $\mu\text{gC/L}$ ).

Likewise, these stations were dominated by diatoms throughout the year, with increased chlorophyte biomass during summer. Across all collections in the upper James, diatoms made up an average of 76% of total algal biomass, with chlorophytes and cyanobacteria contributing 16% and 6% respectively (Figure 5, Table 3). Unlike the lower James River, species composition remained relatively constant across varying chlorophyll levels, with similar distribution of biomass between major groups at both lower and elevated chlorophyll concentrations (Figure 5).



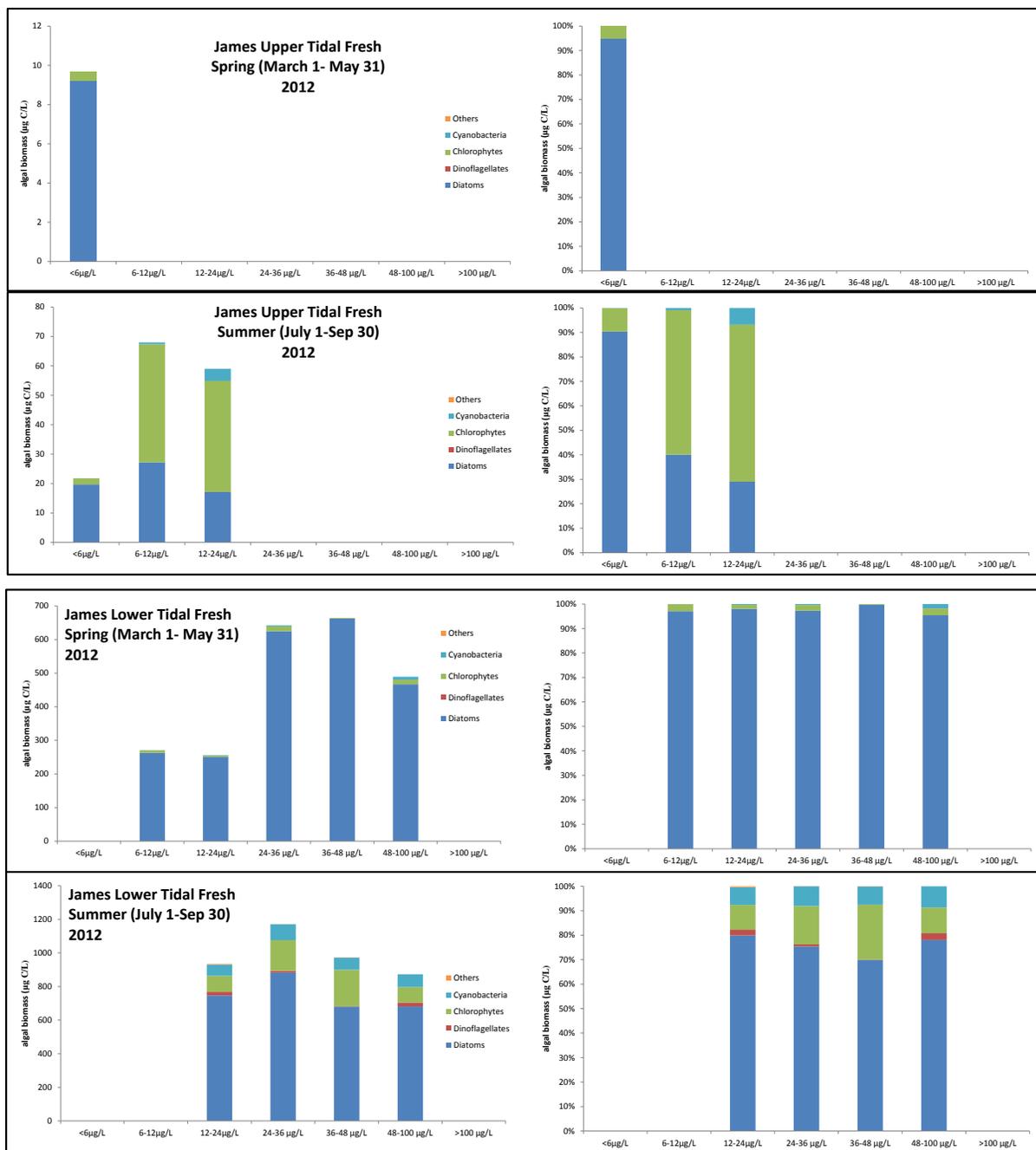
**Figure 4:** Weekly phytoplankton biomass and chlorophyll concentrations at four stations in the Upper James River. Note difference in Y axes between TF5.3 and other stations.

**Table 2:** Upper James River segments: average phytoplankton composition in terms of *biomass* ( $\mu\text{g C/L}$ ) of major taxonomic groups compared to the corresponding chlorophyll concentration. Number of samples for each segment/season shown in left column. Upper JMSTF= station TF5.3 (JMS99), Lower JMSTF= stations TF5.5 (JMS75) and TF5.5A (JMS69). Spring=March 1-May 31, Summer=July 1-September 30. Total number of samples examined for entire upper James River=89 .

river segment/season	Chlorophyll range ( $\mu\text{g/L}$ )	Diatoms ( $\mu\text{g C/L}$ )	Dinoflagellates ( $\mu\text{g C/L}$ )	Chlorophytes ( $\mu\text{g C/L}$ )	Cyanobacteria ( $\mu\text{g C/L}$ )	Others ( $\mu\text{g C/L}$ )
Upper JMSTF Spring n=4	<6	9.20	0	0.48	0	<0.01
	6-12					
	12-24					
	24-36					
	36-48					
	48-100					
	>100					
Upper JMSTF Summer n=10	<6	19.70	0	2.08	0.02	<0.01
	6-12	27.23	0	40.16	0.62	<0.01
	12-24	17.13	0	37.80	4.05	<0.01
	24-36					
	36-48					
	48-100					
	>100					
Lower JMSTF Spring n=8	<6					
	6-12	262.88	0	7.69	0.20	<0.01
	12-24	250.91	0	4.16	0.84	<0.01
	24-36	624.95	0	14.62	2.84	<0.01
	36-48	662.18	0	1.92	0	<0.01
	48-100	466.81	0	13.60	8.95	<0.01
	>100					
Lower JMSTF Summer n=20	<6					
	6-12					
	12-24	746.66	21.60	95.25	67.23	3.98
	24-36	882.88	10.26	183.65	94.27	0.29
	36-48	680.29	1.71	218.26	72.30	1.16
	48-100	681.27	23.94	91.68	76.00	<0.01
	>100					

**Table 3:** Upper James River segments: average phytoplankton composition in terms of *percent biomass* of major taxonomic groups compared to the corresponding chlorophyll concentration. Number of samples for each segment/season shown in left column. Upper JMSTF= station TF5.3 (JMS99), Lower JMSTF= stations TF5.5 (JMS75) and TF5.5A (JMS69). Spring=March 1-May 31, Summer=July 1-September 30. Total number of samples examined for entire upper James River=89 .

river segment/season	Chlorophyll range ( $\mu\text{g/L}$ )	Diatoms % biomass	Dinoflagellates % biomass	Chlorophytes % biomass	Cyanobacteria % biomass	Others % biomass
Upper JMSTF Spring n=4	<6	95.0	0	5.0	0	<0.1
	6-12					
	12-24					
	24-36					
	36-48					
	48-100					
	>100					
Upper JMSTF Summer n=10	<6	90.3	0	9.5	0.1	<0.1
	6-12	40.0	0	59.0	0.9	<0.1
	12-24	29.0	0	64.1	6.9	<0.1
	24-36					
	36-48					
	48-100					
	>100					
Lower JMSTF Spring n=8	<6					
	6-12	97.1	0	2.8	0.1	<0.1
	12-24	98.0	0	1.6	0.3	<0.1
	24-36	97.3	0	2.3	0.4	<0.1
	36-48	99.7	0	0.3	0.0	<0.1
	48-100	95.4	0	2.8	1.8	<0.1
	>100					
Lower JMSTF Summer n=20	<6					
	6-12					
	12-24	79.9	2.3	10.2	7.2	0.4
	24-36	75.4	0.9	15.7	8.0	0.0
	36-48	69.9	0.2	22.4	7.4	0.1
	48-100	78.0	2.7	10.5	8.7	0.0
	>100					



