

# **ERRATA SHEET**

## **Causes and Consequences of Algal Blooms**

### **In the Tidal Freshwater James River**

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DEQ considers all chlorophyll data to be provisional. Those data collected prior to 2014 were generated using analytical methods not certified through the Virginia Environmental Laboratory Accreditation Program (VELAP) as required by Regulations of the Dept. of General Services (1VAC30-45 & 1VAC30-46). Any use of those data shall be considered provisional pending results of a comparison study between method used prior to that date and VELAP methods.

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in the Tidal Freshwater James River

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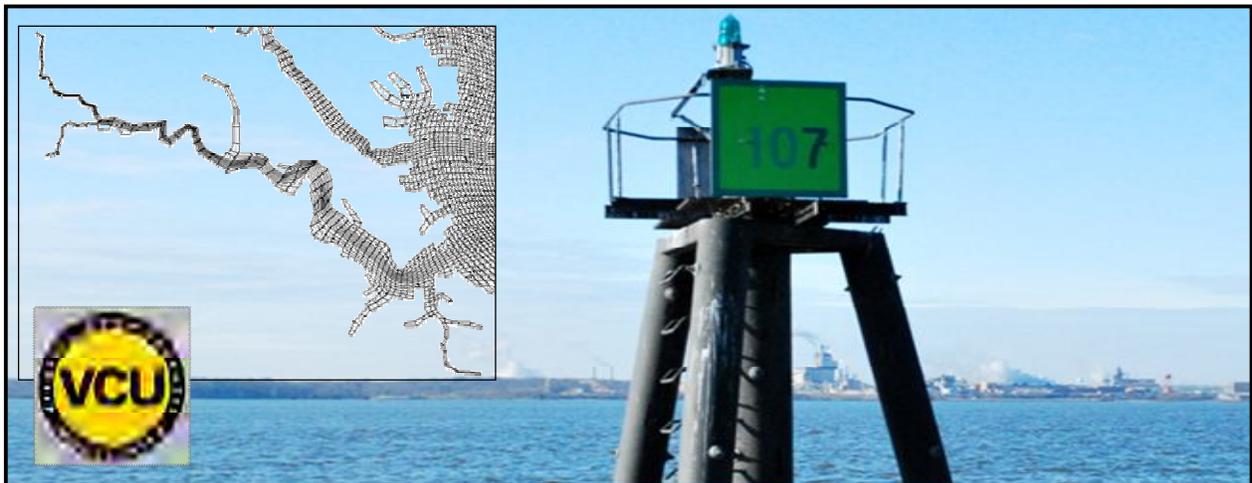
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Report to Virginia Department of Environmental Quality  
September 2014

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[Contract #15433](#)

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## Background

VCU is assisting the VA DEQ in their review of the numeric Chlorophyll-*a* (CHL<sub>a</sub>) criteria and associated modeling framework for the tidal James River. The objective is to provide a scientific basis for a potential water quality standards rulemaking process, which may result in revisions to the nutrient allocations for the Chesapeake Bay TMDL. This report describes results from research and monitoring activities conducted by VCU during 2013 for DEQ Contract #15433. The findings are organized into three topic areas: (1) studies of food-web ('top-down') effects on algal blooms in the tidal-fresh James, (2) toxicity studies on the effects of microcystin on living resources of the James, and (3) results from weekly monitoring of CHL<sub>a</sub> and Microcystin (MC) in the tidal fresh James. The views expressed herein are those of the authors and do not necessarily represent those of the funding agency. CHL<sub>a</sub> data presented in this report were collected prior to accreditation of the VCU Environmental Analyses Laboratory by VELAP.

## Part One: Consumers and Their Influence on the Trophic State of the James

### *Introduction*

Under Subtask 1.2 (Environmental factors favoring algal blooms) the Science Advisory Panel (SAP) identified two areas where data were needed to understand bloom formation and to support modeling efforts for CHLa attainability. The first of these concerned factors that regulate phytoplankton growth, and specifically, the forms of nutrients that sustain algal blooms. This data need was addressed in 2012 by performing algal bioassay experiments to assess the role of light vs. nutrient limitation and the occurrence of N vs. P limitation among phytoplankton communities in the tidal-fresh James (Wood and Bukaveckas 2014). A second issue to be addressed was the role of consumers in influencing algae-nutrient relationships in the tidal freshwater James. Consumers can potentially influence algal-nutrient interactions in two ways: first, by directly consuming algae and secondly, through consumer-mediated nutrient recycling (Vanni et al. 2013). Thus, their effects may be negative (suppression of CHLa through grazing) or positive (fueling algal blooms through nutrient re-generation). At present, we have little basis for inferring the importance of either of these top-down forces on phytoplankton in the tidal fresh segment of the James. It has been argued for Chesapeake Bay that loss of oyster populations has resulted in historically low grazing rates and that this may preclude attainability of lower CHLa even with reductions in nutrient loads. While oysters do not occur in the tidal freshwater James, other consumers may play an equally important role in regulating algal abundance. The food web of the James has likely undergone historical changes, in part due to fisheries introductions, but there is little recent or historical data on grazing rates. The most recent study (Bukaveckas et al. 2011) reported grazing rates by zooplankton of ~5% CHLa/d, which were low in comparison to other estuaries (typically ~30%/d). This study did not consider other potential consumers such as benthic filter-feeders and planktivorous fish or their role in nutrient recycling. Recent work has suggested that internal recycling provides 60% of the nutrients sustaining algal blooms in the James (Bukaveckas and Isenberg 2013; Wood and Bukaveckas 2014), though the contribution of consumer-mediating re-cycling is unknown.

Lack of information on grazing and nutrient recycling by consumers diminishes our ability to forecast CHLa under various environmental conditions and nutrient loading scenarios. For example, increased water residence time is expected to favor high CHLa due to diminished advective losses. However in systems where benthic filter-feeders are abundant, expected relationships between residence time and CHLa may not be apparent as grazing effects intensify with longer residence time (Lucas and Thompson 2012; Peierls et al. 2012). While a detailed food-web study was beyond the scope of this project, the SAP recommended that some effort should be devoted to assessing top-down effects on CHLa in the James. The first step is to identify the important consumers of phytoplankton as determined by (1) their numerical abundance, (2) their consumption (ingestion) rate, and (3) the proportion of algae in their diet. In addition to zooplankton and benthic filter-feeders (*Rangia*), the James has large resident populations of planktivorous fishes including Threadfin Shad, Gizzard Shad and Atlantic Menhaden. The potential role of planktivorous fishes in ingesting phytoplankton and recycling nutrients in the James is not known. Studies of eutrophic Midwestern reservoirs have shown that Gizzard Shad can play an important role in internal nutrient regeneration by feeding on sedimented particulate matter (detritivory) and releasing nutrients into the water column

(Shostell et al. 2004; Vanni et al. 2013). The numerically-abundant Blue Catfish may also contribute to CHLa removal and nutrient regeneration in the James. Juveniles are benthivorous and their consumption of sedimented phytodetritus may decrease suspended CHLa owing to active sedimentation and re-suspension in this segment of the James.

In the context of understanding how environmental conditions, and specifically food webs, influence algal abundance in the James, we estimated grazing and nutrient recycling rates by primary consumers including fish, *Rangia* and zooplankton for the tidal-fresh segment of the James. For nutrient recycling, we focused on nitrogen as our recent studies have shown that phytoplankton in the tidal fresh segment are N- or co- (N-P) limited (Wood and Bukaveckas 2014). Preliminary work was completed in 2012 and involved the analysis of CHLa and PON in fish gut contents to identify those fishes that may be important grazers and/or recyclers. This work was conducted in conjunction with a study of microcystin in fish tissues, the results of which were described in last year's report. Here, we present an analysis of the sources and fate of CHLa in the tidal fresh James which takes into account the role of grazers. We also consider the role of grazers in N cycling relative to other sources and sinks of N. Data collected in 2013 include estimates of fish abundance which we use to derive estimates of CHLa and PON ingestion by the dominant fish species of the James. We compare fish consumption and N recycling rates with similar values for *Rangia* (Wedge Clams) and zooplankton. *Rangia* ingestion rates of CHLa and PON were measured monthly in 2012 and we combine these results with estimates of their abundance obtained from Chesapeake Bay Program (CBP) to derive population-scale estimates of their contribution to grazing and N cycling. Similarly, zooplankton grazing rates were derived from twice-monthly measurements of their abundance obtained in 2013 and literature-derived estimates of species-specific clearance rates. Lastly, we compare CHLa losses due to grazing with other loss processes (respiration and downstream export) and compare N recycling via consumers to external inputs from the watershed and nutrient regeneration via microbial decomposition.

