Executive Summary

First Year Findings from the James River Chlorophyll-a Study

Prepared for the DEQ Science Advisory Panel

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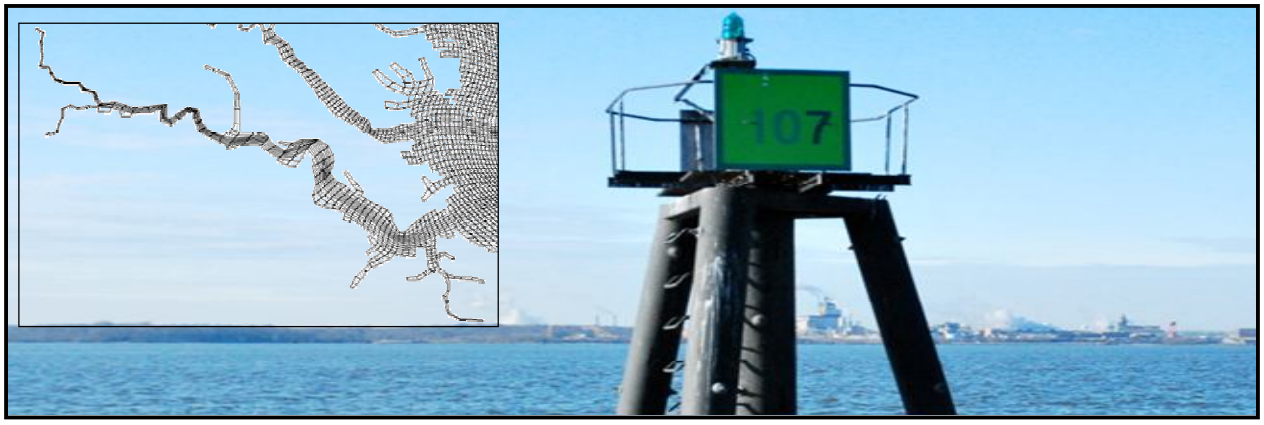


INTRODUCTION

CHLa concentrations are typically the most responsive parameter to elevated nutrient inputs and are widely used for monitoring and regulatory purposes due to their ease and reliability of measurement (Harding et al. 2013). The EPA recommends that numeric CHLa criteria should be adopted where harmful algal blooms may cause undesirable ecological consequences even in the absence of other water quality impairments. Such areas include, but are not limited to, waters which do not experience oxygen depletion for hydrodynamic reasons (e.g., shallow, well-mixed estuaries) and those in which reduced water clarity results primarily from suspended sediment rather than algae. The Virginia Department of Environmental Quality established numeric Chlorophyll-*a* (CHLa) criteria for the James River Estuary (DEQ 2004) to foster balanced algal communities and protect against the over-abundance of nuisance or potentially harmful algal species. A compilation of factors including historical concentrations, reference conditions, presence of harmful algae, attainability and recommendations provided by EPA were used to derive seasonal and salinity-specific standards for the James.

The VA DEQ is now undertaking a review of the CHLa standards and associated modeling framework. This effort will provide the scientific basis for a potential water quality standards rulemaking process, which may result in revisions to nutrient allocations contained in the Chesapeake Bay TMDL. The Commonwealth of Virginia initiated a multi-year study of the James to better understand the causes and consequences of algal blooms and to refine existing numeric models linking CHLa to nutrient inputs. The DEQ has established a Science Advisory Panel to support these efforts. The Panel's role is to guide data collection and model evaluation activities, and to make recommendations to the DEQ based on their outcomes. The Panel has developed a workplan for data collection which is being implemented over a 3-year period (2012-2014). The workplan is being carried out through contracts awarded by DEQ to researchers with regional expertise on harmful algal blooms and water quality issues. The investigators are required to submit annual reports on their findings. This report is a summary of the principal findings obtained in the first year of data collection (2012).

BACKGROUND

Over-abundance of phytoplankton due to anthropogenic nutrient enrichment is one of the primary causes of poor water quality in the nation's estuaries, including Chesapeake Bay (Howarth et al. 2000; Kemp et al. 2005). Nutrients from point and non-point sources stimulate phytoplankton production and lead to secondary impacts which include reductions in water clarity and submersed aquatic vegetation, depletion of dissolved oxygen and alterations in food web structure (Cooper and Brush 1993; Kemp et al. 2001). Nutrient enrichment also contributes to harmful algal blooms (HABs); the outbreak of species which produce biochemicals harmful, and sometimes toxic, to humans and aquatic life. Though the general mechanisms linking nutrient inputs to adverse effects on aquatic systems are well known, establishing quantitative relationships between nutrient loads, algal abundance and impairments remains a challenge.