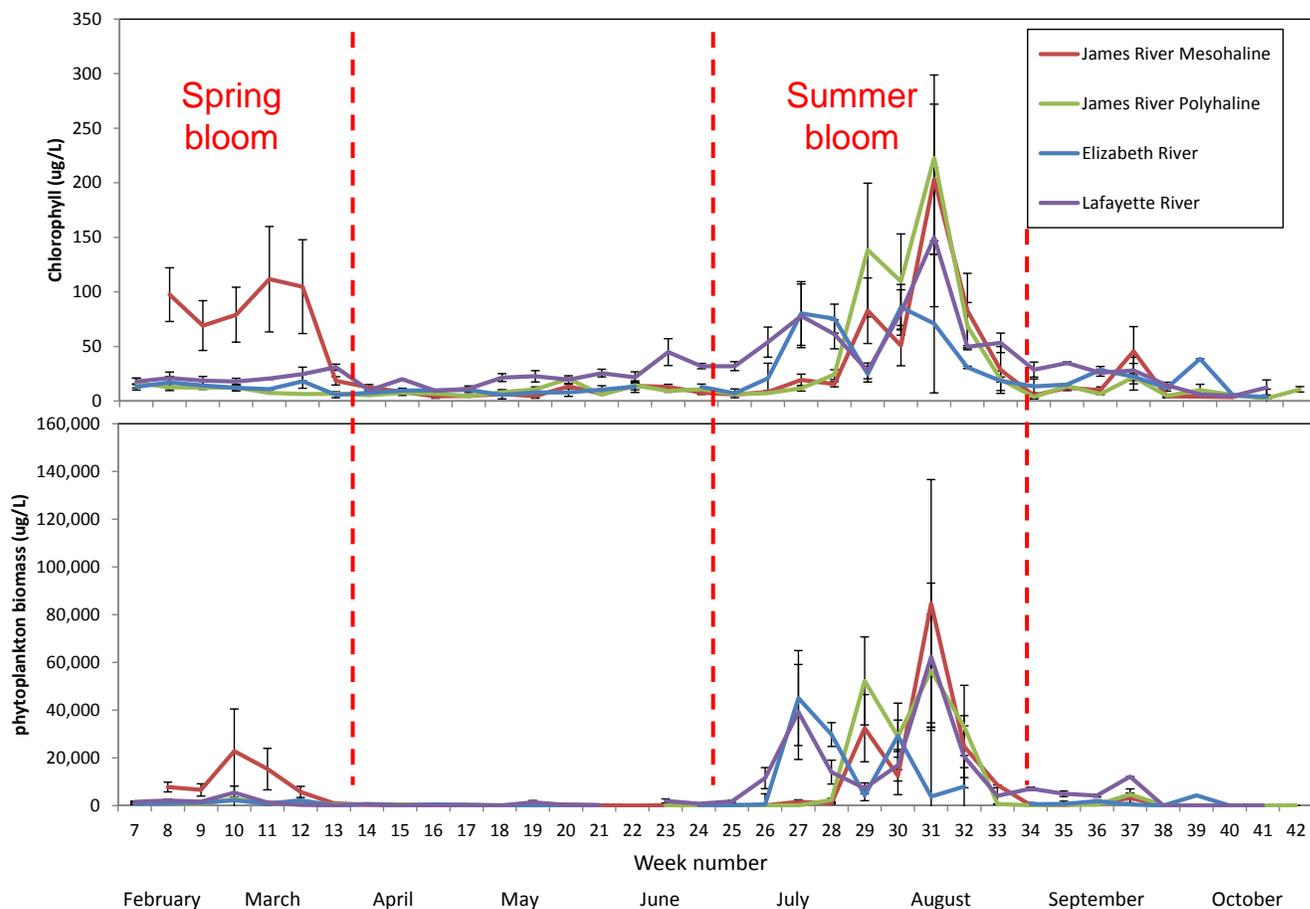
**Figure 5:** Algal biomass (left: concentrations, right: percent composition) of collections made in the upper (TF5.3) and lower (TF5.5, TF5.5A) tidal fresh James River in relation to chlorophyll concentration.

### Characterization of cell abundance and biomass during 2012 blooms in lower James River:

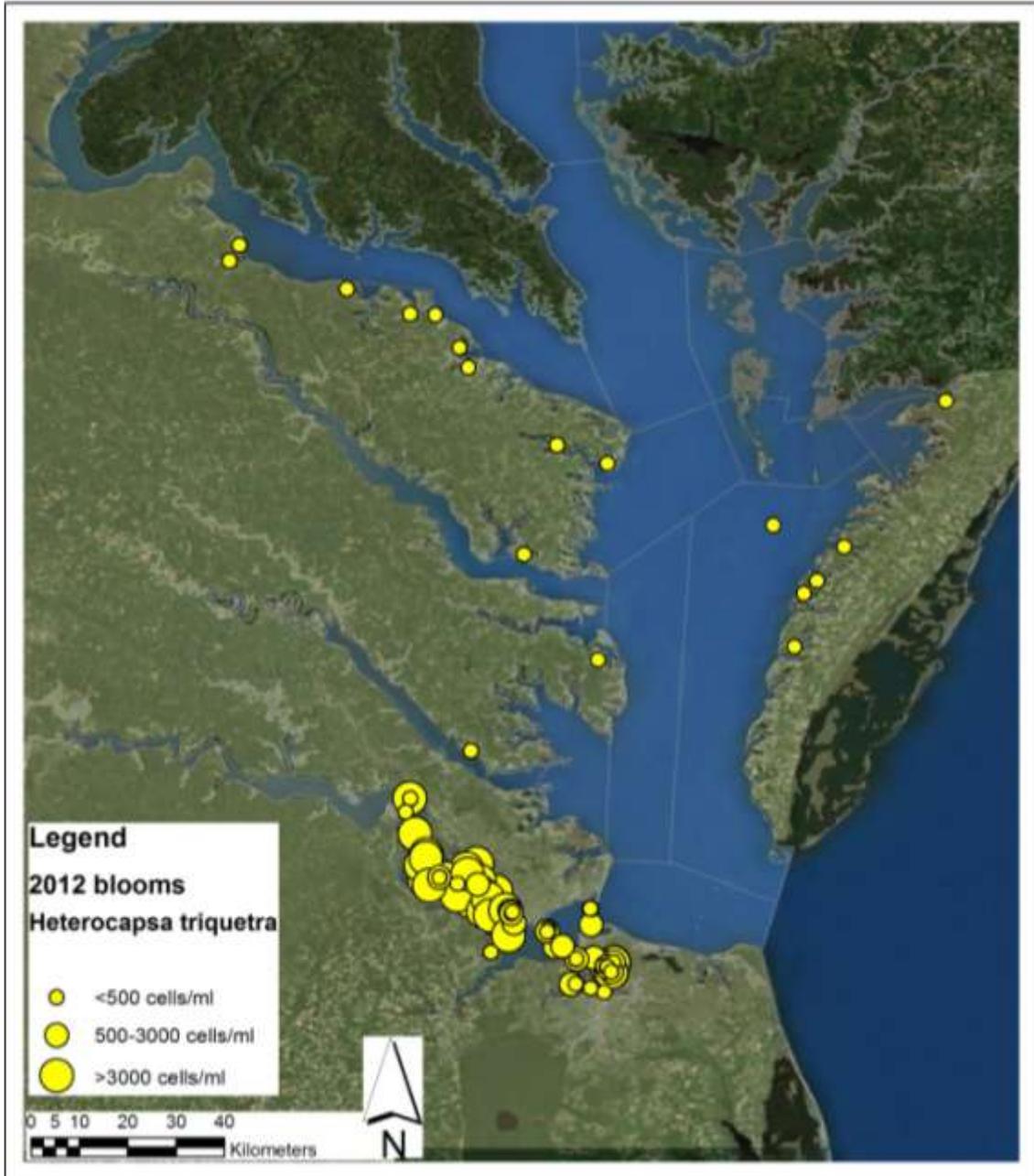
Two major seasonal dinoflagellate blooms occurred in the meso- and polyhaline regions of the James River during 2012 (Figure 6). The spring bloom, lasting 5 weeks during February and March, and was dominated by *Heterocapsa triquetra* (a non-HAB species). Maximum

bloom development was located within the mesohaline waters, with highest cell densities above 190,000 cells/ml, associated with chlorophyll concentrations extending above 400  $\mu\text{gC/L}$  and biomass estimates of  $1.2\text{-}7.2 \times 10^4$   $\mu\text{gC/L}$  (Table 4). The *H. triquetra* bloom was found throughout the lower James, with the highest concentrations in the JMSMH segment (Figure 7).