## Methods

### **Study Site and Data Collection**

Our analysis of top-down effects is based on data collected from the lower half of the tidal fresh segment (designated JMSTF1; as per CBP segmentation scheme). This is the portion of the tidal-fresh segment between Hopewell, VA and the confluence with the Chickahominy which is characterized by persistent, elevated CHLa concentrations in summer months (Bukaveckas et al. 2011). This segment includes three of our weekly sampling locations, which are also monthly sampling sites for the CBP monitoring (JMS75, JMS69 and JMS56). Data collected for this analysis of top-down effects include CHLa and PON in fish gut contents (monthly in 2012), fish abundance (3 surveys in 2012-2013), *Rangia* grazing rates (monthly in 2012), and zooplankton abundance (twice-monthly in 2013). Other data included in this analysis were daily river discharge (to determine CHLa export), primary production and respiration rates (to derive CHLa and algal N demand) and annual benthic surveys of *Rangia* abundance.

### **Fish Abundance and Dietary Analyses**

Fish abundance was estimated using low and high frequency electrofishing. Five to ten randomly located 500 m transects were sampled in each of three areas: City Point, Rice Center, and Tar Bay (all within 1 km of JMS75). The transects encompassed both main channel and

shallow water habitats with separate transects for high vs. low frequency electrofishing. Low frequency electrofishing was used to estimate Blue Catfish abundance; high frequency electrofishing was used for all other species. The electrofishing boat traversed the 500 m transect equipped with a fixed 5-m cross-section to delineate the sampling area. During high frequency electrofishing, all fish within the reference area were collected for identification. For low frequency electrofishing, catfish appearing within the 5-m span were counted and assigned to one of three size categories (<20 cm, 20-40 cm, and > 40 cm). For each transect, 10 randomly selected fish of each species were measured for length and weight. Sampling was conducted in September 2012, June 2013 and August 2013. Fish abundances were estimated based on sampling area (2500 m<sup>2</sup> per transect) and volume (the product of transect area and depth).

We analyzed gut contents of numerically-dominant fish species of the tidal-fresh James: Gizzard Shad (YOY and adult), Threadfin Shad, Atlantic Menhaden and juvenile Blue Catfish (<20 and 20-40 cm). Gut contents were removed surgically for determination of wet weight, dry weight (60°C for 48-72 h), C, N and CHLa. C and N content was determined on acid-fumed samples using a Perkin-Elmer CHN Analyzer. Samples for CHLa analyses were extracted overnight in 90% buffered acetone prior to analysis on a TD-700 fluorometer. Approximately 500 fish were obtained for gut contents analyses from monthly collections during April to October 2012. The number and types of fish obtained were sufficient to derive 34 monthly, taxa-specific estimates of a possible 42 taxa-month combinations for the 6 taxa-size groupings. We derived monthly estimates of fish CHLa and PON consumption based on fish abundance, gut contents analysis and assumed gastric evacuation rates. We used the PON content of gut materials to estimate fish N consumption. For CHLa, we did not use the CHLa content of gut materials to directly estimate consumption because prior work has shown appreciable degradation (loss) of CHLa from the foregut to the hindgut (Friedland et al. 2005). Instead, we used the measured organic matter content of dietary materials (C%) along with the measured CHLa:C ratio of suspended particulate matter in the James. The CHLa:C ratio was derived from the slope of a regression relating paired measurements of CHLa and POC using bi-monthly data from three stations (JMS75, JMS69 and JMS56) obtained during March-October 2012-13 (CHLa:C =  $12.2 \pm 0.8 \mu\text{g}:\text{mg}$ ; N = 108,  $R^2 = 0.70$ ,  $p < 0.0001$ ). The CHLa content of suspended particulate matter was appreciably higher than that of gut contents from planktivorous fishes (0.17 – 0.23  $\mu\text{g}:\text{mg}$ ) thereby resulting in higher estimates of the amount of CHLa consumed than those derived from CHLa in gut contents. For benthivorous fishes we substituted the C:CHLa ratio of surficial sediments (0.27  $\mu\text{g}:\text{mg}$ ) to estimate CHLa ingestion. In addition to fish abundance and gut contents data, estimation of ingestion rates requires knowledge of gut clearance or turnover times, which are known to vary among species and food conditions. We considered a range of gastric evacuation rates (4-18 d<sup>-1</sup>; Shepherd and Mills 1996) to derive a potential range of CHLa consumption and N recycling rates.

### ***Grazing by Zooplankton and Rangia***

Wedge clams (*Rangia cuneata*) are the dominant benthic filter-feeders in the tidal freshwater zone of the James and other major sub-estuaries of Chesapeake Bay (Gerritsen et al. 1994; Cerco and Noel 2010). We derived estimates of CHLa and PON consumption rates due to grazing by *Rangia* as a point of comparison for similar data from fish. *Rangia* grazing estimates are based on measured clearance rates derived using clams and food sources obtained from the James and abundance estimates for the James provided by the CBP. Methods for determining *Rangia* clearance rates were described in our previous report and briefly summarized here.

Water and clams used in these experiments were collected from the James at a site near the VCU Rice Center. Clams were obtained using oyster tongs and water was transported in 20 L carboys. The experimental design followed Wong et al. (2010) whereby we monitored CHLa and PON concentrations (at 0, 2 and 4 h) in 20 L mesocosms with and without clams. In the presence of clams, concentrations decline faster such that differences in the slopes of regression lines (concentration vs. time) between mesocosms with and without clams can be used to estimate the clearance rate. This rate is a theoretical value representing the volume of water cleared of CHLa or PON based on the mass removed by consumers and average concentration in the water (Coughlan 1969). Monthly experiments were performed during March-October 2012. Clams were kept overnight for acclimation to experimental conditions which included two temperature treatments: a standard reference of 20°C and ambient (river) which ranged from 14° to 32° C. Six mesocosms were used for each temperature treatment (3 with, 3 without clams). Each mesocosm contained a similar mass of clams (3-10 individuals depending on size; average body mass of 2.6 g ind<sup>-1</sup>; range = 0.5 to 5.0 g ind<sup>-1</sup>). Clearance rates were expressed per unit of body mass. Mesocosms were kept in dark conditions (to minimize phytoplankton growth during the experiment) and equipped with a circulating pump to maintain particulates in suspension. Samples for CHLa analysis were filtered thru Whatman GF/A glass filters (0.5 µm nominal pore size) and analyzed as per methods for fish gut contents. PON was determined on a Perkin-Elmer CHN analyzer. The abundance of wedge clams in the James is determined annually through benthic surveys conducted by CBP. We used the long-term (2001-2010) annual average abundance and the average of the monthly measured clearance rates to derive the average CHLa and PON ingestion rate by *Rangia*.

Zooplankton consumption of CHLa and PON was determined based on measured abundance and previously-published species-specific per capita clearance rates. Zooplankton abundance in the James was measured twice per month during March-September 2013 (total = 13 collections). Triplicate vertical net tows were collected at a single station (JMS75) and retained for subsequent identification and enumeration (within 1 week of collection). For nauplii, copepods and cladocerans, samples were obtained with a 64 µm mesh size, 0.5 m diameter net. Dead animals, as indicated by aniline blue staining, were not included in final abundance estimates used to calculate grazing (Elliott et al. 2009; 2010). For rotifers, a 20 L depth-integrated sample was passed through a 20 µm mesh net and preserved in Lugol's until analysis. Clearance rates were obtained from the literature for the dominant species occurring in the James: rotifers = 0.38 ml ind<sup>-1</sup> d<sup>-1</sup> (Sierzen and Frost 1990), *Bosmina* = 1.63 ml ind<sup>-1</sup> d<sup>-1</sup> (Sierzen and Frost 1990), *Eurytemora* adults and copepodids = 9.67 ml ind<sup>-1</sup> d<sup>-1</sup> (Sierzen and Frost 1990), and copepod nauplii = 2.00 ml ind<sup>-1</sup> d<sup>-1</sup> (Bogdan and Gilbert 1984).

### ***Comparisons to Other Fluxes***

To place top-down effects in the broader context of factors regulating CHLa and N in the James, we compared losses due to grazing with other input and output fluxes. We compared CHLa production rates to losses via respiration, grazing and downstream export (advective loss). Over the course of a growing season, CHLa production by phytoplankton should balance losses due to respiration, grazing and advection, assuming no net change in the CHLa content of sediments. Determination of whether these independent estimates of production and loss are in balance provides a means for assessing our understanding of sources and fate of phytoplankton production in this system. For N, we compared DIN demand to support phytoplankton

production against external inputs (from the watershed and direct point source inputs) and internal re-generation via microbial- and consumer- mediated nutrient recycling.