The James River is the third largest tributary of Chesapeake Bay based on discharge and nutrient loads. The James River Estuary extends 177 km from the Fall Line at Richmond, VA to its confluence with Chesapeake Bay. The nature, timing and frequency of algal bloom events differ between the Upper (freshwater) and Lower (saline) segments. The Upper Estuary, defined here as the tidal-freshwater segment from the Fall Line to the Chickahominy River, is characterized by chronic algal blooms in the region near Hopewell, VA (river miles 69-75). CHLa is persistently elevated during May –October due to favorable conditions of light and water residence time, and to proximal nutrient inputs from riverine and local point sources (Bukaveckas et al. 2011; Bukaveckas and Isenberg 2013). In late summer, these blooms are dominated by cyanobacteria (blue-green algae) including harmful species capable of producing cyanotoxins (Marshall et al. 2005). The Lower James River Estuary (inclusive of the oligo-, meso- and poly- haline regions) experiences algal blooms that are ephemeral in nature and unpredictable in their timing, location and duration (Mulholland et al. 2009; Morse et al. 2011). The blooms are transported by currents such that sites of initiation may be geographically distinct from areas where blooms develop and cause detrimental effects on water quality and living resources. Blooms in the Lower James are comprised of dinoflagellates which are known to cause harmful effects, though specific toxins are unknown.

The Panel reviewed existing data resources and modeling capacity to identify knowledge gaps in characterizing the occurrence of algal blooms in the tidal James River and associated impairments to designated uses. The panel considered data needs inclusive of supplemental monitoring (i.e., in addition to on-going programs) as well as research activities addressing specific questions and supporting model development. The data needs were organized under three broad objectives: (1) characterizing the magnitude, frequency and duration of algal blooms, (2) understanding the conditions which favor the development of algal blooms, and (3) identifying and quantifying impairments to designated uses associated with algal blooms. Data presented in this report were collected by investigators associated with Old Dominion University (Harold Marshall and Margaret Mulholland), Virginia Commonwealth University (Paul Bukaveckas, Greg Garman and Stephen McIninch) and the Virginia Institute of Marine Science (Iris Anderson, Ken Moore, Kim Reece and Wolfgang Vogelbein). A study of sediment nutrient fluxes was initiated in 2012 but will not be completed until 2013 and therefore will be presented in the Year 2 Summary Report.

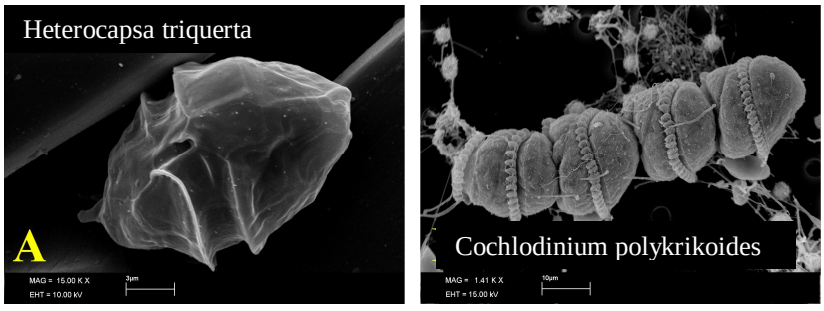
PRINCIPAL FINDINGS

Magnitude, Duration and Composition of Algal Blooms

Seasonal and spatial dynamics of algal blooms during 2012 followed expected patterns with persistent elevated CHLa (> 20 µg/L) observed during summer in the tidal fresh segment and sporadic outbreaks occurring in various locations in the lower saline estuary. Weekly monitoring in the tidal fresh segment showed that the region of elevated CHLa extended over 30 km (encompassing long-term monitoring stations JMS75, JMS69 and JMS56). Annual average CHLa values at JMS75 during 2012 (24.1 ± 2.5 µg L^{-1}) were similar to 2011 (25.6 ± 3.6 µg L^{-1}) though peak values were lower (79.7 and 66.7 µg L^{-1}, 2011 and 2012, respectively). The number of days when CHLa exceeded 20 µg/L was >170 in each year; elevated CHLa persisted somewhat later in 2012 due to the absence of Fall high discharge events. During the period of elevated CHLa, diatoms made up an average of 76% of total algal biomass, with chlorophytes and cyanobacteria contributing 16% and 6%, respectively. Peak cyanobacteria biomass did not exceed 8.7% of total algal biomass. Unlike the lower James River, species composition remained relatively constant over a range of chlorophyll levels, with similar distribution of biomass between major groups at both lower and elevated chlorophyll concentrations. In the saline estuary. dinoflagellates constituted 70% of algal biomass with diatoms contributing 25%. During blooms, dinoflagellates comprised over 90% of total algal biomass in the lower James. Two major dinoflagellate blooms occurred in the meso- and polyhaline regions of the James River during 2012. A spring bloom, lasting 5 weeks during February and March, was dominated by *Heterocapsa triquetra* (a non-HAB species). Maximum bloom development was located in the mesohaline, with highest densities above 190,000 cells/ml. The summer bloom lasted approximately 13 weeks from late June to mid-September. During this period, densities of several dinoflagellate species were elevated, with *Cochlodinium polykrikoides* being the dominant taxon. Maximum *Cochlodinium* densities reached 75,000 cells/ml. The bloom extended throughout the mesohaline and polyhaline segments, including the Lafayette and Elizabeth rivers.