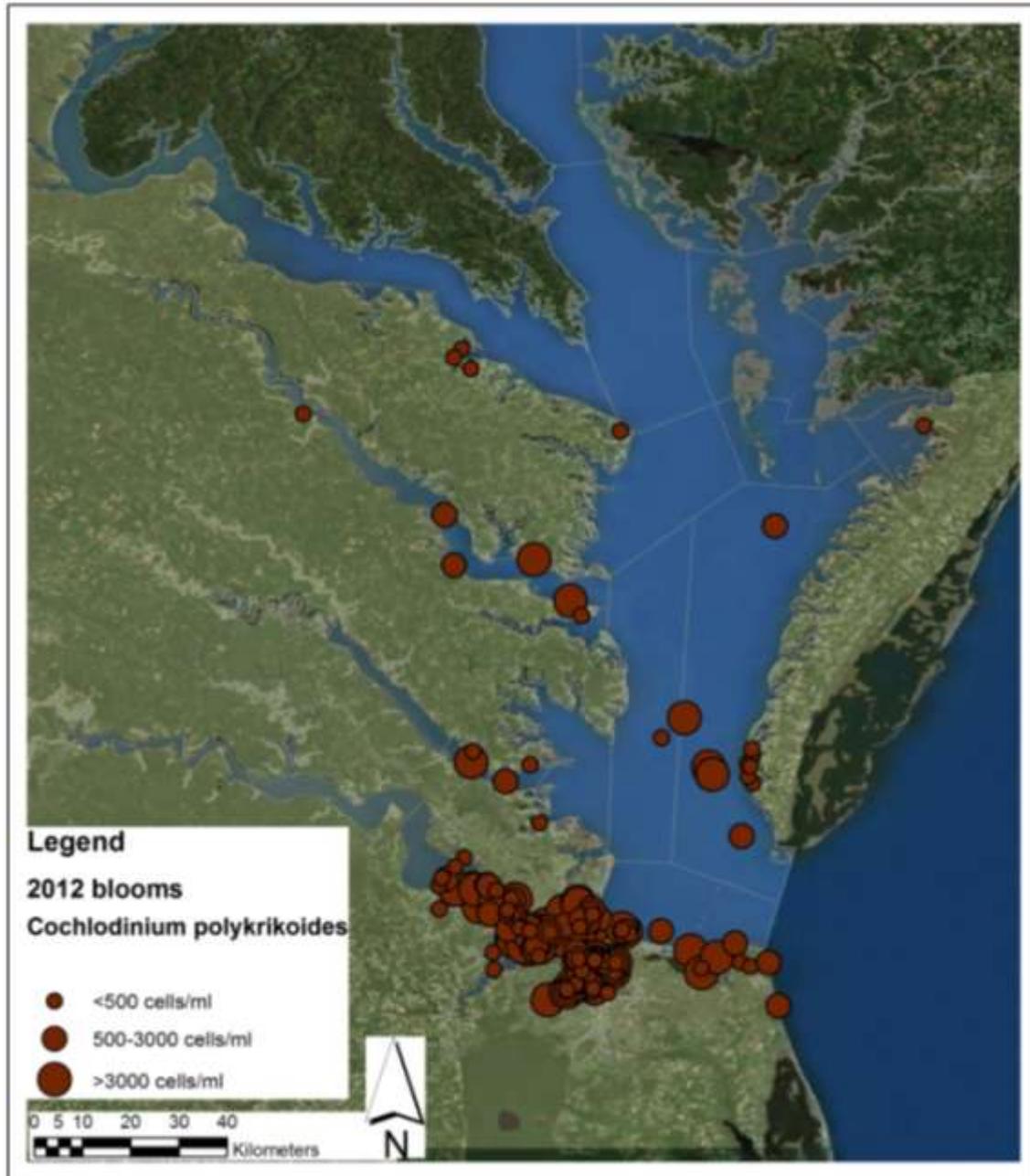
The summer bloom lasted approximately 13 weeks from late June to mid-September. During this time period, densities of several dinoflagellate species were elevated, with *Cochlodinium polykrikoides* being the dominant taxon. Maximum *C. polykrikoides* bloom concentrations reached 75,000 cells/ml, corresponding with chlorophyll concentrations above 400  $\mu\text{gC/L}$ , and biomass estimates of  $2.6 \times 10^5$   $\mu\text{gC/L}$ . Bloom concentrations were present throughout the estuary, that included the Lafayette and Elizabeth rivers, and the meso- and polyhaline regions of the James River. In addition, the *C. polykrikoides* bloom extended into much of lower Chesapeake Bay and its tributaries (Figure 8). Subdominant to *C. polykrikoides* were additional dinoflagellates including *Alexandrium monilatum* which was observed in the James River for 3 weeks in August and September with a maximum density of 1000 cells/ml (Figure 9).



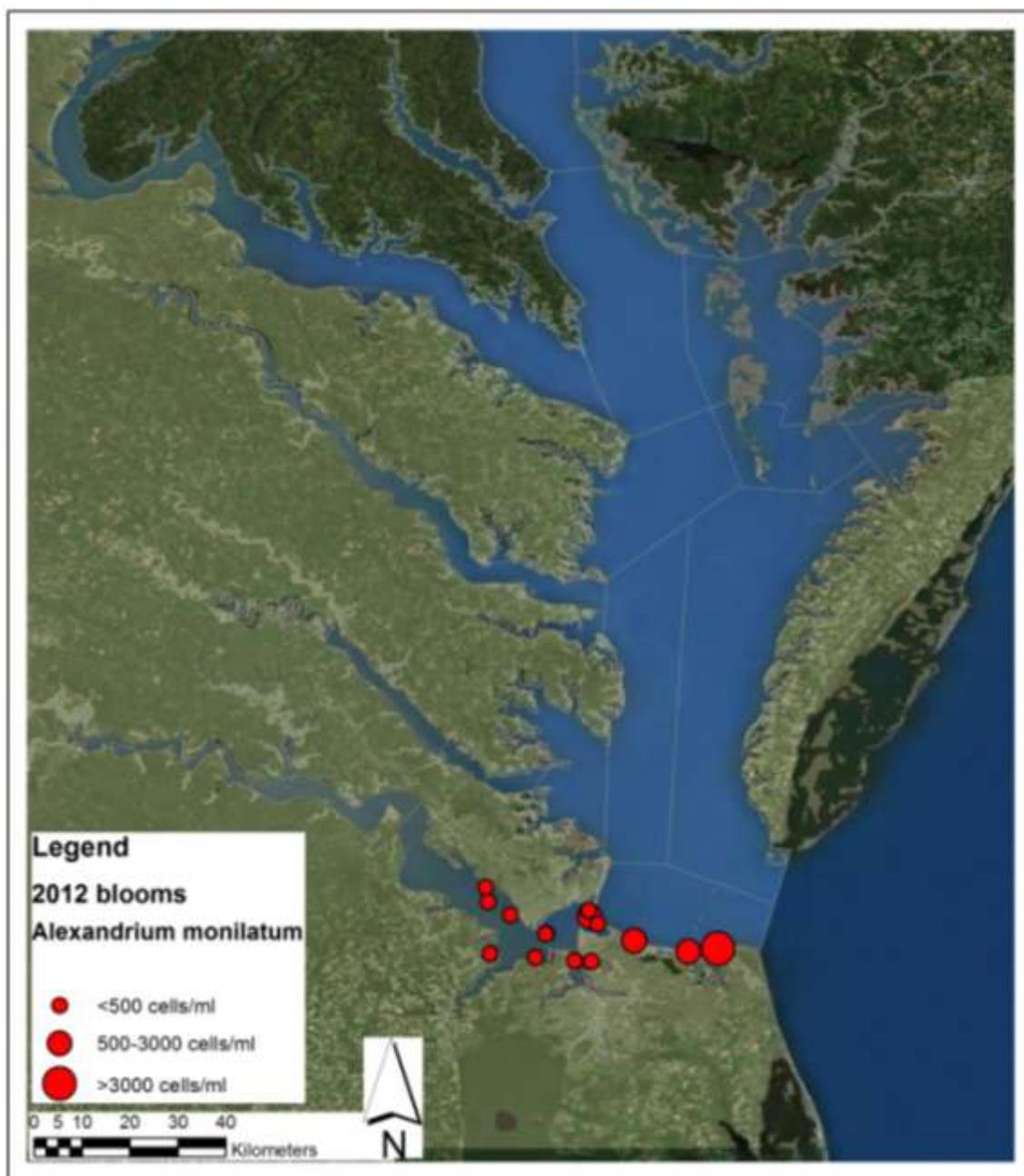
**Figure 6:** Weekly mean chlorophyll and algal biomass values from the meso- and polyhaline James, Elizabeth and Lafayette Rivers in 2012. Seasonal algal blooms occurred in Spring (Feb-March) and Summer (June-September)