CHLa production ( $\mu\text{g L}^{-1} \text{d}^{-1}$ ) was derived from measurements of daily NPP and an empirically-determined CHLa:C ratio for the James (see above). Phytoplankton DIN demand was similarly derived from NPP and the Redfield C:N ratio. Daily primary production was calculated from diel oxygen data measured at the VCU Rice Center (near JMS75) during March–November of 2012 and 2013. Use of a single-station diel method to derive metabolism estimates is potentially complicated by tidal influences on local  $\text{O}_2$  concentrations. After de-trending the diel data for a 24-h cycle we did not find that tidal intervals explained a significant fraction of the residual variation and therefore concluded that a single-station method was appropriate for this site. Methods of computation were previously described by Bukaveckas et al. (2011) and follow those used by Caffrey (2003, 2004) for National Estuarine Research Reserve sites. Dissolved  $\text{O}_2$  concentrations recorded at 15-min intervals were smoothed to 30-min averages for flux analyses. We derived daily NPP from the sum of daytime  $\text{O}_2$  fluxes, daily R from the sum of nighttime  $\text{O}_2$  fluxes extrapolated to 24 h, and daily GPP from NPP plus R occurring during daytime. Derivation of NPP and R from diel data requires correction for atmospheric exchange. Air–water  $\text{O}_2$  fluxes were calculated assuming that atmospheric exchange varied only in response to dissolved  $\text{O}_2$  saturation. This approach uses a fixed exchange coefficient which yields a potential range of  $-0.5$  to  $+0.5 \text{ g O}_2 \text{ m}^{-2} \text{ h}^{-1}$  for 0–200% saturation. We had previously compared these fixed estimates to those corrected for variable wind speed (Marino and Howarth 1993) and found that the latter yielded a similar range of exchange values ( $-0.3$  to  $+0.3 \text{ g O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) for wind speeds observed at this site.  $\text{O}_2$ -based NPP and R values were converted to C assuming a photosynthetic and respiratory quotient of 1.0.

Determination of respiratory losses of CHLa requires partitioning total respiration into fractions supported by autochthonous production and allochthonous inputs. We used the method of del Giorgio and Peters (1994) whereby the y-intercept for the regression of R vs. NPP is taken to represent the fraction of R that is supported by allochthonous inputs (i.e., R at NPP=0). The regression was based on pooled 2012 and 2013 data and yielded an estimate for allochthonous-R of  $2.0 \pm 0.2 \text{ mg O}_2 \text{ m}^{-2} \text{ d}^{-1}$  ( $R^2 = 0.51$ ,  $p < 0.0001$ ). When subtracted from total-R (mean =  $5.6 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$ ) this provided an estimate of autochthonous-R of  $3.6 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$ . This value was used along with the previously-referenced C:CHLa ratio to estimate daily loss of CHLa due to respiration. Internal re-generation of DIN via microbial decomposition was derived similarly except that we used the C:N content of autochthonous (Redfield = 6.6) and allochthonous (C:N = 15.1) of organic matter in combination with autochthonous-R and allochthonous-R. The allochthonous C:N ratio was determined from seston samples collected during periods of high discharge and low CHLa at stations JMS75, JMS69 and JMS56. We estimate that autochthonous contributions to these samples are less than 10% based on POC and C:CHLa.

Lastly, we considered export losses of CHLa via advection downstream and DIN inputs from external sources. DIN loads to the tidal fresh segment include riverine inputs at the Fall Line as well as local point source discharges; these were previously estimated to be  $0.125 \text{ mg DIN L}^{-1} \text{ d}^{-1}$  (Bukaveckas and Isenberg 2013). CHLa export losses were derived as the net difference between input and output fluxes. We used discharge data from USGS gauges on the James and Appomattox Rivers (#2037500 and #2041650, respectively) and measured CHLa concentrations at a station located above our study reach (JMS99) to derive input fluxes. We used the average CHLa concentrations of three stations located within our study reach (JMS75,

JMS69 and JMS56) in combination with the discharge data to estimate output losses. Concentrations were measured ~weekly at all stations during March-November of 2012 and 2013. We interpolated daily values for concentration based on regressions relating CHLa to discharge. Daily input and output fluxes were summed and converted to average daily volumetric values ( $\mu\text{g L}^{-1} \text{d}^{-1}$ ) that represent the net gain or loss of CHLa from the study reach.

## Results

### *Fish CHLa Consumption and N Cycling*

Our analysis of fish abundance and their diet shows that planktivorous fishes, particularly Threadfin Shad, were the dominant consumers of CHLa among fishes in the tidal fresh James (Figure 1). Threadfin Shad had a high CHLa content in their diet ( $175 \pm 21 \mu\text{g CHLa g DW}^{-1}$ ) as did other pelagic-feeding species (YOY Gizzard Shad =  $198 \pm 24 \mu\text{g CHLa g DW}^{-1}$ ; Atlantic Menhaden =  $77 \pm 12 \mu\text{g CHLa g DW}^{-1}$ ). By comparison, benthivorous fishes had low CHLa in their diet (adult Gizzard Shad =  $44 \pm 6 \mu\text{g CHLa g DW}^{-1}$ ; two size classes of juvenile Blue Catfish: 34 and  $45 \mu\text{g CHLa g DW}^{-1}$ ). Threadfin Shad were also the most abundant taxa accounting for almost half of total fish density. Threadfin Shad accounted for 65% of the total CHLa consumption by fish despite their small individual size ( $6.5 \text{ g ind}^{-1}$ ) and low population biomass ( $0.26 \text{ g m}^{-3}$ ). YOY Gizzard Shad were the second most important contributor accounting for 25% of fish CHLa consumption. The combined CHLa consumption by benthivorous fishes was less than 3% of the total, despite accounting for 90% of fish biomass. Though not important as consumers of CHLa, benthivorous fishes, particularly adult Gizzard Shad, accounted for a large proportion of PON ingestion (Figure 2). N content of diet materials was similar among all fishes (mean =  $36.2 \text{ mg PON g DW}^{-1}$ ; range = 31.1 to  $49.1 \text{ mg PON g DW}^{-1}$ ) and therefore relative contributions to N recycling were largely determined by per capita ingestion rates (a function of body size) and numerical abundance. The large body size of adult Gizzard Shad (mean =  $386 \text{ g ind}^{-1}$ ) yielded a proportionally large mass of gut contents and the highest per capita PON ingestion rate. For the range of gut turnover times considered (4 - 18  $\text{d}^{-1}$ ), we estimate that adult Gizzard Shad consume 330 to  $1,480 \mu\text{g PON ind}^{-1} \text{d}^{-1}$ . Adult Gizzard Shad were also the most abundant group by biomass ( $3.9 \text{ g m}^{-3}$ ) accounting for 60% of total fish biomass. This resulted in a population-scale estimate of PON consumption that was highest among the 6 taxa-size groups.

### *Grazing by Rangia and Zooplankton*

Rotifers and wedge clams (*Rangia*) were the dominant consumers of CHLa and PON among invertebrates (Figure 3). Rotifers (principally *Brachionus calyciflorus*) were important consumers despite their low per capita grazing rates ( $0.38 \text{ ml ind}^{-1} \text{d}^{-1}$ ) due to their high densities (mean =  $154,000 \pm 13,500 \text{ ind m}^{-3}$ ) which yielded consumption estimates of  $1.1 \mu\text{g CHLa L}^{-1} \text{d}^{-1}$  and  $20 \mu\text{g PON L}^{-1} \text{d}^{-1}$ . *Rangia* abundance was orders of magnitude lower (mean =  $10 \pm 2 \text{ ind m}^{-3}$ ) but their clearance rates were correspondingly higher ( $7,400 \text{ ml ind}^{-1} \text{d}^{-1}$ ) which resulted in similar consumption rates ( $1.4 \mu\text{g CHLa L}^{-1} \text{d}^{-1}$  and  $21 \mu\text{g PON L}^{-1} \text{d}^{-1}$ ) to rotifers. Other zooplankton (*Bosmina*, *Eurytemora*, copepod nauplii) did not contribute appreciably to CHLa or PON consumption. Combined rates of CHLa consumption for invertebrates ( $2.7 \mu\text{g L}^{-1} \text{d}^{-1}$ ) were higher than those for fish ( $0.36 \mu\text{g L}^{-1} \text{d}^{-1}$ ) even when maximal gut clearance rates were used for fish. The combined PON consumption by invertebrates ( $45 \mu\text{g L}^{-1} \text{d}^{-1}$ ) was also higher than that of fish ( $27 \mu\text{g L}^{-1} \text{d}^{-1}$ ).