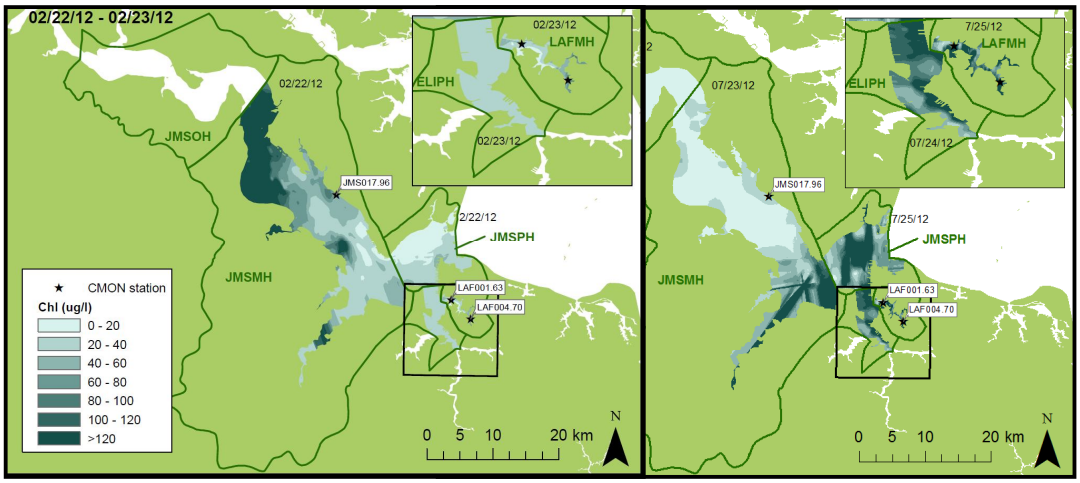
Segment	2012 Bloom Events
Tidal-fresh	Summer lasting 26 weeks (May thru Oct) with CHLa > 20 µg/L - comprised of diatoms (76%), chlorophytes (16%) and cyanobacteria (6%).
Mesohaline	Spring lasting 5 weeks (Feb & Mar) by the non-HAB dinoflagellate *Heterocapsa triquetra with* CHLa >40 µg/L covering 18% of area.
Polyhaline	Summer lasting 13 weeks (June thru Sept) of *Cochlodinium polykrikoides* with CHLa >40 µg/L covering 60% of area.

A method of on-board and underway CHLa monitoring (DATAFLOW) was used in the saline segments of the James to locate and map the spatial extent of algal blooms. Cruises were performed at weekly to monthly intervals by VIMS (oligohaline) and HRSD (meso- and poly- haline). The cruises detected more variability and higher CHLa in comparison to the monthly, fixed-station monitoring. For example, weekly DATAFLOW monitoring at a polyhaline site (LE5.5) indicated CHLa in excess of 40 µg/L, while monthly DEQ sampling indicated an annual maximum of 15.8 µg/L. In addition, the greater spatial coverage of the DATAFLOW approach captured localized concentrations which in some cases exceeded the manufacturer listed maximum values (>400 µg/L). By comparison, the highest CHLa recorded in the James during the long-term monthly monitoring was 189 µg/L. This discrepancy is due to both the spatial patchiness and ephemeral nature of bloom events in the saline portion of the estuary. During the