**Figure 7:** 2012 distribution of *Heterocapsa triquetra* blooms in James River and lower Chesapeake Bay.

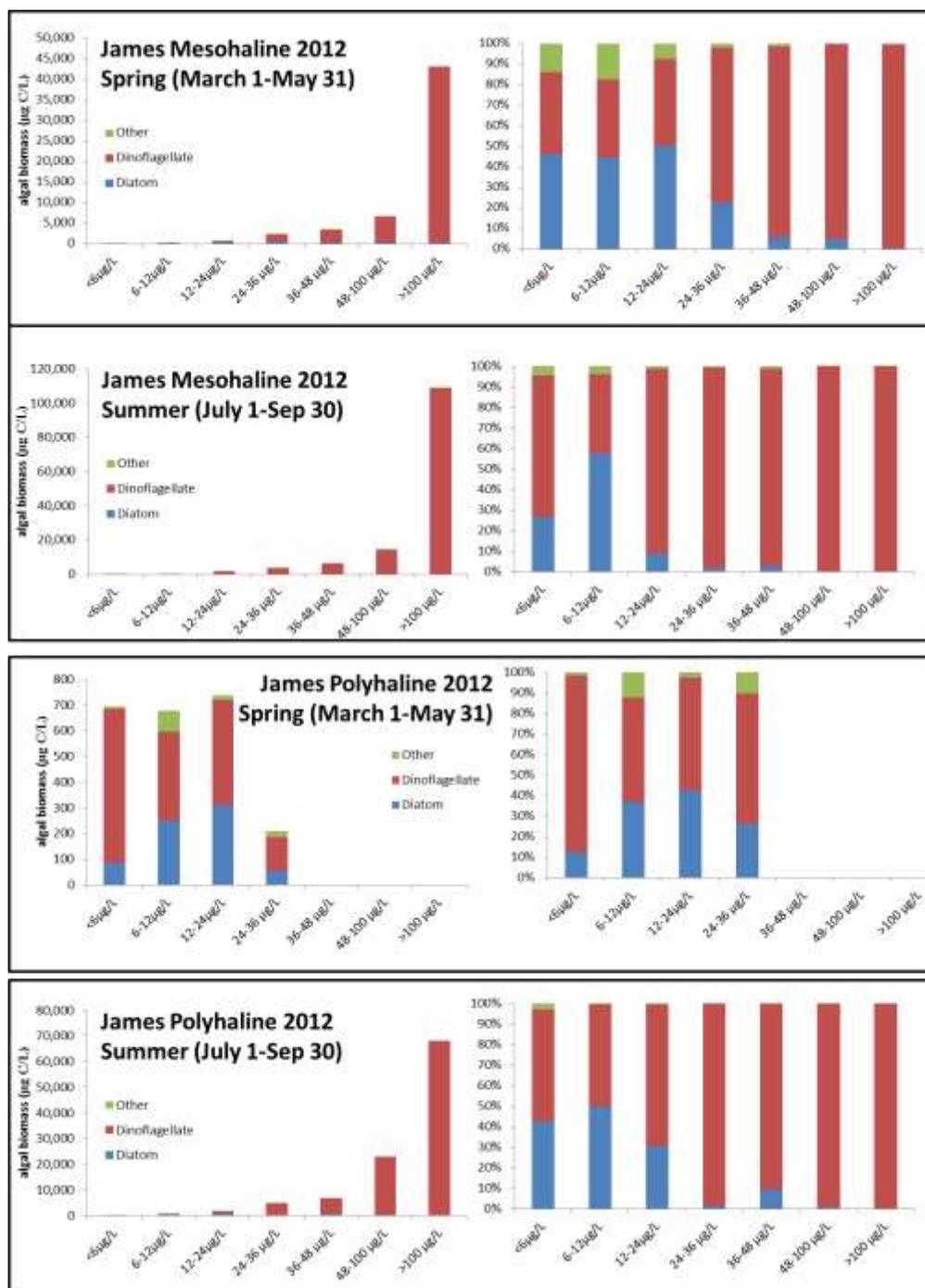


**Figure 8:** 2012 distribution of *Cochlodinium polykrikoides* blooms in James River and lower Chesapeake Bay.



**Figure 9:** 2012 distribution of *Alexandrium monilatum* blooms in James River and lower Chesapeake Bay.

On average, dinoflagellates constituted 70% of algal biomass in the lower James River. During bloom periods when chlorophyll concentrations were elevated, dinoflagellates composed over 90% of total algal biomass (Figure 10, Tables 4-5). Diatoms also represented a significant fraction of total algal biomass, with an average of (25%).



**Figure 10:** Algal biomass (left: concentrations, right: percent composition) of collections made in the meso and polyhaline James River in relation to chlorophyll concentration in 2012.

**Table 4:** Lower James River segments: average phytoplankton composition in terms of *biomass* ( $\mu\text{g C/L}$ ) of major taxonomic groups compared to the corresponding chlorophyll concentration. Number of samples for each segment/season shown in left column. Spring=March 1-May 31, Summer=July 1-September 30. Total number of samples examined for entire lower James River region=439 .

river segment/season	Chlorophyll range ( $\mu\text{g/L}$ )	Diatoms ( $\mu\text{g C/L}$ )	Dinoflagellates ( $\mu\text{g C/L}$ )	Chlorophytes ( $\mu\text{g C/L}$ )	Cyanobacteria ( $\mu\text{g C/L}$ )	Others ( $\mu\text{g C/L}$ )
JMSMH Spring n=62	<6	42.13	8.36	4.97	0	7.61
	6-12	126.78	48.53	41.52	1.05	6.73
	12-24	372.46	269.61	45.38	0	9.05
	24-36	342.83	859.22	44.32	0	2.26
	36-48	203.74	3,135.20	41.89	0	0.46
	48-100	320.95	6,169.77	3.01	0	1.79
	>100	64.63	27,620.40	8.66	0.03	6.52
JMSMH Summer n=71	<6	9.80	25.61	0	0	1.60
	6-12	110.48	74.25	0.25	0.83	6.18
	12-24	134.82	1,378.52	0.10	0	19.15
	24-36	55.02	3,342.92	0.29	0.10	23.00
	36-48	170.96	5,960.67	0	0	72.61
	48-100	18.76	14,323.97	0.01	0	8.22
	>100	78.36	108,756.55	0	0	13.46
JMSPH Spring n=30	<6	85.04	119.51	0.11	0	9.89
	6-12	55.00	109.86	0.05	0	15.54
	12-24	62.42	315.03	0.08	0	18.61
	24-36	54.69	132.70	0	0	21.24
	36-48					
	48-100					
	>100					
JMSPH Summer n=49	<6	26.67	34.21	0	0	1.71
	6-12	234.27	235.92	0	0	2.02
	12-24	502.30	1,134.67	0	0	3.32
	24-36	63.95	4,726.58	0	0	3.32
	36-48	628.97	6,114.97	0	0	0
	48-100	135.38	22,605.27	0	0	2.01
	>100	116.11	67,725.44	0	0	2.41

**Table 5:** Lower James River segments: average phytoplankton composition in terms of *percent biomass* of major taxonomic groups compared to the corresponding chlorophyll concentration. Number of samples for each segment/season shown in left column. Spring=March 1-May 31, Summer=July 1-September 30. Total number of samples examined for entire lower James River region=439 .

river segment/ season	Chlorophyll range ( $\mu\text{g/L}$ )	Diatoms % biomass	Dinoflagellates % biomass	Chlorophytes % biomass	Cyanobacteria % biomass	Others % biomass
JMSMH Spring n=62	<6	66.8	13.3	7.9	0	12.1
	6-12	56.4	21.6	18.5	0.5	3.0
	12-24	53.5	38.7	6.5	0	1.3
	24-36	27.5	68.8	3.5	0	0.2
	36-48	6.0	92.7	1.2	0	0.0
	48-100	4.9	95.0	<0.1	0	0.0
	>100	0.2	99.7	<0.1	<0.1	0.0
JMSMH Summer n=71	<6	26.5	69.2	0	0	4.3
	6-12	57.5	38.7	0.1	0.4	3.2
	12-24	8.8	89.9	<0.1	0	1.2
	24-36	1.6	97.7	<0.1	<0.1	0.7
	36-48	2.8	96.1	0	0	1.2
	48-100	0.1	99.8	<0.1	0	0.1
	>100	0.1	99.9	0	0	0.0
JMSPH Spring n=30	<6	39.6	55.7	0.1	0	4.6
	6-12	30.5	60.9	<0.1	0	8.6
	12-24	15.8	79.5	<0.1	0	4.7
	24-36	26.2	63.6	0	0	10.2
	36-48					
	48-100					
	>100					
JMSPH Summer n=49	<6	42.6	54.7	0	0	2.7
	6-12	49.6	50.0	0	0	0.4
	12-24	30.6	69.2	0	0	0.2
	24-36	1.3	98.6	0	0	0.1
	36-48	9.3	90.7	0	0	0
	48-100	0.6	99.4	0	0	0
	>100	0.2	99.8	0	0	0

### **The initiation, composition, and progressions of bloom development in the James River in 2012 in comparison to blooms occurring in 2011.**

The spring dinoflagellate bloom of *Heterocapsa triquetra* within the mesohaline James began in mid-February during 2012, 7 weeks earlier than in 2011. Maximum bloom conditions of cell densities of *H. triquetra* were greater than 15,000 cells/ml and chlorophyll measurements of at least 100 µg/L were present for 5 weeks (Feb. 21- Mar. 20) in 2012. In comparison, these same conditions were present for only 2 weeks in 2011 (April 6-13). Maximum 2012 *H. triquetra* concentrations were 191,200 cells/ml, and approximately 3x greater than that observed in 2011 (65,000 cells/ml). In both years, the blooms appeared to be initiated in the mesohaline segment of the James, in the vicinity of Burwell Bay, with maximum concentrations developing downstream of the Warwick River (Figure 5).