### *Grazing in Relation to Other Sources and Sinks*

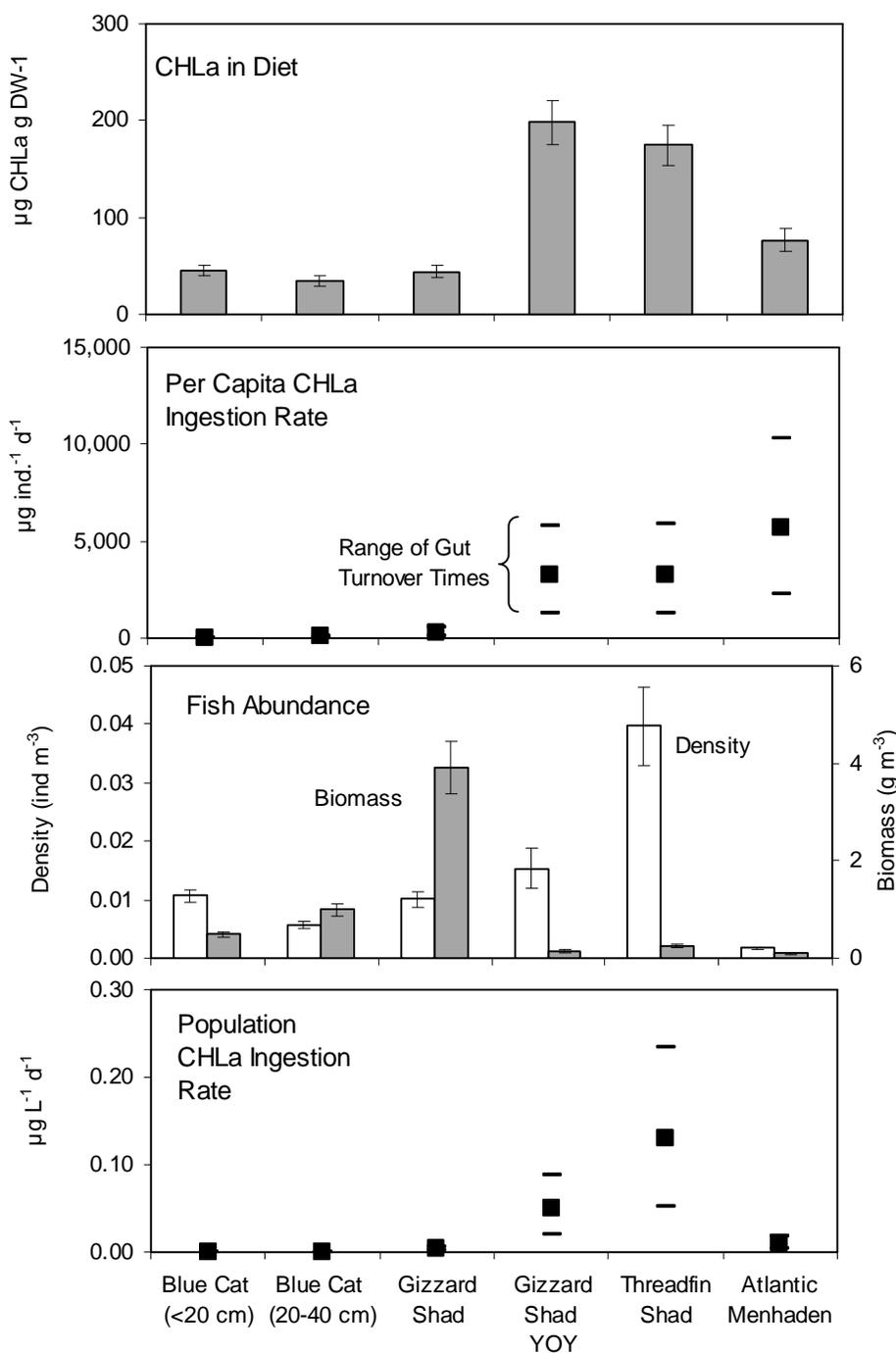
Daily average CHLa production in the James was  $20.1 \mu\text{g L}^{-1} \text{d}^{-1}$  during March–November of 2012 and 2013 (Figure 4). The dominant fate of this production was microbial decomposition with respiratory losses accounting for  $14.8 \mu\text{g L}^{-1} \text{d}^{-1}$  (74% of production). By comparison, losses due to the combined grazing of fish, zooplankton and *Rangia* were  $3.1 \mu\text{g L}^{-1} \text{d}^{-1}$  (15% of production). Export losses were also small ( $0.9 \mu\text{g L}^{-1} \text{d}^{-1}$ ) corresponding to 4% of production. The sum of all three losses accounted for 93% of the estimated CHLa production. For N, daily phytoplankton demand was  $290 \mu\text{g L}^{-1} \text{d}^{-1}$ . The combined PON ingestion rate for all grazers was  $72 \mu\text{g L}^{-1} \text{d}^{-1}$  suggesting that consumer-mediated recycling could contribute 25% of daily phytoplankton N demand (assuming all ingested PON was excreted in available form). By comparison, microbial-mediated N cycling ( $291 \mu\text{g L}^{-1} \text{d}^{-1}$ ) could potentially account for 100% of daily phytoplankton N demand. External inputs of DIN were previously estimated as  $125 \mu\text{g L}^{-1} \text{d}^{-1}$  (Bukaveckas and Isenberg 2013) and were equivalent to 43% of daily phytoplankton demand.

### *Conclusions*

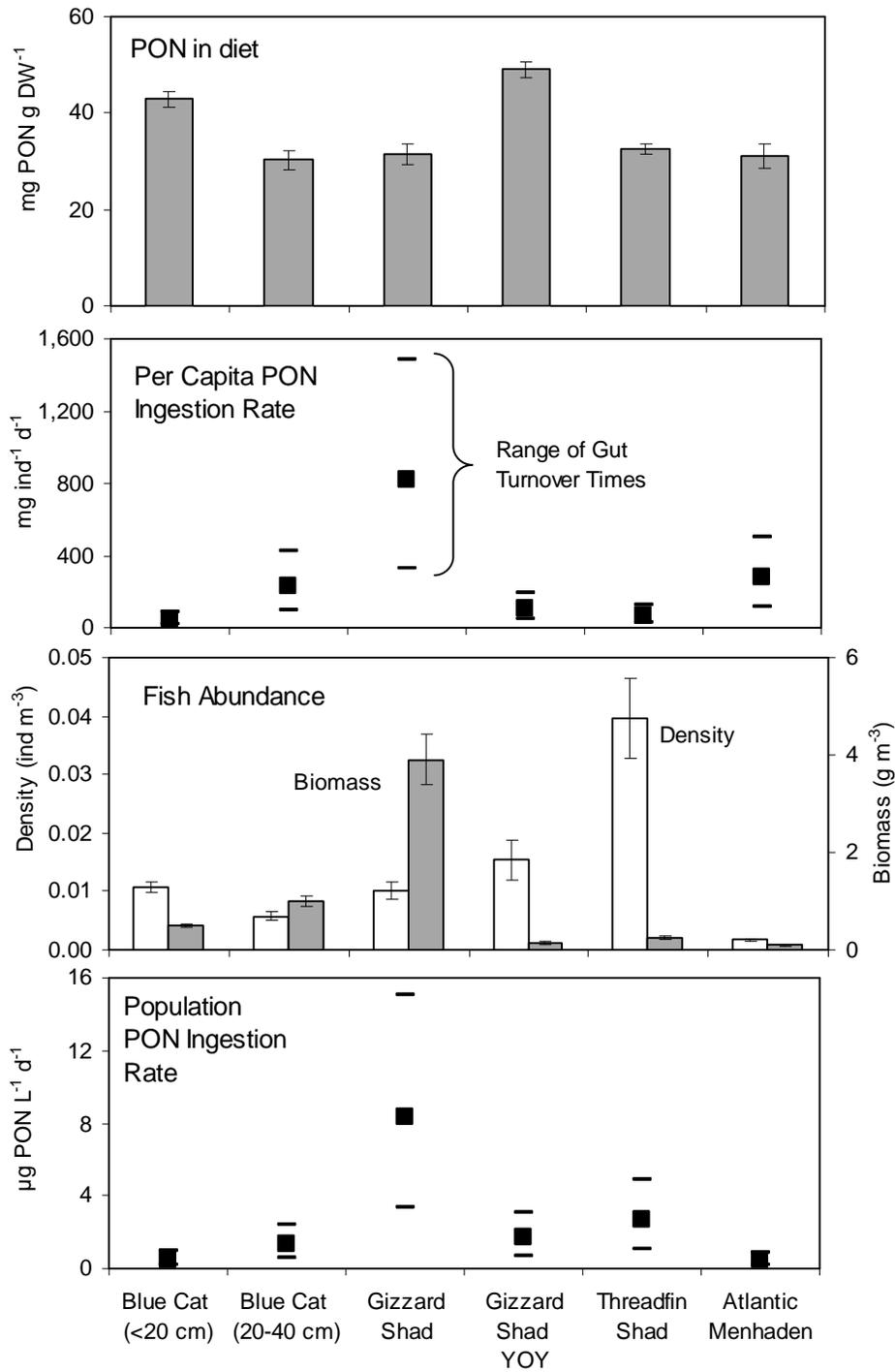
Our findings suggest that the direct effect of grazers as consumers of CHLa in the tidal fresh James are weak. The combined grazing rate by zooplankton, benthic filter-feeders and fish was small ( $3.1 \mu\text{g L}^{-1} \text{d}^{-1}$ ) corresponding to 15% of average daily CHLa production. Assigning error to this estimate of CHLa consumption is problematic given the difficulties in deriving propagated errors from multiple sources. For fish, potential sources of error include intra-specific variation in algal contributions to diet, variation in fish abundance and uncertainty regarding gut turnover rates. Our largest source of uncertainty was gut turnover time for which we lack empirical data specific to this study and therefore incorporated a large range (~4-fold) to assess the potential affect on CHLa consumption values. However, our results are robust in that CHLa ingestion by fish was low ( $0.36 \mu\text{g L}^{-1} \text{d}^{-1}$ ) even when a maximal turnover rate was used ( $18 \text{d}^{-1}$ ). Our data suggest that the other two sources of error were small. Intra-specific variation in algal contributions to diet was low as indicated by the small standard error (<20%) relative to the means derived from monthly values for each of the species. Similarly, standard errors for fish abundance estimates were within 20% of the mean derived for the three sampling periods. Our three fish sampling events were conducted over a period of 12 months and therefore may not be representative of longer-term variability in fish abundance that might be associated with year-to-year variation in recruitment or survivorship. There is little comparative data with which to assess the accuracy of our fish abundance estimates except that our estimate of Blue Catfish density ( $511 \text{ind. ha}^{-1}$ ) showed agreement with an estimate derived through mark-recapture methods ( $681 \text{ind. ha}^{-1}$ ; R. Greenlee, VDGIF, pers. comm.).