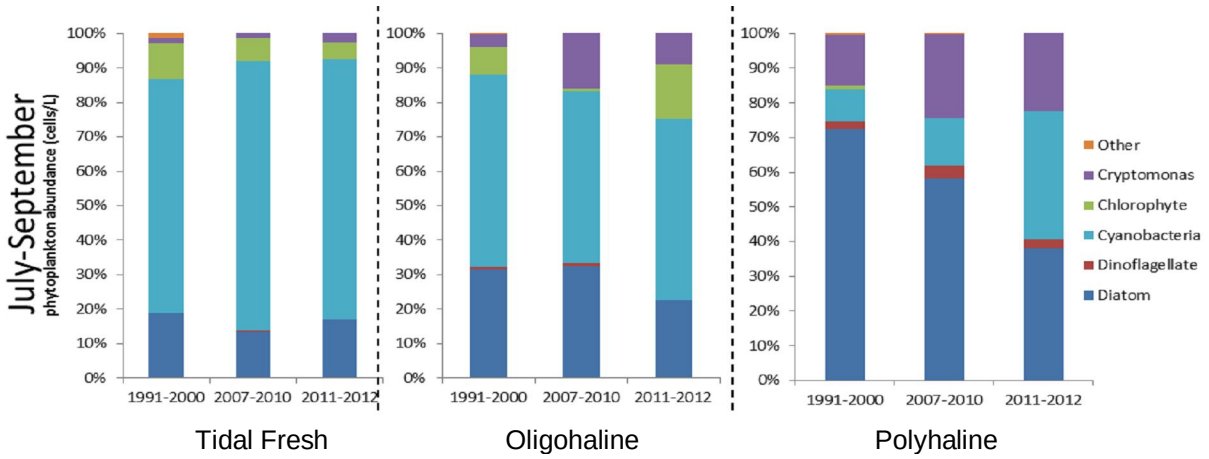


spring *Heterocapsa* bloom, median concentrations in the mesohaline were <25 µg/L, although individual measurements exceeded 300 µg/L. Segment-wide CHLa concentrations exceeding 40 µg/L, covered approximately 18% of the segment area during March. The spring bloom of *Heterocapsa* likely extended into the oligohaline though no data are available for this time period as DATAFLOW sampling was initiated in May. Overall, the oligohaline was characterized by low CHLA with less than 10% of the segment exceeding 10 µg/L. Higher CHLa (>40 µg/L) was observed during August but covered only 1-2% of the segment area. The spring bloom of *Heterocapsa* did not appreciably affect CHLa in the polyhaline with less than 10% of the segment exceeding 10 µg/L and no measurable area exceeding 20 µg/L. CHLa concentrations in the polyhaline during the August *Cochlodinium* bloom were markedly greater than those observed in the mesohaline. Median concentrations measured over the entire segment area reached ~25 µg/L during August with individual patches exceeding 300 µg/L. During the period of maximum bloom intensity in late July, ~60% of the segment area exceeded 40 µg/L with nearly 80% of the surface area exceeding 10 µg/L.

CHLa concentrations in the saline waters of the James River Estuary during the Spring *Heterocapsa* bloom (left) and the Summer *Cochlodinium* bloom (right).



To assess historical changes in phytoplankton community composition, recent data (2011-2012) were compared to earlier time periods used for the calibration of the water quality model (1991-2000) and the HAB model (2007-2010). The analyses was based on proportional contributions by major algal groups to total cell densities based on samples collected at the plankton monitoring stations in the tidal fresh (JMS75, TF5.5), oligohaline (JMS43, RET5.2) and polyhaline (JMS0.4, LE5.5). There was little to no significant difference in algal abundance or composition among the three time periods. 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 and use of DATAFLOW tracking employed in 2011 and 2012.



In summary, an expanded monitoring effort in 2012 provided enhanced spatial and temporal resolution to characterize the magnitude, duration and composition of algal blooms in the James River Estuary. Persistent elevated CHLa concentrations (>20 µg/L) were observed in the tidal fresh segment during May to September in a region spanning 30 km. High CHLa concentrations were not associated with a specific bloom-forming species, but rather were comprised of a mixture of phytoplankton that included diatoms, chlorophytes and cyanobacteria. In the saline estuary, two discrete bloom events were captured in 2012 – a spring *Heterocapsa* bloom in the mesohaline and a Summer *Cochlodinium* bloom in the polyhaline. The 2012 blooms in the lower James were larger and more extensive than in 2011. During the *Cochlodinium* bloom, median segment-wide CHLa concentrations reached ~25 µg/L in the polyhaline with some individual measurements exceeding 300 µg/L.

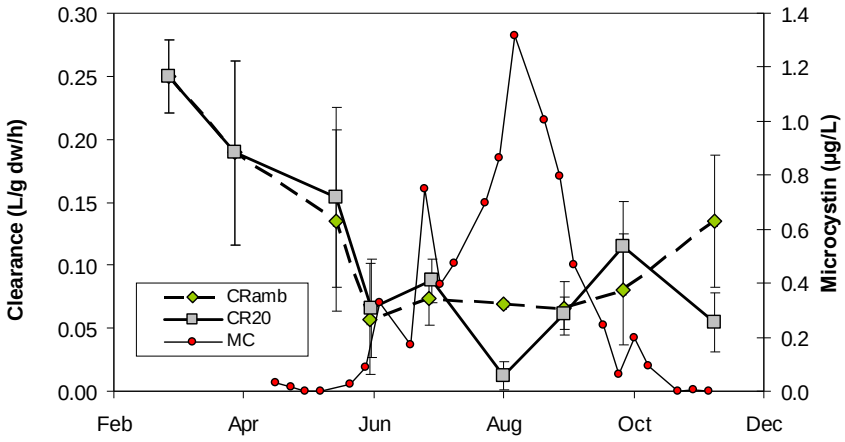
Conditions Favoring Bloom Development

The development of algal blooms is controlled by a variety of factors which include resource conditions (light and nutrient availability) as well as those which constrain nutrient utilization and biomass accrual (temperature, advective transport and grazing effects). A range of research activities were carried out to investigate the influence of these factors on algal bloom development in the James, and where possible, to derive quantitative relationships linking these to CHLa.