The regional summer/autumn *Cochlodinium polykrikoides* bloom have been documented to originate in the Lafayette River for several years (Marshall and Egerton 2009, Mulholland et al. 2009, Morse et al. 2011, Egerton et al. 2012). Following excystment and bloom initiation, *C. polykrikoides* is transported into the Elizabeth River and subsequently into the poly and mesohaline regions of the James River, and potentially into Lower Chesapeake Bay (Figure 6). In 2012, *C. polykrikoides* was first observed in the upper branches of the Lafayette River on June 20. Bloom concentrations of  $\geq 3000$  cells/ml were present for 7 weeks (June 26-Aug. 8), compared to 5 weeks in 2011 (July 27-Aug. 23). The maxima observed for *C. polykrikoides* were approximately 17x greater in 2012 (75,780 cells/ml) than 2011 (4350 cells/ml).

### **Comparison of 2011/2012 James River phytoplankton abundance and composition to time periods 1991-2000 and 2007-2010.**

This preliminary comparison of Chesapeake Bay Monitoring program phytoplankton data is to relate the recent algal data to data utilized in calibration of the water quality model (1991-2000), and the HAB model (2007-2010). This comparison is depicted in Fig. 11 for spring (March, April, May) and summer (July, August, September) months during the three periods at the three CBP phytoplankton monitoring stations, TF5.5, RET5.2 and LE5.5. At the tidal freshwater station TF5.5, there were no significant difference ( $p > 0.05$ ) in total phytoplankton abundance between time periods in either spring or summer. At the oligohaline station RET5.2, the average total phytoplankton abundance during spring was significantly greater ( $p = 0.008$ )

during 1991-2000, than during 2007-2010. However, the 2011-2012 spring algal abundance was not significantly different than either of these time periods. During the summer at station RET5.2, the total abundance was significantly less during 2007-2010 than during the other two time periods. At the polyhaline station LE5.5, there were no significant difference ( $p>0.05$ ) in total phytoplankton abundance between time periods in either spring or summer. The phytoplankton abundance at these stations fluctuates seasonally, as well as inter-annually, with the percentage representation of the major constituents presented in Figure 11. While variable, the phytoplankton composition at each station was also largely similar between time periods in both spring and summer. Diatoms were the most abundant group at all three stations during spring months. In summer, there is a greater representation of cyanobacteria, particularly in the upstream stations (RET5.2 and TF5.5). Dinoflagellates and cryptophytes make up a larger fraction of the algal community in the downstream site (LE5.5). These data correspond closely with historical records regarding phytoplankton composition, major bloom producers, and seasonal expression of bloom development (Marshall et al. 2005).

### **Established Chlorophyll a, microcystin, and *Microcystis* standards recognized in Virginia**

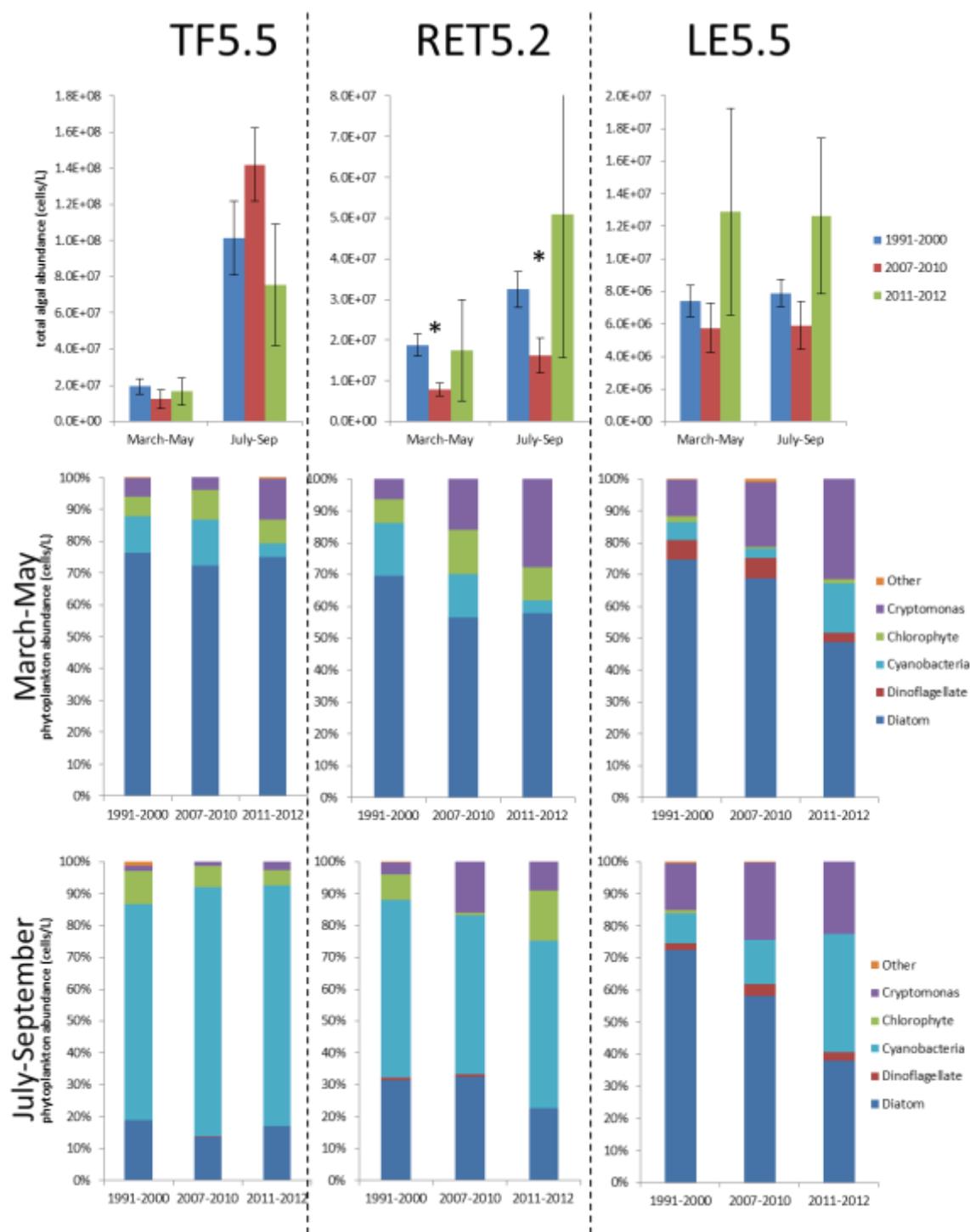
*Microcystis aeruginosa*, is a common freshwater species that has produced blooms in the tidal fresh regions of Virginia rivers, with major past development occurring in the Potomac River and its associated inlets. Often these blooms enter the lower saline regions and may persist temporarily in their transport downstream. Health concerns are associated mainly with the ingestion of these cells, or water containing the toxin by animals or humans, with health alerts released when specific levels of toxin are present. The Commonwealth of Virginia follows water quality standards for chlorophyll a, microcystin levels, and *Microcystis* abundance regarding health alerts and concerns during bloom occurrences of this taxon (USEPA 2004). The threshold levels indicating potential health concerns are: chlorophyll a  $>27 \mu\text{gL}^{-1}$ , microcystin  $>10 \mu\text{gL}^{-1}$ , and *Microcystis aeruginosa* concentrations  $> 50,000 \text{ cells ml}^{-1}$ . These criteria were established regarding human health risks and the issuance of health alerts regarding recreational usage of waters meeting these levels. Our ODUPAL has consistently used these criteria regarding our bloom analysis reports when they were exceeded to DEQ and the Virginia Department of Health. As noted previously, neither high *M. aeruginosa* abundance, nor microcystin levels have developed in the lower James River complex polyhaline regions. *Microcystis aeruginosa* is

present in the upper James River tidal fresh regions, but has been represented by low levels of these criteria during 2011-2012 at these sites. During 2012, *M. aeruginosa* concentrations did not exceed 10,000 cells/ml, with a mean density of less than 1000 cells/ml (Table 6). This species composed a maximum of 2.7% of algal biomass, with an average of just 0.4%, and did not represent a significant contributor of phytoplankton Carbon during the study.