Grazers were more important for their role in recycling nitrogen as their estimated contribution ( $72 \mu\text{g L}^{-1} \text{d}^{-1}$ ) was large in comparison to external inputs of DIN ( $125 \mu\text{g L}^{-1} \text{d}^{-1}$ ). Consumer-mediated N cycling could supply 25% of daily phytoplankton N demand. Their greater contribution to N recycling vs. direct consumption of CHLa stems from the fact that phytoplankton account for a relatively small proportion of particulate matter in the James. Non-algal materials have low CHLa content but are relatively rich in N; therefore grazers ingesting a mixed diet will consume more N relative to CHLa. This was most apparent in benthivorous fishes which accounted for a greater proportion of PON ingestion (37%) than for CHLa (11%). Benthivorous fishes are important in the James (90% of biomass) and their detritus-based diet was low in CHLa but comparable to planktivores in N content. Thus our findings suggest that

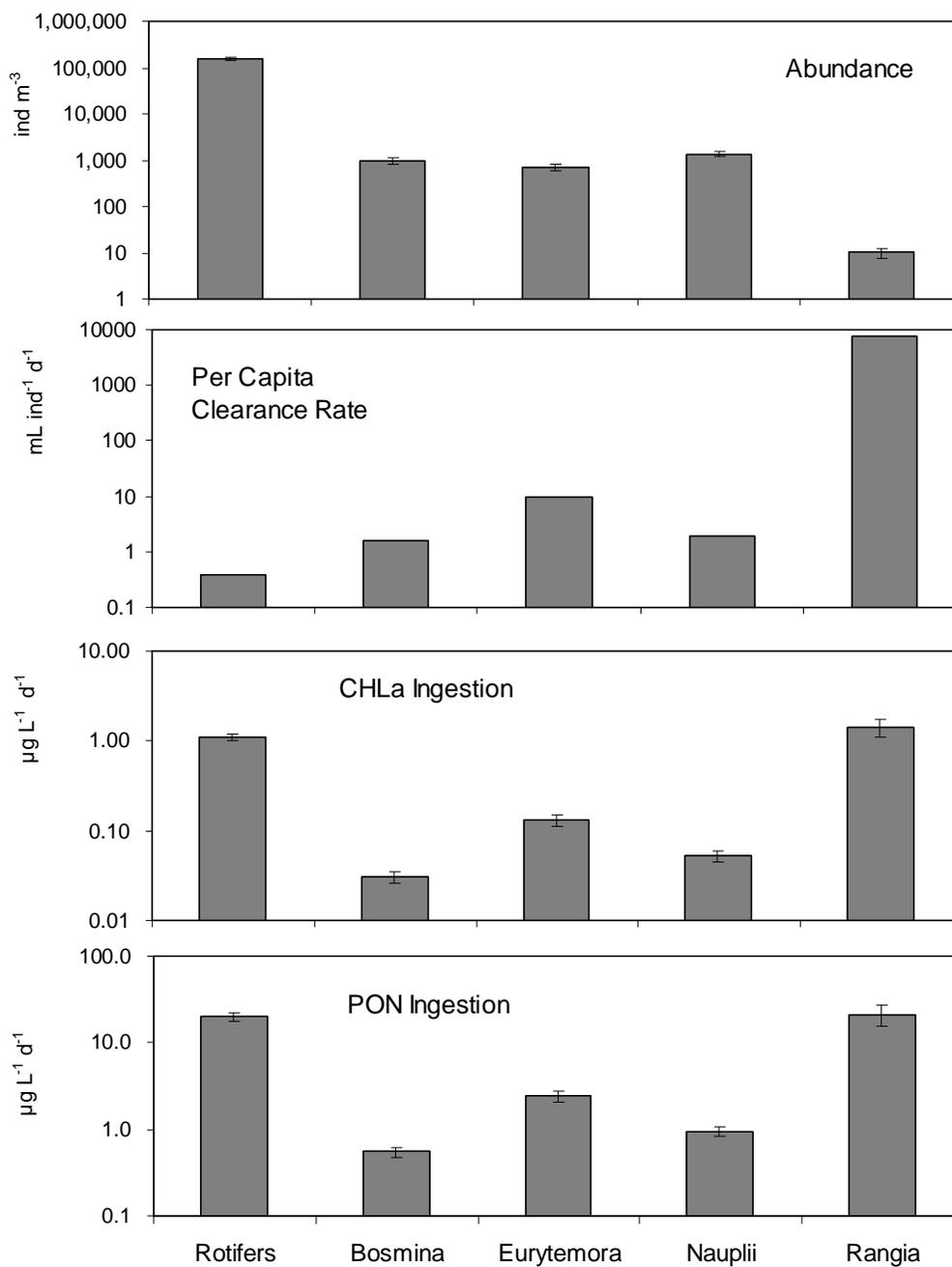
top-down effects promote eutrophic conditions in the James because consumers play a more important role in recycling of N than in removing CHLa through grazing. The dominant fate of algae production in the tidal fresh segment of the James was microbial decomposition with a small fraction entering the grazer food chain and a smaller fraction lost through downstream export.



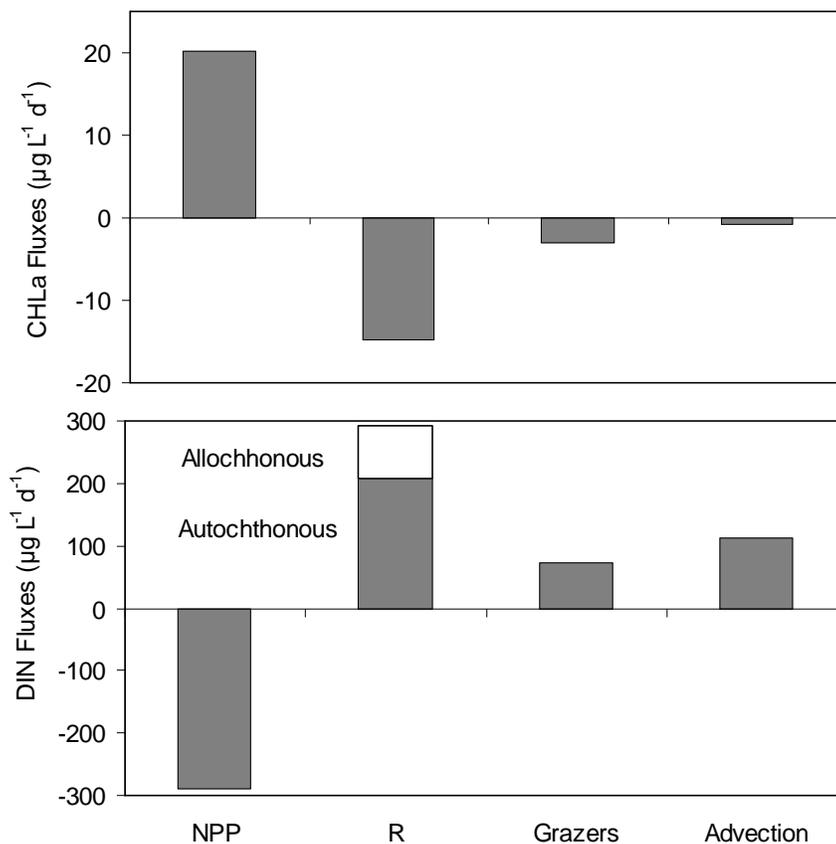
**Figure 1.** Fish as consumers of CHLa in the tidal freshwater James River. Data shown are (top) CHLa in fish gut contents, (2<sup>nd</sup>) per capita CHLa ingestion for a range of gut turnover rates, (3<sup>rd</sup>) fish biomass and density and (bottom) population-scale estimates of CHLa ingestion.