Specific objectives for research conducted in the tidal fresh segment included (1) characterizing light and nutrient constraints on phytoplankton growth in the region where CHLa is persistently elevated, and (2) assessing the potential role of grazers in controlling algal blooms. Light and nutrient effects were investigated using James River phytoplankton incubated in laboratory experiments. Key questions of interest were: (a) Under what conditions are phytoplankton growth rates constrained by mineral nutrients? (b) Which nutrients are limiting (N vs. P)? and (c) To what extent are different forms of N (NO_3, NH_4 & DON) utilized by phytoplankton?. The most striking finding was the prevalence of nutrient limitation (observed in 11 of 12 experiments), given that a prior study concluded that phytoplankton in the tidal fresh James were exclusively light limited (Fisher et al. 1999). The prior study was conducted at the same station (JMS75) and reported no detectable response to nutrient additions based on 11 bioassay experiments during 1992-1993. We compared nutrient conditions in the James during the 2012 bioassay experiments with historical data (from CBP monthly monitoring) and found that summertime values of DIN and PO_4 have declined at the site where bioassay experiments were performed. Declines in nutrient concentrations are consistent with results from a recent mass balance analyses showing that point source inputs of N and P to this segment of the James have been reduced by one-third and one-half (respectively) since the early 1990's (Bukaveckas and Isenberg 2013). Together, the monitoring and bioassay data suggest that these reductions have fostered a shift toward greater nutrient limitation of phytoplankton

production. In the James, direct point source inputs account for a large proportion of the external nutrient load, particularly for dissolved inorganic fractions during summer (e.g., 93% of NH_4 and 75% of NO_3 and PO_4 ; Bukaveckas and Isenberg 2013). We contend that phytoplankton in the James are responding to changes in the concentration of nutrients in inflow, rather than to loading rates associated with riverine fluxes. An important implication of these findings is that nutrient limitation of phytoplankton in the tidal fresh James is principally determined by local point source nutrient inputs, and the extent to which these are diluted by watershed (riverine) runoff. Secondary findings from the bioassay studies indicate that phytoplankton in the tidal fresh segment are co-limited by N and P, though responses to N addition alone were observed on occasion. The various forms of N tested (NO_3, NH_4 & DON) had comparable effects on phytoplankton growth rates suggesting that all three forms are utilized. Experiments performed by ODU using stable isotopes to measure uptake rates found that Urea and NH_4 were the dominant forms of N taken up. These findings suggest that wastewater and regenerated N compounds were important for fueling phytoplankton growth in the tidal fresh portion so the estuary.

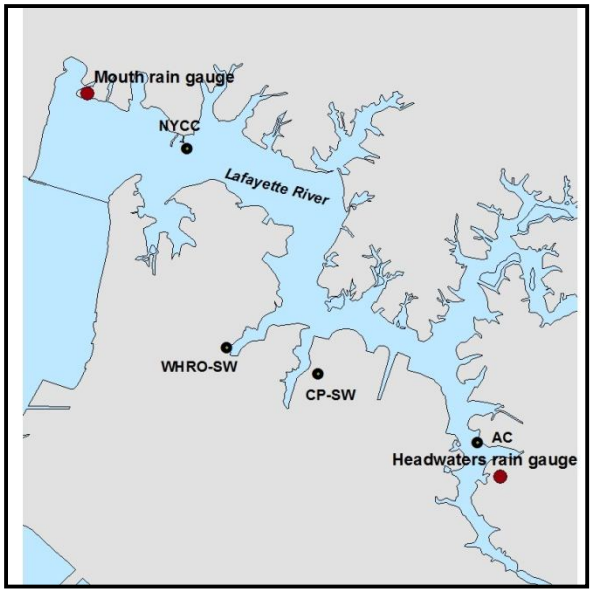
A second objective for research conducted in the tidal fresh segment was to assess the role of benthic and pelagic grazers in controlling algal blooms. Consumers have complex influences on phytoplankton abundance and community composition: grazing can mitigate eutrophication effects by reducing phytoplankton biomass (Cohen et al. 1984, Cloern and Alpine 1991, Ibáñez et al. 2012), or exacerbate problems by increasing nutrient cycling and favoring cyanobacteria through selective removal of their competitors (Schaus et al. 2002, Vanni et al. 2006, Friedland et al. 2011). Based on the Chesapeake Bay Program's benthic macroinvertebrate survey data (2001-2010), *Rangia cuneata* (common wedge clam) was identified as the dominant benthic filter-feeder in the tidal fresh segment of the James. A recent modeling study indicated that incorporating grazing by *Rangia* can improve Chesapeake Bay chlorophyll models because the clams impose an appreciable loss rate on phytoplankton (Cerco and Noel, 2010). Their modeling study relied on grazing rates of American Oysters for parameterization due to the lack of available data on *Rangia* grazing rates. In order to improve our understanding of these filter feeders, and to improve model depictions of their effects on CHLa, we undertook a study to measure *Rangia* grazing rates. *Rangia* grazing rates were measured monthly from March to November within 20 L mesocosms containing water and clams obtained from the James River (near JMS75). Biomass-specific clearance rates were highest in Spring (March-May) and lowest in Summer (Jun-Sep). Clams incubated at standardized (20°C) and in situ (river) temperatures exhibited similar clearance rates. Low clearance rates in summer coincided with elevated Microcystin concentrations in the water column. Microcystin was a significant predictor of clearance rates with a non-linear model explaining 66% of the variation. Based on an average density of 30 ind m^{-2} (CBP monitoring, D. Dauer, ODU) and an average depth of 1.3 m in the region where algal blooms occur, we estimate that *Rangia* can remove up to 28% of CHLa per day. This estimate is based on maximal (Spring) filtration rates which declined to 10% CHLa d^{-1} during the period of summer algal blooms.