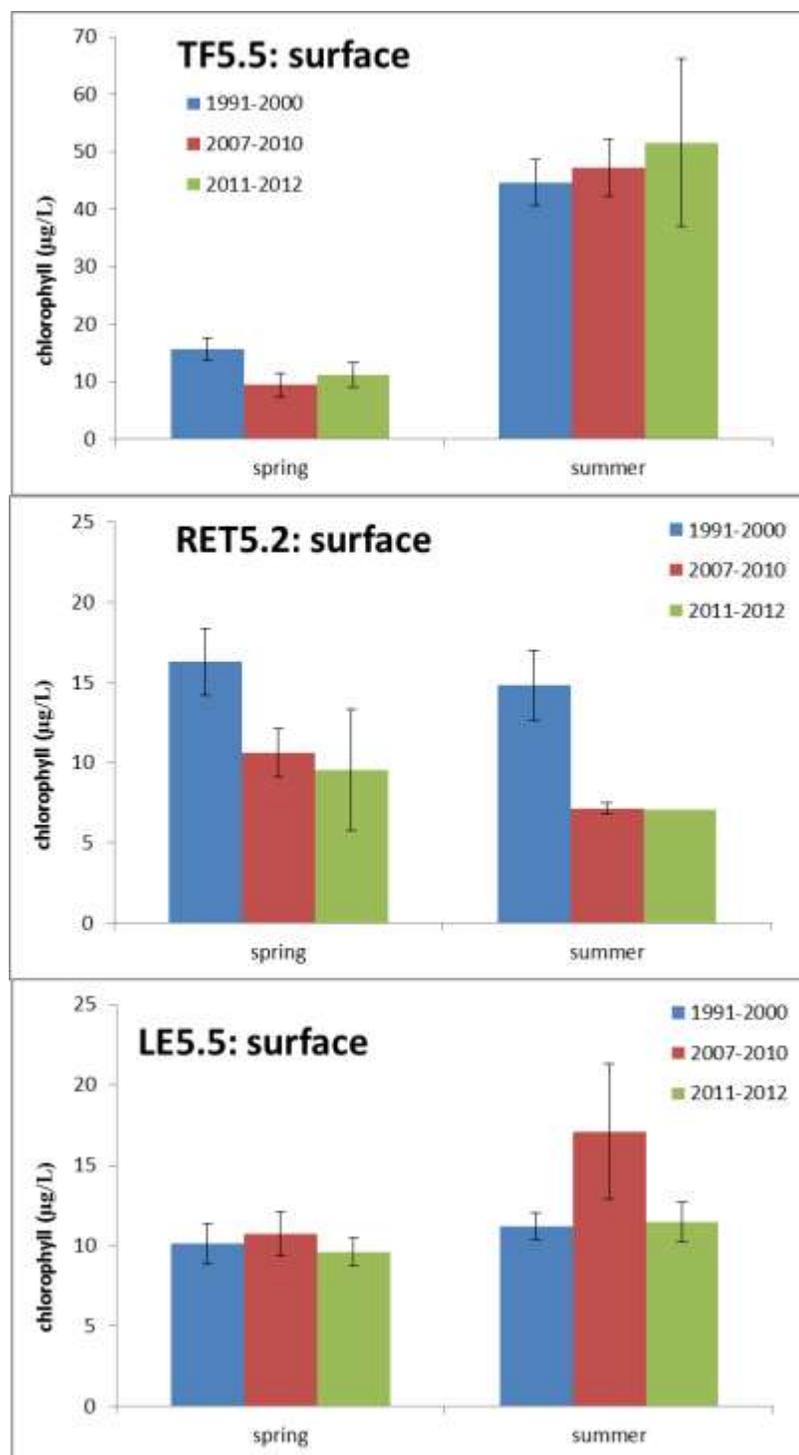
### **Comparison to historical chlorophyll values in the James River**

Chlorophyll concentrations at the three CBP phytoplankton stations within the James River were compared between the time periods (1991-2000, 2007-2010, and 2011-2012) associated with the three models (Figure 12). Similar to the analysis of total phytoplankton abundance, ANOVA was used to compare spring and summer values between time periods. While there was seasonal and inter-annual variability in values, there were no significant differences ( $p > 0.05$ ) between the time periods at any station.

During 2011 and 2012, weekly DATAFLOW cruises were conducted in the meso/polyhaline segments which included chlorophyll measurements. These cruises detected more variability, including higher chlorophyll values than monthly collections, even at fixed stations. For example, weekly DATAFLOW monitoring at station LE5.5 indicated chlorophyll values in excess of 40  $\mu\text{g/L}$ , while monthly DEQ sampling indicated an annual maximum of 15.8  $\mu\text{g/L}$  (Figure 13). In addition, the additional spatial coverage of the DATAFLOW approach captured the spatial variability associated with algal blooms, including concentrations in excess of the manufacturer listed maximum values ( $>400\mu\text{g/L}$ ). These values are far in excess of those measured by historical CBP monthly monitoring at fixed stations. For comparison, the highest chlorophyll concentration recorded in the James River by the CBP is 189  $\mu\text{g/L}$ . This is largely due to both the spatial patchiness and ephemeral nature of algal blooms, which are difficult to monitor using solely a network of fixed stations.



**Figure 11:** Mean abundance and composition at the three CBP James River (above pycnocline) stations during three time periods (1991-2000, 2007-2010, 2011-2012). Significant differences in total abundances (\*) between time periods were identified in station RET5.2, described in text.



**Figure 12:** Spring (March-May) and summer (July-September) surface chlorophyll concentrations at the three James River phytoplankton monitoring stations during three time periods (1991-2000, 2007-2010, 2011-2012). No significant differences between time periods.

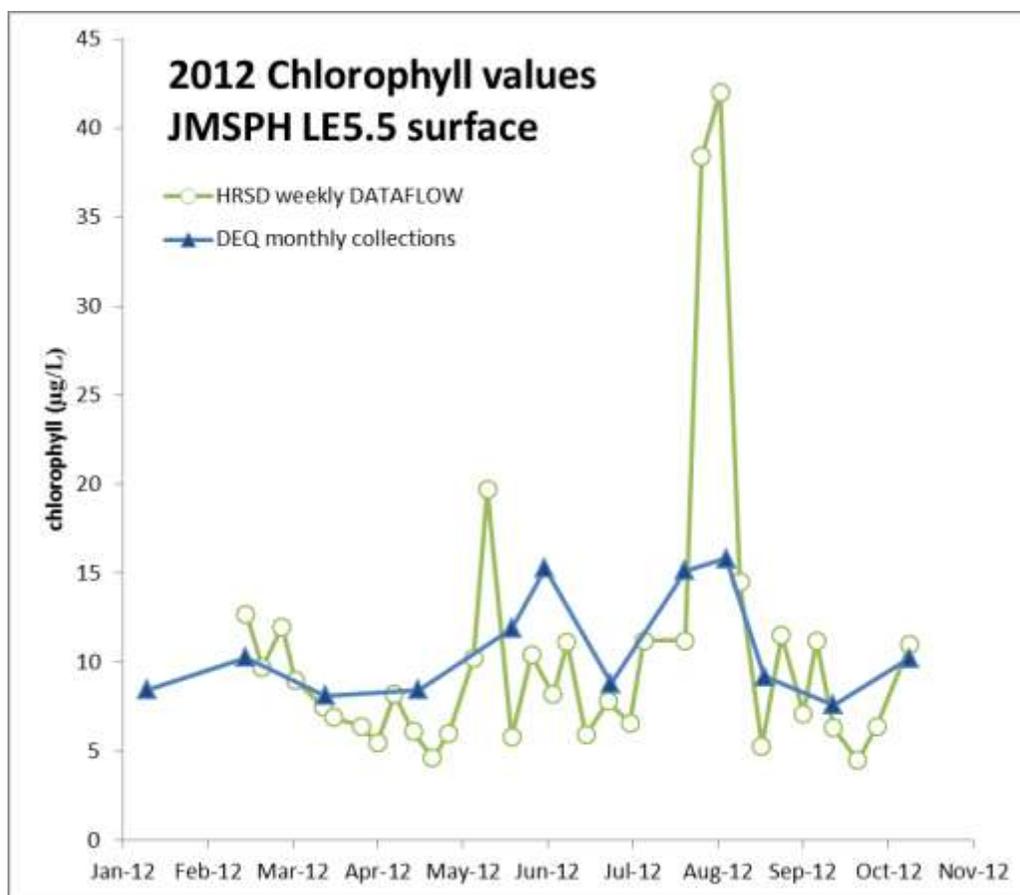


Figure 13: Monthly CBP/DEQ (blue) and weekly HRSD DATAFLOW (green) measurements of chlorophyll at station LE5.5 during 2012.