**Figure 2.** Fish as recyclers of N in the tidal freshwater James River. Data shown are (top) PON in fish gut contents, (2<sup>nd</sup>) per capita PON ingestion for a range of potential gut turnover rates, (3<sup>rd</sup>) fish abundance and (bottom) population-scale estimates of PON ingestion.



**Figure 3.** Abundance, per capita clearance rates and CHLa and PON ingestion by dominant zooplankton and benthic filter-feeders (*Rangia*) of the tidal freshwater James River. Zooplankton abundances are based on bi-monthly collections during March-September 2013. *Rangia* abundance is the average for 2001-2010 based on annual surveys by CBP.



**Figure 4.** (Top) CHLa production by phytoplankton (NPP) compared to losses via respiration (R), grazing and advection (export). (Bottom) Phytoplankton demand for DIN compared to inputs via respiration (bacterial re-mineralization), consumer-mediated N cycling (grazing), and advection (external inputs). Data shown are average daily values for March-November of 2012 and 2013. Grazing losses include contributions by zooplankton, benthic filter-feeders and fish.

## Part Two: Effects of Microcystin on Aquatic Living Resources of the James

### *Introduction*

This work addresses Subtask 2.1 of the DEQ Science Advisory Panel Workplan which focuses on the effects of the cyanotoxin Microcystin (MC) on aquatic living resources of the tidal freshwater James River. MC is a hepatotoxin whose presence in waterbodies has raised concerns for human exposure through drinking water, recreational contact and fish consumption, as well as toxic effects on living resources (Ueno et al. 1996, Chorus and Bartram 1999, Best et al. 2001, Bláha et al. 2004, Malbrouck and Kestemont 2006, Ibelings and Havens 2008). Human exposure to Microcystin raises concerns regarding impairment of designated uses such as swimability and fishability and therefore requires an assessment of the magnitude and duration of HAB events and toxin propagation in food webs (Tango and Butler 2008, Davis et al. 2010, Davis and Gobler 2011). For living resources, toxicity is thought to occur primarily through dietary consumption but little is known about the factors which contribute to variable exposure and toxin accumulation (Kozlowsky-Suzuki et al. 2012). In the James River, exposure to Microcystin may also pose a threat to the federally endangered Atlantic sturgeon, which uses tidal freshwater reaches as spawning and nursery habitats.

The tidal fresh segment of the James River Estuary shares a number of features in common with systems where cyanotoxins have been found including large anthropogenic nutrient loads, shallow depths, and high CHLa concentrations (Bukaveckas et al. 2011; Bukaveckas and Isenberg 2013; Wood and Bukaveckas 2014). As part of a broader effort to characterize harmful algal blooms in the James, we undertook a study in 2012 to monitor the occurrence of Microcystin in water, sediments and biota in the tidal freshwater segment. Results from this study were the first comprehensive assessment of Microcystin in the James. Microcystin was consistently found in the water column (99% of samples tested). The toxin was already present in May when sampling was initiated and persisted through late November. Microcystin concentrations in the James were similar to those reported in other systems where elevated tissue concentrations were observed in fish and macroinvertebrates (e.g., Wilson et al. 2008; Garcia et al. 2010). We found widespread occurrence of toxin contamination in all species of fish and shellfish sampled from the James. Peak occurrence of toxin contamination (~80% of individuals) was observed in months with highest Microcystin in the water column (July-September). Microcystin was measurable in liver tissues even in May (e.g., 71% of 20-40 cm blue catfish and 100% of blue crabs). These findings suggest that health effects associated with the toxin may occur outside of bloom periods when the toxin is produced. An important and novel finding from our study was that dietary exposure to Microcystin within food webs was linked to feeding habits. We found that pelagic-feeding, planktivorous fishes had higher levels of CHLa in their gut contents as well as higher concentrations of Microcystin in their tissues compared to benthic-feeding detritivores. This finding suggests that at-risk species for toxic effects include Atlantic Menhaden, juvenile Gizzard Shad, Threadfin Shad and anadromous shad and herring (*Alosa*) species. We also found that clearance rates by the dominant benthic suspension-feeder (*Rangia*) declined in summer coinciding with elevated levels of Microcystin in the water column. These findings suggest a potential negative feedback of harmful algal blooms via suppression of consumer grazing.

In summary, the 2012 assessment of Microcystin in the tidal fresh James revealed widespread occurrence of the toxin in water and living resources. The presence of the toxin was

directly related to the magnitude and duration of algal blooms as evidenced by elevated Microcystin and CHLa concentrations in the region of JMS56-75 during July-September. A key unanswered question is: What are the health effects on living resources associated with toxin exposure? To address this question, we performed a series of experiments to measure lethal and sub-lethal effects on native living resources, including economically and ecologically important fishes. Our objective was to determine acute and chronic effect thresholds for toxin concentrations that can be related to CHLa levels and thereby used to assess current regulatory standards for the James River. We conducted experiments to establish Microcystin dose-response relationships for selected species native to the tidal freshwater James River. Test species include zooplankton (*Bosmina*), suspension-feeding bivalves (*Rangia*), anadromous fish (juvenile Blueback Herring) and the endangered Atlantic Sturgeon. These species are primary consumers (excluding sturgeon) and therefore are exposed to Microcystin in the water as well as dietary ingestion. Working with a suite of species allows for a more robust assessment of toxin effects on the food web and a wide range of response parameters including survivorship (all species), growth (zooplankton, herring), reproduction (zooplankton) and grazing (*Rangia*). Experiments involving *Rangia* and Blueback Herring have been completed; experiments with Atlantic Sturgeon are in progress. *Bosmina* experiments involving exposure to dissolved MC have been completed; dietary experiments are in progress.

## Methods

### ***Effects of Microcystin on Zooplankton Feeding, Survivorship and Reproduction***

Zooplankton play an important role in estuarine food-webs by transferring energy from primary producers to higher trophic levels (Wong et al. 2003). Prior work has demonstrated that toxin-producing strains of *Microcystis* cause mortality of zooplankton (DeMott et al. 1991; Ger et al. 2010) as well as sub-lethal effects which include reductions in longevity and reproductive output (Lampert 1981; Hanazato and Yasuno 1984; Ferrao-Filho and Azevedo 2003). This results in a negative feedback mechanism whereby the toxin suppresses grazing and exacerbates bloom conditions. In addition, zooplankton are among the few organisms known to bioaccumulate Microcystin thereby transferring the toxin to higher trophic levels (Kozlowsky-Suzuki et al. 2012). While there is a large body of literature on dietary effects of cyanobacteria on zooplankton, less is known regarding the specific effects of Microcystin. Further, much of this work has focused on zooplankton common to lake environments, such as *Daphnia*. We studied the effects of Microcystin on the life history characteristics of one of the common zooplankton in the tidal freshwater James River, the cladoceran *Bosmina longirostris*. This taxa was selected based on prior work characterizing zooplankton communities in the tidal freshwater segment (Park and Marshall 2000; Bukaveckas et al. 2011) and concurrent sampling of zooplankton conducted as part of the study of ‘top-down’ effects (see Part One). Lethal and sub-lethal effects were assessed by monitoring feeding, survival and fecundity of animals in laboratory incubations. Exposure to microcystin was performed in a dosage-dependent manner to facilitate detecting thresholds for deleterious effects. In addition to testing for the direct effects of MC dissolved in the water, we are developing techniques for conducting dietary exposure experiments.

*Bosmina* used in these experiments were collected from the tidal freshwater James River (at VCU Rice Center) and used to establish laboratory cultures. Cultures were maintained under

controlled environmental conditions and fed a diet of *Chlamydomonas reinhardtii*. Newly hatched juveniles were used for experiments. To evaluate the effects of dissolved Microcystin we incubated *Bosmina* in water treated with 0.0, 0.4, 0.8, and 1.6  $\mu\text{g L}^{-1}$  dissolved Microcystin (as Microcystin-LR, available from Abraxis). Toxin concentrations in the James reached 1.2 and 4.5  $\mu\text{g L}^{-1}$  (2011 and 2012, respectively). Note that these are total concentrations (inclusive of particulate-bound MC); the dissolved fraction is expected to be <30% of the total. Thus, our experimental treatments encompassed the range observed in the James. Experiments were performed in January (effects on survivorship and fecundity) and March (effects on feeding) 2014. All experiments were conducted in a Conviron growth chamber set to 20° C and a 14 h light: 10 h dark cycle. For the survivorship and fecundity experiment, animals were incubated in 75 mL tissue culture flask (3 ind per flask) with 0.45  $\mu\text{m}$  filtered James River water and appropriate food. To ensure consistent dissolved MC and food concentrations throughout the duration of the experiment, individuals were transferred into containers with fresh water and algae every 1-3 d. Development time from birth to reproductive maturity (first brood) was determined from daily monitoring under a dissecting microscope. Survival, as total lifespan (longevity, days), was also monitored daily. In addition to quantification of development and survival, we measured fecundity as the number of offspring produced by each female during the experiment. Experiment measuring MC effects on feeding were performed in 75 ml culture flasks containing 10-15 individuals each. CHLa concentrations were measured at the start and end of the 48-h grazing period to determine per capita clearance rates. Three replicates were used for each of the treatments (0.0, 0.4, 0.8, 1.6 and 3.2  $\mu\text{g MC L}^{-1}$ ). All experimental procedures and measurements comply with accepted and best practice methodology according to the ICES Zooplankton Methodology Manual (Harris et al. 2000).