In addition to the *Rangia* studies, we considered the potential effects of grazing by benthic and pelagic fishes in removing CHLa. The tidal fresh James River has large resident fish populations (e.g., gizzard and threadfin shad, blue catfish) as well as transient populations of Atlantic menhaden. Their feeding

habits include pelagic filter-feeding (Atlantic menhaden, threadfin shad and young gizzard shad) and benthic detritivory (catfish, adult gizzard shad). We measured CHLa concentrations in gut contents obtained from dominant fish species to assess their role as phytoplankton consumers. Small, pelagic fishes (YOY gizzard shad, threadfin shad and Atlantic menhaden) consumed 7 times as much CHLa on a per body mass basis in comparison to benthic detritivores (adult gizzard shad and blue catfish). Based on these results, we derived an average clearance rate based on per capita CHLa in gut contents (26 µg CHLa ind^{-1}), average CHLa in the water column (28.7 µg CHLa L^{-1} at sites JMS56, 69 and 75) and assuming a gut turnover time of 2 h (Gottlieb 1998, Friedland et al. 2005). The average per capita clearance rate was 11 L ind^{-1} d^{-1} which is comparable to the value reported by Lynch et al. (2010; 14 L ind^{-1} d^{-1}) for Chesapeake Bay menhaden feeding at CHLa concentrations similar to those observed in the tidal fresh James during our study period. Clearance rates based on CHLa are likely to be conservative as CHLa is known to degrade during passage from the fore-gut to hind-gut (Friedland et al. 2005). In considering their relative importance to controlling algal blooms, it should be noted that CHLa in fish gut contents was maximal in June-July, whereas *Rangia* grazing declined in summer suggesting that fish may exert a stronger influence on summer phytoplankton blooms. In Year-2 (2013) we will be measuring the abundance of dominant fish species in order to estimate areal removal rates of CHLa by fishes. CHLa values for gut contents were also found to be useful for interpreting inter-specific variation in Microcystin contamination of fish tissues (see following section).

A challenge to establishing linkages between nutrient loading, CHLa and impairments in the lower estuary lies in understanding the effects of physical forcing events on the timing and location of algal blooms. Physical factors such as freshwater input, surface heating, and both wind and tidally-driven mixing influence the development of algal blooms by altering resource conditions (nutrient and light availability). Meteorological events result in localized nutrient inputs via overland flow and wet deposition during rainfall events, and via wind-induced mixing of nutrients from the sediments into the water column. These nutrient inputs can trigger the initiation of blooms in the lower James River estuary (Mulholland et al. 2009, Morse et al. 2011) but are not adequately captured by watershed and estuarine monitoring programs. An intensive monitoring and mapping program was conducted in 2011 and 2012 to identify the causal factors initiating and sustaining algal blooms, examine the distribution and transport of CHLa in the lower James River, and assess the influence of meteorological events on physical-chemical conditions in the saline estuary. The monitoring program measured stratification, nutrient concentrations, phytoplankton biomass and diagnostic pigments at high temporal resolution to capture the initiation of bloom events and identify potential triggers. Between March and October 2012, two fixed stations equipped with continuous water quality sensors and automated samplers were placed in the Lafayette River at Ashland Circle (AC) and the Norfolk Yacht and Country Club (NYCC). Event sampling was also conducted from land by installing automated samplers adjacent to two storm drains. Using the combination of *in situ* sensors, automated sampling and DATAFLOW mapping, we were able to capture the initiation of *Cochlodinium* blooms in July 2011 and June 2012. In both years the bloom initiated in the Lafayette River, a sub-tributary of the lower James River, and extended into the Elizabeth and lower James River through August. During the 2012 bloom, as temperatures approached 24 to 26°C, CHLa concentrations increased. Blooms of *Cochlodinium* were first observed in the headwaters at AC during the end of June, preceded by a rain event (6/22, ~ 8 cm), while more intense bloom development began at