### **Relationships between seasonal phytoplankton cell abundance and biomass to chlorophyll levels in the James, Elizabeth, and Lafayette rivers.**

Cell abundance alone may not indicate the major contributors to biomass and chlorophyll present at these river sites. Many of the cyanobacteria noted at high abundance levels consist of cells less than 2 microns in size, and although their accumulative abundance numbers may be high, their total biomass and the chlorophyll content in their cells may be considerably less than less abundant (but larger sized) diatoms or dinoflagellates in the water column. For this reason, algal biomass values are calculated based on cell biovolume and compared to measured chlorophyll values.

In contrast, dinoflagellates make up a much smaller percentage of the algal community in the upper James. In the tidal fresh segments (JMSTF), diatoms account for ~76% of algal biomass, with chlorophytes (green algae) and cyanobacteria making up ~16% and 6% of biomass

respectively (Figure 5, Table 3). In this region, centric diatoms including *Aulacoseira granulata* were the dominant taxa, accounting for up to 85% of total cell C. The composition in the current study is consistent with our review of historical data from this region of the James. There were significant positive relationships between algal biomass and chlorophyll concentration for total phytoplankton, diatoms, chlorophytes and cyanobacteria (Figure 14).

In the lower James, including meso and polyhaline segments and the Elizabeth and Lafayette Rivers, the major algal groups in abundance, biomass, and chlorophyll levels are diatoms and dinoflagellates. In these waters, the dinoflagellates account for 71% of algal biomass, with diatoms accounting for 25% (Figure 10, Table 5). Both of these algal groups contain species highly responsive to water quality conditions and nutrient levels favorable to their development and in several, major bloom production. There were significant positive relationships between algal biomass and chlorophyll concentration for total phytoplankton and dinoflagellates (Figure 15). Of the dinoflagellates, the two major bloom species (*H. triquetra* and *C. polykrikoides*) were the dominant species, accounting for up to 99% of total cell C in some bloom cases.

Based on historical data bloom occurrences in these rivers and Chesapeake Bay, the timing, general composition, and magnitude of blooms these past two years are generally comparable. Exceptions involve the occurrence of pulses of growth from various species that can occur and are unpredictable.

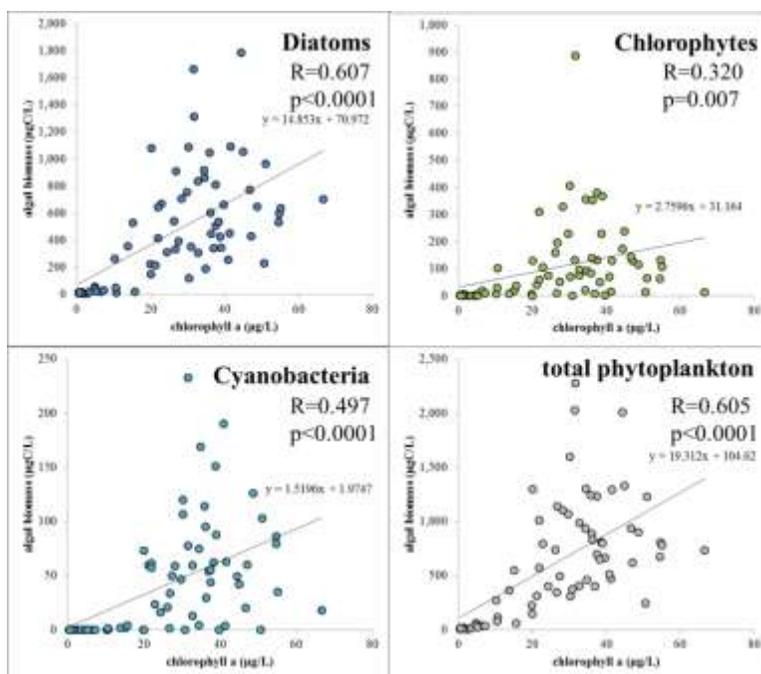


Figure 14: Significant positive correlation between tidal freshwater algal biomass and chlorophyll a concentrations. No significant relationship was observed in other algal groups (not shown).

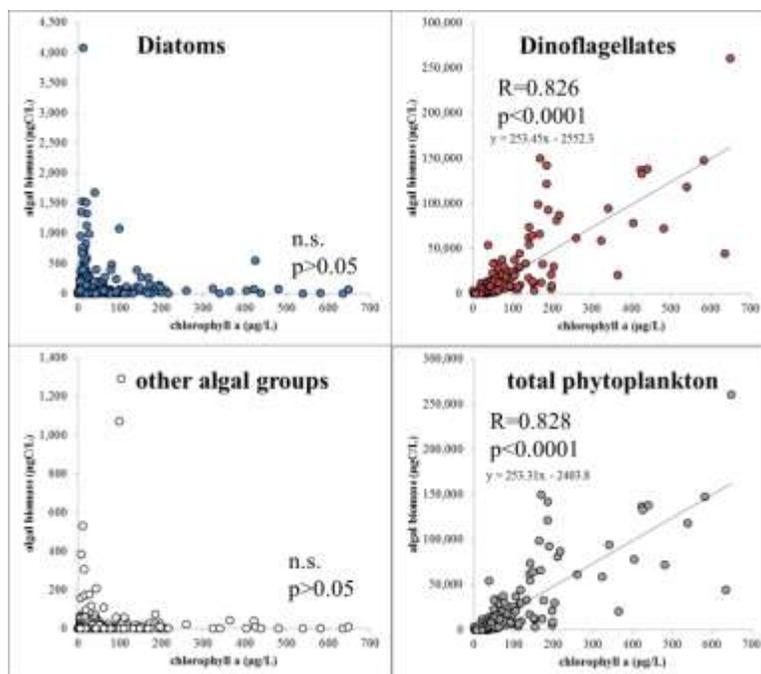


Figure 15: Correlations between meso-polyhaline algal biomass and chlorophyll a concentrations. Other MH/PH algal groups include all phytoplankton besides diatoms and dinoflagellates.

### **HABs and other bloom producers regarding their contributions to biomass and chlorophyll levels in the James, Elizabeth, and Lafayette rivers.**

Due to the favorable growth conditions (e.g. adequate nutrients, warm summer temperatures, etc.) in the James River estuarine system, high seasonal levels of algal biomass and chlorophyll are predictable from the diverse phytoplankton species that are present, and this outcome is substantiated by historical records in these rivers. Accentuating these favorable growth conditions are the bloom events occurring in these rivers. The major contributors to algal biomass and chlorophyll are an assemblage of HAB and non-HAB species, plus an array of pelagic and benthic diatoms, with other algal components playing a contributing, but lesser role.

HAB species, and blooms in general, did not appear to play as large of a roll in the contribution to biomass and chlorophyll in the tidal freshwater James. There were seasonal periods of increased growth, but no distinct bloom events, and the community was very diverse throughout the freshwater segment, including high chlorophyll and high biomass collections (Figures 4-5, Tables 2-3). The HAB species *Microcystis aeruginosa*, was present in the lower tidal fresh segment (JMSTF) during summer at comparable, sub bloom densities (avg.  $1.9-3.9 \times 10^3$  cells/ml) between chlorophyll levels of 12 to  $>48 \mu\text{g/L}$ . (Table 6).

In the saline waters of the lower James system, seasonal monospecific dinoflagellate blooms play a large role in the overall biomass of the phytoplankton community, and at times may account for up to 99% of total cell C. There is indicated here a strong linkage between algal bloom biomass and chlorophyll concentrations. Algal biomass increased with increased chlorophyll, and was highest when chlorophyll was  $\geq 100 \mu\text{g/L}$  in segments JMSMH and JMSPH (Figure 10, Table 4). When chlorophyll concentrations were elevated, specifically above  $24 \mu\text{g/L}$ , dinoflagellates made up the vast majority of algal biomass (~80-100%), with a more even mixed community (diatoms and dinoflagellates) at concentrations below  $24 \mu\text{g/L}$  (Figure 10, Table 5). Cell densities of the bloom forming dinoflagellates *H. triquetra* and *C. polykrikoides* increased with chlorophyll concentrations during their respective blooms, with average densities  $>1000$  cells/ml generally associated with chlorophyll  $>24 \mu\text{g/L}$ , and the highest densities occurring with maximum chlorophyll measurements (Table 7).

Algal blooms in the James, and the region in general are linked to adequate nutrients entering the river system, favorable temperatures and salinity range, plus adequate periods of increased residency time in the rivers during many of the summer periods of bloom

development. The present study, due to its increased coverage of these waters, is able to capture a more realistic scenario of the number, duration, and significance of these bloom producers to a higher presence of chlorophyll throughout this time period than was previous and more accurately analyzed and determined for these rivers. These relationships will provide a more realistic data set for determining a chlorophyll standard for these rivers.