### ***Effects of Microcystin on Rangia Grazing Rates***

In the tidal fresh James, the wedge clam (*Rangia cuneata*) is the dominant benthic suspension feeder accounting for >80% of benthic secondary production based on CBP surveys. Our prior work has documented reductions in *Rangia* clearance rates during periods when elevated Microcystin concentrations were observed. We conducted controlled experiments to measure *Rangia* clearance rates in response to Microcystin dissolved in water and ingested in particulate matter.

We tested the effects of dissolved MC on *Rangia* by measuring clearance rates at varying levels of the toxin. *Rangia* were collected from the James (near JMS75) and acclimated to laboratory conditions for 12 h. Individual clams were placed in 3 L mesocosms containing water from the James and held at in situ (river) temperature at the VCU Aquatics Facility. The length of each individual was measured to determine biomass from a previously-developed (2012) length-weight regression. Mesocosms were treated with dissolved Microcystin (Microcystin-LR, Abraxis) at concentrations of 0.0, 0.4, 0.8, 1.6 and 3.2  $\mu\text{g L}^{-1}$  (similar to zooplankton experiments). Aqueous dissolved microcystin was added at the beginning of the incubation and water samples collected at the end of the 4-h exposure showed that MC was within 10% of intended concentrations. Each dosage was performed in triplicate and the experiment was replicated twice (May and June).

Assessing the effects of dietary exposure to Microcystin is complicated by confounding factors which influence food quality such as the proportions of algal and non-algal particulate

matter and the relative contributions by algal groups which differ in their nutritional properties (e.g, greens, diatoms, cyanobacteria). We assessed dietary effects of MC by comparing *Rangia* grazing rates on seston from two sources: the James River, where we have documented elevated MC in summer, and the Pamunkey River, where MC concentrations are expected to be low (Sin et al. 1999; Marshall 2009; Reay 2009). The Pamunkey site was selected on the basis of having similar salinity as the James. Water was collected on the day of the experiment from sampling locations in the tidal freshwater segment (Pamunkey TF 4.2, James at VCU Rice Pier). As prior work has shown that bivalve clearance rates are influenced by food concentrations, we used a 50% dilution (with filtered river water) of water obtained from the James, in order to attain CHLa levels more similar to those observed in the Pamunkey. For each experiment, twelve 3 L mesocosms were established with three replicates each for water from the two sources, and with/without clams. The dietary experiment was replicated four times (April, May, June and September) to compare grazing rates on the two food sources before and after the expected rise in MC in the James. We hypothesize that grazing rates of James River clams fed James River or Pamunkey River seston would be the same when MC concentrations were low at both sites, but that grazing rates on James seston would decline relative to the Pamunkey during periods with elevated MC concentrations in the James.

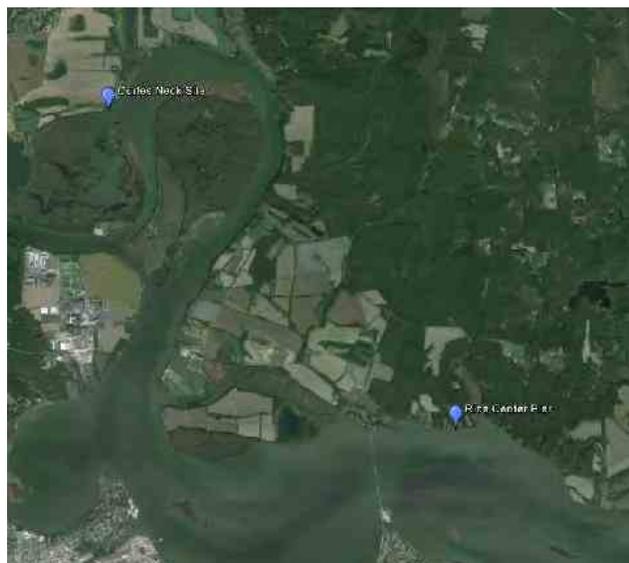
Our response variable for both the dissolved and dietary exposure experiments was clearance rate, which we have previously measured for *Rangia* from the James. Our method for measuring clearance rates was the same as used previously (described in last year's report) and follows Wong et al. (2003). The clearance rate is derived from changes in CHLa (at 0, 2 and 4 h) in mesocosms with and without clams. In the presence of clams, CHLa declines faster such that differences in the slopes of regression lines (concentration vs. time) between mesocosms with and without clams can be used to estimate the clearance rate. This rate is a theoretical value representing the volume of water cleared of particulate material based on the mass removed by consumers and average concentration in the water (Coughlan et al. 1969). Mesocosms were kept under low light conditions (to minimize phytoplankton growth during the experiment) and equipped with a circulating pump to maintain particulates in suspension. CHLa was measured by the same methods as for the weekly monitoring (see Part 3).

### ***Effects of Microcystin on Fish Survivorship and Toxin Accumulation***

Our studies of the effects of Microcystin on fish included three components. First, we documented acute effects of MC on early life stages of native and ecologically important species in laboratory experiments using wild-collected eggs and larvae of American Shad (*Alosa sapidissima*) and Blueback Herring (*Alosa aestivalis*). These species are anadromous clupeid fishes that spawn in the tidal James River and reside in-system through much of their first year. VCU collaborated with USFWS personnel (Michael Odom, Manager) at the Harrison Lake National Fish Hatchery (HLNFH) to develop methods for the culture and rearing of larvae from local, wild stock. Early life stages are hypothesized to be the most susceptible to acute effects of dissolved Microcystin because eggs and larvae are relatively permeable to water and may, therefore, accumulate higher relative burdens from extracellular sources (Sotton et al. 2012). Secondly, we performed sentinel experiments using juvenile Gizzard Shad obtained from HLNFH to determine rates of MC accumulation in fish tissues. Juvenile Gizzard Shad are planktivorous and are therefore exposed to Microcystin through dietary sources. Thirdly, we evaluated the effect of dietary ingestion of Microcystin on juvenile Atlantic Sturgeon. Atlantic

Sturgeon are a federally endangered, anadromous species that resides in the upper tidal James River for the first several years of life and then returns to the river as an adult to spawn. We conducted Microcystin exposure studies on captive early juveniles from Canadian sources at VCU's Aquatics Facility. The investigators hold all necessary university IACUC permits for the experimental procedures described below. VCU holds all necessary federal and state permits for collecting and holding the species listed above.

We exposed eggs and pre-larvae of American Shad and Blueback Herring to known concentrations of commercially available Microcystin (Microcystin-LR, Abraxis). The experiments were performed at HLNFB using fertilized eggs incubated in fabricated tanks dosed with MC concentrations of 0.0, 0.4, 0.8, 1.6 and 3.2  $\mu\text{g L}^{-1}$  (as per zooplankton and *Rangia* experiments). Following *in vitro* fertilization, eggs were rested for 24 h. This lag time allowed embryos to develop so that viable eggs could be identified visually (20X magnification) and selected for experiments. Egg and larval exposures were accomplished within a closed circulation-filtration system that was modified from a design developed by HLNFB personnel and tested by VCU. The egg containment capsules were comprised of PVC and 200  $\mu\text{m}$  Nitex screen. The combination of these elements provided a chamber (51 mm diameter) in which eggs could be held and later recovered. Five egg containment capsules were placed in each of two 20 L enclosures that were used for each treatment. Ten eggs were placed into each containment capsule such that 100 eggs were used for each treatment including the Control. After 48 or 72 h, eggs were sorted for viability and counted. One experiment was performed with eggs from American Shad (May 13-15) and three experiments were performed with eggs of Blueback Herring (April 18-20, May 17-20, and May 22-25). Experiments conducted on cohorts of pre-larvae (prior to yolk sac absorption) followed the same design with 50 larvae used in each of two replicates for all treatments. Because only yolk-sac larvae were used in exposure studies, feeding prior to or during experimental trials was not necessary. One experiment was performed with larvae of American Shad (May 7-9) and three experiments were performed with larvae of Blueback Herring (April 22-24, May 20-22, and May 25-28). Larval survivorship was determined after 48 h. Water used for the larval exposure experiment performed with American Shad was tested at the conclusion of the experiment and MC concentrations were found to be within 20% of expected values. All experiments (eggs and larvae) were performed at 17° C.