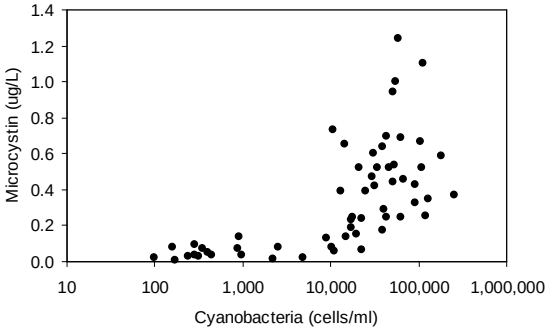


NYCC during the beginning of July following a lesser rain event (6/22 – 6/25; ~5 cm). The bloom peaked at AC at the end of July, and at NYCC at the beginning of August, but persisted up-river at higher densities compared to those at the mouth throughout August and September. Low DO concentrations followed the bloom, with concentrations decreasing as the bloom persisted. The lowest concentrations of DO (< 2 mg/L) were observed following the bloom in August at AC. Precipitation events introduced a freshwater influence that provided nutrients and buoyancy, two factors that were key in promoting the dinoflagellate bloom. DIN concentrations were high during bloom initiation but these were rapidly drawn down and for the majority of the bloom period DIN concentrations were low. The dominant forms of N taken up were urea and NH_4^+ which together comprised about 90% of the observed N uptake during the *Cochlodinium* bloom. These findings suggest that local weather events can exert impacts on the lower estuary through their influence on nutrient availability and bloom development. Blooms appear to initiate in the Lafayette River where they are able to grow in response to localized nutrient inputs. CHLa, particularly during summer months, is highest in the headwaters of the Lafayette River prior to transport and proliferation into a river-wide bloom. The headwaters reaches are shallow (< 2m), leading to earlier stratification compared to the main stem of the river and have stagnant areas where residence times are high. Meteorological forcing and precipitation introduces nutrients and buoyancy – these appear to be causal factors in bloom initiation during summer.

Deleterious Effects of Algal Blooms

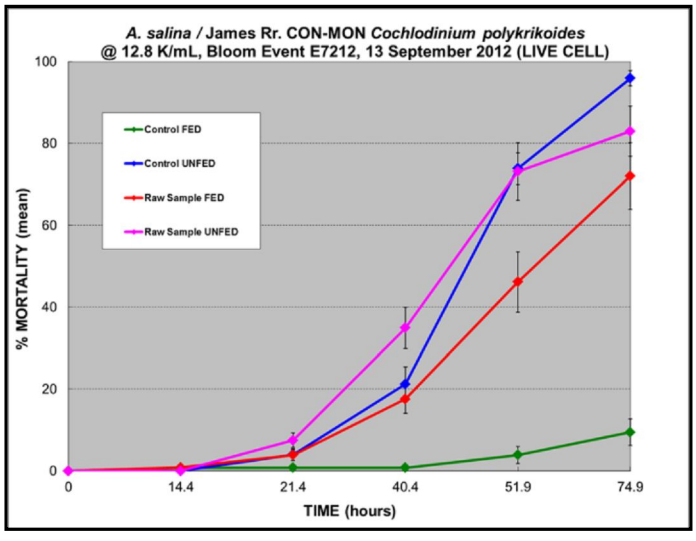
Harmful Algae Blooms (HABs) are a growing worldwide concern particularly in coastal areas with large anthropogenic nutrient loads. Harmful algae produce secondary metabolites which act as toxins and therefore pose threats to human health and living resources (De Figueiredo et al. 2004). Site-specific information on the occurrence of HABs, coupled with information documenting their impacts on living resources and human health, may provide a basis for deriving HAB-based CHLa criteria for the James. This approach necessitates the development of quantitative relationships between nutrient loading, CHLa, and the abundance of harmful algal species as well as an assessment of the toxicity of harmful species.

In fresh and brackish waters, the most common harmful algae are the cyanobacteria which are capable of producing toxins. Microcystin is a hepatotoxin produced by multiple cyanobacteria species (including *Microcystis;* Graham et al. 2010), which was detected in all cyanobacteria bloom samples collected in CB tidal-fresh and oligohaline waters during 2003 and 2005 (EPA 2007). *Microcystis* cell densities and Microcystin concentrations showed significant positive relationships with CHLa on a Bay-wide scale as well as for individual tributaries (Tango and Butler 2008). There is a growing literature on the impacts of cyanotoxins on designated uses, including effects on human health and aquatic life (Goleski et al. 2010; Jonasson et al. 2010; Lehman et al. 2010). Microcystin is not routinely measured in the James and therefore little is known of its spatial and temporal distribution. Furthermore, no prior work has been done to assess its presence in the food web. Research undertaken in 2012 documented the presence of the toxin in water, sediment and biota in the tidal freshwater segment of the James. Microcystin was detected in 104 of 105 water samples with highest concentrations measured at sites in the CHLa maximum (JMS69 and JMS75). Two seasonal peaks in Microcystin occurred on July 17 (mean = 0.92 µg/L) and August 28 (mean = 0.78 µg/L). Concentrations exceeded the 1 µg L^{-1} drinking water standard (WHO) on these dates at stations JMS56 and JMS69. None of the samples collected in proximity to the Hopewell water intake (APP1.53) exceeded the WHO drinking water guideline. By November 27, Microcystin was undetectable at all stations. CHLa, total phytoplankton, cyanobacteria and *Microcystis*