Table 6: Upper James River segments: average abundance of HAB species compared to the corresponding chlorophyll concentration. Number of samples for each segment/season shown in left column. Upper JMSTF= station TF5.3 (JMS99), Lower JMSTF= stations TF5.5 (JMS75) and TF5.5A (JMS69). Spring=March 1-May 31, Summer=July 1-September 30. Total number of samples examined for entire upper James River=89 .

river segment/ season	Chlorophyll range ( $\mu\text{g/L}$ )	<i>M. aeruginosa</i> (cells/ml)
Upper JMSTF Spring n=4	<6	0
	6-12	
	12-24	
	24-36	
	36-48	
	48-100	
	>100	
Upper JMSTF Summer n=10	<6	0
	6-12	0
	12-24	150.0
	24-36	
	36-48	
	48-100	
	>100	
Lower JMSTF Spring n=8	<6	
	6-12	0
	12-24	0
	24-36	0
	36-48	0
	48-100	0
	>100	
Lower JMSTF Summer n=20	<6	
	6-12	
	12-24	3,975.0
	24-36	1,964.3
	36-48	2,307.1
	48-100	2,670.0
	>100	

Table 7: Lower James River segments: average abundance of bloom species compared to the corresponding chlorophyll concentration. Number of samples for each segment/season shown in left column. Spring=March 1-May 31, Summer=July 1-September 30. Total number of samples examined for entire lower James River region=439 .

river segment/season	Chlorophyll range (µg/L)	<i>H. triquetra</i> (cells/ml)	<i>C. polykrikoides</i> (cells/ml)	<i>A. monilatum</i> (cells/ml)	<i>K. veneficum</i> (cells/ml)	<i>P. minimum</i> (cells/ml)
JMSMH Spring n=62	<6	0.9	0	0	0	1.8
	6-12	45.8	0	0	0	16.3
	12-24	647.1	0	0	0	5.7
	24-36	1,850.0	0	0	0	80.0
	36-48	7,880.0	0	0	0	0
	48-100	15,670.0	0	0	0	43.3
	>100	72,300.0	0	0	0	12.9
JMSMH Summer n=71	<6	0	1.5	0	0	0.8
	6-12	0	0.8	0.4	0	0.8
	12-24	0.7	236.7	0	0	2.7
	24-36	0	586.7	0	0	8.3
	36-48	0	1,293.3	0	0	0.0
	48-100	0	3,782.5	0.8	0	6.7
	>100	0	31,293.3	13.3	0	0.0
JMSPH Spring n=30	<6	15.0	0	0	76.7	48.3
	6-12	50.0	0	0	28.5	53.8
	12-24	95.6	0	0	21.1	371.1
	24-36	0	0	0	0	0
	36-48					
	48-100					
	>100					
JMSPH Summer n=49	<6	0	0	1.5	0	0
	6-12	0	49.1	5.2	0	0
	12-24	0	267.1	4.5	0	0
	24-36	0	1,320.0	0.0	0	0
	36-48	0	1,086.7	443.3	0	0
	48-100	0	6,458.5	0.0	0	0
	>100	0	19,562.0	0.0	0	0

## Summary statements

1. The phytoplankton analysis identified species (and algal groups) representing the major (>90%) source of algal biomass and chlorophyll in the James, Elizabeth, and Lafayette rivers. There are two distinct algal populations represented in the James River, each representing the major contributors in biomass and chlorophyll values. In the upper James the dominant taxa are freshwater diatoms and chlorophytes, whereas, in the lower James the dominant flora are estuarine dinoflagellates and diatoms. Six HAB species were among the taxa present.
2. The 2012 algal development also differed between the upper and lower James.
  - a.) Phytoplankton in the upper James represented a general expression of increased algal development from winter into spring that extended into the summer and autumn months. There was no breaking-out from this pattern by a species that would be considered a major bloom producer beyond its general pattern of growth. Diatoms and chlorophytes prevailed as the major biomass and chlorophyll source in spring and summer (Tables 2-3).
  - b.) In contrast, phytoplankton in the lower James River also followed the seasonal patterns of algal development, but within this cycle, also occurred excessive and rapid blooms by several dinoflagellates that dominated in abundance and biomass for extended periods of time. The major biomass and chlorophyll source during spring came from diatoms and chlorophytes, with the summer contributors mainly dinoflagellates and diatoms (Tables 4-5). Algal blooms are common seasonal events in these waters that have been reoccurring annually for several past years.
  - c.) Variations to algal development and occurrence of algal blooms described above for 2012 may vary both seasonally and annually in relation to environmental conditions and the algal response.
3. There were two extended periods of peak chlorophyll concentrations in the lower James the result of specific algal blooms. The first occurred during 5 weeks in spring and was produced by the non-HAB dinoflagellate *Heterocapsa triquetra* (reaching 191,000 cells/ml). The other occurred in summer throughout the lower James River complex and was caused by the HAB *Cochlodinium polykrikoides* that persisted for 7 weeks attaining maximum concentrations of 75,000 cells/ml. The 2012 blooms had

longer durations, greater intensity (cell abundance and chlorophyll concentrations), and larger spatial coverage than those observed in 2011 with comparable monitoring efforts. Throughout the year, the lower James also contained a stable assemblage of other phytoplankton constituents, including a variety of non-HAB dinoflagellates and diatoms that were also significant contributors to the chlorophyll values in these waters.

4. Two HAB species, both cyanobacteria, were identified in the upper James. *Microcystis aeruginosa*, a common freshwater species, was in the VCU station samples, with *Limnothrix redekei* present in samples provided by the Hopewell Regional Waste Water Treatment Facility. Both taxa had low abundance levels and non-bloom status. *Microcystis aeruginosa* concentrations did not exceed 6,900 cells ml<sup>-1</sup>, and had average concentrations of 2,129 cells ml<sup>-1</sup>. (Table 6). During the 2012 spring and summer collections the cyanobacteria biomass did not exceed 8.7% of the total algal biomass present, nor was it a significant contributor to chlorophyll present (Table 3). Bloom levels of concern for *Microcystis aeruginosa* and microcystin concentrations generally begin at >50,000 cells ml<sup>-1</sup> (USEPA 2004, Marshall and Egerton 2009a).
5. HABs present in the lower James River complex were the dinoflagellates *Alexandrium monilatum*, *Cochlodinium polykrikoides*, *Karlodinium veneficum*, and *Prorocentrum minimum*. Only *C. polykrikoides* had a major influence on total biomass and chlorophyll levels as noted above (# 3). The others were not major contributors to either biomass or chlorophyll in 2012.
6. When present, a chlorophyll level >15µg/L was used as an alert standard for monitoring HABs potential harmful impact to living resources in the lower James River. Greater chlorophyll levels in this section of the river were positively related to bloom status, or beginning bloom status among various species. Elevated chlorophyll can be associated with blooms forming non-HAB algal species, as well as HAB species, or a combination of both occurring together. The use of a chlorophyll standard as a HAB alert system alone has numerous limitations (e.g. a response to a non-HAB taxon). However, the combination of a bloom threshold chlorophyll level standard as an alert warning sign, is feasible, when followed by microscopic analysis of the water sample to determine an HAB presence, or threat to water quality. The

2012 results suggest that in the lower James, chlorophyll levels greater than 24 µg/L were most indicative of a phytoplankton community that was in bloom status (ie. elevated biomass, reduced community evenness dominated by a single species) (Table 5). This relationship between elevated chlorophyll and impact to the phytoplankton community composition was not apparent in the upper James during 2012 (Table 3). The impact of increased chlorophyll and algal blooms on other living resources is yet to be determined, and requires the monitoring of other trophic levels (ie. zooplankton, fish, shellfish, etc.).

7. There is little to no significant difference in algal abundance or composition data between the time periods associated with the different models (1991-2000, 2007-2010, 2011-2012). However this is based solely on the routine phytoplankton data collected monthly from the fixed stations as part of the DEQ/CBP monitoring program. The 2011 and especially 2012 algal blooms represent considerably higher cell abundances, biomass, and chlorophyll concentrations than that shown by routine monitoring alone. This is largely due to the increased sampling effort (weekly DATAFLOW collections) employed in 2011 and 2012. This may have significant implications as to the ability of the model to predict bloom conditions in the lower James.
8. In comparison to historical data, the major algal groups are consistent with those of previous years, with similar seasonal patterns of development. However, the 2012 blooms in the lower James were larger and more extensive than in 2011 and previous years. Annual differences in bloom initiation, duration, and areal coverage are under the influence of numerous environmental factors that may fluctuate year to year and produce these differences in bloom expression. High levels of regional storm activity in late 2011, and elevated temperatures in 2012 may have contributed to more favorable growth conditions in 2013 for certain taxa. Constants in these growth patterns are the spring diatom pulse accompanied by dinoflagellates, and the increased blooms of dinoflagellates during the summer/autumn months in the lower James River. In comparison to past years, there are also indications that several of the HABs have increased their presence in this river and region (e.g. *C. polykrikoides*, *K. veneticum*), plus an apparent new HAB of significance (*Alexandrium monilatum*) is occurring in these waters.

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