were all found to be significant predictors of variation in Microcystin ($p < 0.01$). Microcystin was detected in 11 of 60 sediment samples collected from three stations with peak concentrations (Tar Bay in September) coinciding with highest sediment CHLa concentrations. Microcystin was detected in liver/viscera tissues of 67% of fish and shellfish collected during May-October 2012. Highest incidence of toxin contamination in liver/viscera was observed in August (94%) and September (83%). The proportion of individuals with measureable toxin levels in muscle tissue was lower (mean = 14% for all species and months). Muscle tissues also exhibited consistently lower concentrations of Microcystin in comparison to liver/viscera. Highest incidence of Microcystin contamination occurred in blue crabs (viscera = 100%; muscle = 64%). Our estimates of Microcystin concentrations in blue crab muscle tissue (0.018 $\mu g\ g^{-1}$ DM) were similar to those previously reported by Garcia et al (2010) for a eutrophic Louisiana estuary (0.021 $\mu g\ g^{-1}$ DM). Laboratory studies by Dewes et al. (2006) on an estuarine burrowing crab (*Chasmagnathus*) demonstrated that tissue concentrations of Microcystin exceeding 0.013 $\mu g\ g^{-1}$ induced physiological and biochemical imbalances. We observed 10-fold higher concentrations in the James (viscera = 0.118 $\mu g\ g^{-1}$) suggesting that cyanobacteria blooms may adversely affect blue crab populations. Eleven of 65 blue crabs collected for Microcystin analyses exhibited muscle Microcystin concentrations above the WHO TDI guidelines for human consumption. Microcystin accumulation in fish was higher among planktivores (Threadfin Shad, YOY Gizzard Shad, Atlantic Menhaden) in comparison to benthic detritivores (Blue Catfish, Adult Gizzard Shad). CHLa concentrations in fish gut contents were found to be a significant predictor of inter-specific differences in liver Microcystin concentrations. To our knowledge, this is the first study linking consumer feeding habits with Microcystin exposure. No fish exceeded the WHO TDI consumption guidelines. Year-2 studies (2013) will focus on documenting effects of toxin exposure on growth, survivorship and reproduction of locally-important species.

There is a growing body of literature on HABs in saline waters (e.g., Turner and Tester 1997; Landsberg et al. 2008; Kudela and Gobler 2012), including studies in Chesapeake Bay and the Lower James River Estuary (Morse et al. 2011). Development of HAB-based CHLa criteria in marine waters has lagged because mechanisms of toxicity and links to human health are poorly understood. However, a large number of studies have documented the occurrence and causes of marine HABs and a subset of these have documented impairments of aquatic life. The most relevant is the study by Mulholland et al. (2009) of a dinoflagellate (*Cochlodinium*) bloom in the James which was coupled with laboratory bioassay experiments documenting mortality effects on oysters and larval fishes. These findings and similar experiments to be performed as part of the James River CHLa study may provide a basis for determining whether existing CHLa criteria are protective of aquatic life designated uses. In 2012, samples were obtained from the James during bloom events for use in laboratory toxicity bioassays and to establish clonal isolate cultures. The objective was to assess adverse health impacts through quantitative measurements of morbidity and mortality using brine shrimp, *Artemia salina*, nauplii exposed to whole cells or lysates. Samples were analyzed to determine phytoplankton community composition and cell density via microscopy and/or molecular-genetic approaches. Bioassays with *Artemia* were conducted using samples collected during the *Cochlodinium* bloom. *Artemia* mortality was directly related to *Cochlodinium* cell concentrations and CHLa levels in the samples. Thus, bioassays exposing *Artemia* nauplii to low cell concentrations (e.g., 2,500 - 4,000 cells/ml) and CHLa (<120 µg/L)



exhibited final mortality levels < 20%. By comparison, exposure to *Cochlodinium* densities > 10,000 cells/ml and CHLa >150 µg/L resulted in higher mortality (60-100%).

FUTURE WORK

Data collection activities in 2013 will continue in each of the three target areas: (1) characterizing the magnitude, frequency and duration of algal blooms, (2) understanding the conditions which favor the development of algal blooms, and (3) identifying and quantifying impairments to designated uses associated with algal blooms. For the tidal fresh, objective #1 will entail weekly monitoring of CHLa, Microcystin and phytoplankton community composition. Other activities in the tidal fresh will focus on Objective #3, using exposure studies conducted in the field and laboratory to assess the effects of Microcystin on various components of the James River food web. In the lower estuary, DATAFLOW cruises will be used to map bloom events and trigger sampling for phytoplankton composition and toxicological studies. Continuous fixed station monitoring and automated sampling will be used to characterize physical-chemical conditions associated with the initiation of bloom events. Lastly, the sediment nutrient flux study will be completed in Spring 2013.

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