We conducted sentinel (*in-situ*) experiments using field enclosures deployed at two locations in the tidal fresh segment of the James. *In-situ* enclosures have long been used in fish exposure studies, including the effects of *Microcystis* blooms on fish (Dejenie et al. 2009). Such enclosures allow experimental fish to feed normally on plankton that pass through the enclosure, while allowing the researcher to control exposure duration and location. In this way, fish are exposed to Microcystin in water and food for known periods. Fish used in these studies (YOY Gizzard Shad) were sourced from nearby pond stocks (HLNFB; mean length = 108 mm, mean weight = 12.8 g). A subset of these (N

= 20) were analyzed prior to the experiment and found to contain no measureable MC in liver tissues. The two-week experiment was conducted during the period when elevated Microcystin concentrations are typically observed (August 9 to 23 2013). Circular enclosures (~0.78 m<sup>3</sup>) constructed of Nitex mesh (500 µm) and PVC pipe were deployed at two sites (Rice Pier and Curles Neck; see photo). The VCU Rice Pier is located near JMS75 where chronic algal blooms and elevated Microcystin concentrations are observed during July-September. The Curles Neck site is located off-channel in an upper constricted segment of the James. VCU maintains continuous water quality monitoring at the Rice Pier (water temperature, dissolved oxygen, CHLa and phycocyanin) and conducts weekly sampling for extracted CHLa and Microcystin at sites proximal to the sentinel locations (Buoy 107/JMS75 and Buoy 127). Fifteen Gizzard Shad were placed in each of two replicate enclosures at both sites. Following a 2-week exposure, fish were removed and analyzed for MC concentrations in their liver tissues. Methods for tissue MC analysis were described in our previous report.

Because of their protected status, it is not possible to directly evaluate MC tissue concentrations and possible effects on James River Atlantic Sturgeon (ATS). However, access to Canadian-source ATS (no protected status) held at VCU's Aquatics Facility provided an opportunity to test hypotheses concerning ATS and MC exposure under laboratory conditions. The goal of this laboratory study was to emulate—to the extent possible—likely dietary consumption of MC by wild, early juvenile (age-0) ATS under natural riverine conditions, based on known toxin concentrations in putative James River prey, such as *Rangia* clams (Wood et al. 2014). The specific objectives of this study were: (1) expose captive ATF to Microcystin-LR in formulated diets, and (2) measure tissue (liver or/and muscle) concentrations of MC across a range of dietary exposures. Given the moderate concentrations of MC proposed for ATS laboratory diets (modeled on MC levels in likely James River sturgeon prey), and the relatively short MC exposure periods possible in the lab, lethal effects in this study were considered unlikely. Hence, a third study objective evaluated possible sub-lethal effects of MC consumption for captive ATS under controlled conditions. Specifically, we hypothesized a positive dose-response relationship between cumulative MC ingestion and MC tissue concentrations, as well as an increase in sub-lethal effects in ATS with higher levels of MC ingestion. Uptake of cyanobacterial toxins by freshwater fish occurs primarily through dietary pathways, rather than across gill epithelia or via ingestion of water (Ernst et al. 2006), hence this study focused on dietary uptake.

Diet Formulation. Pure microcystin-LR was purchased as a dry powder from a commercial source (Abraxis BioScience) and incorporated into a standardized diet for captive ATS. MC powder (0.5 mg) was added to 125 mL of deionized water to yield a 4 µg/mL solution of MC, which was stored at 4°C. Feeding methods used in this study were modified from Hirschfeld, et al. (1970) to allow incorporation of chemical toxins with a semi-solid food matrix. Pellet formulation minimized fragmentation of pellets and MC leaching in closed-system tanks during fish feeding bouts. Four separate diets were prepared corresponding to the following treatment groups: 0.00 (control), 0.025, 0.050, 0.100 µg of MC/g wet weight of food (Table 1). The range in MC treatment concentration is based on MC tissue concentrations in James River benthic invertebrates, including *Rangia* clams (Wood, et al. 2014), which are presumptive prey for wild juvenile Atlantic Sturgeon in the James River. The diets were prepared by thoroughly mixing 250 g of fish feed (Melick Aquafeed sinking No. 1 pellets, Melick Aquafeed, Catawissa PA, USA), 20 g granulated agar, 14 g unflavored gelatin, and MC solution (0 mL, 8 mL, 16 mL or 32 mL, depending on treatment group) to 1000 mL of deionized water heated to 100°C. The

mixture was allowed to cool for 24 h in a flat pan at 4°C and then cut into pellets of a size (1-3 mm<sup>2</sup>) appropriate for ingestion by age-0 ATS. Prepared diets were placed in labeled containers and stored at -20°C for less than 1 month. New batches of experimental pellets were made as necessary during the experiment. Expected concentrations of MC in each dietary treatment were confirmed by ADDA ELISA analysis (Abraxis; Warminster PA, USA) of a sample of pellets (Wood, et al. 2014).

Experimental Animals. Approximately 200 age-0 (40-100 mm FL) Atlantic Sturgeon from a Canadian wild source (St. Lawrence River) were secured under an agreement between VCU and the Maryland Department of Natural Resources, transferred to VCU's Aquatics Facility (1000 W. Cary Street, Richmond VA), and acclimated in freshwater (< 0.5 ppt) at 16°C to an artificial diet with no MC (see above) for at least 1 month prior to the start of the experiment. Care was taken to avoid contamination of sturgeon holding tanks or related equipment with MC. Fish were held in the VCU Aquatics Facility (1000 W. Cary Street, Richmond, Virginia) and used under conditions established by VCU's IACUC committee (protocol AD20127). Superficial fungal infections (suspected *Saprolegnia*) affected a substantial number of ATS after MC exposure trials began. In spite of appropriate treatment, these infections persisted throughout the study, with unknown implications for study conclusions.

Leaching Experiment. A pellet leaching experiment was conducted prior to the exposure study to test the assumption that the loss of MC from pellets during feeding bouts is nil. We placed 5 g of pellets (0.5 µg of MC/g) in 1.0 L of deionized water and removed aliquots (20 ml) of water at regular intervals for a period of 12 h after the food was first submerged. The solution was stirred gently before each sample was taken. Samples were held at 4°C and analyzed for MC (methods described in Wood, et al. 2014) within 24 h. There was no detectable leaching of MC from formulated pellets during soak periods of up to 6 h and this period was greater than fish feeding bouts. Hence, our calculated estimate of MC consumption from pellet diets during the experiment was considered to be accurate and represented the exposure pathway during the experiment.

MC Exposure Experiment. A single experimental unit consisted of a clean, 38-liter glass tank (closed system) with a single age-0 ATS; water was sourced from the VCU Aquatics Lab supply (treated James River water). Treatment groups were fed pellets containing 0.00 (control), 0.025, 0.050, or 0.100 µg MC/g wet weight of food daily for the duration of the trial. All other conditions (e.g. water quality, temperature, photoperiod) were consistent among treatments. Each treatment group consisted of 7 replicates, for a total of n=28 experimental units. Mass (to nearest 0.01 g; blotted wet weight) and fork length (mm) of each fish was measured and recorded during transfer from the stock tank to experimental tanks prior to the initial experiment feeding. Water changes of 50% were completed every 2 d, or as needed, for the duration of the experiment and solid waste was siphoned from the tanks daily. All fish (experimental and control) were fed a daily ration of approximately 3% body weight, based on the mean weight of all fish in the initial trial group, once per day with the appropriate treatment diet. This daily ration is presumed to be a maintenance (zero net growth) ration for juvenile sturgeon (Albert Spells, USFWS, Charles City VA, personal communication) held at the temperature used for this experiment. Preliminary feedings using the MC-free (control) diet demonstrated that age-0 Atlantic Sturgeon consumed a 3% daily ration of pellets within a 30-min feeding bout, after which uneaten pellets were removed from tanks and recorded.

Tanks were checked daily and any dead or moribund fish were removed and stored at  $-80^{\circ}\text{C}$ . Losses were replaced by new fish from the stock tank after the experimental tank was thoroughly cleaned with a 10% Clorox solution and rinsed. Not all fish completed the entire 28-d trial period. Exposure period (d) and daily consumption of pellets were recorded for each fish; these data were used to calculate total (cumulative) MC consumption ( $\mu\text{g}$ ) for each fish in a non-control treatment during its deployment period. The feeding experiment proceeded for consecutive 28 days, after which all remaining fish were euthanized, evaluated, and stored at  $-80^{\circ}\text{C}$ . Samples of liver and muscle tissue were removed from each fish used in the experiment and MC concentrations were analyzed using the methods described in Wood, et al. (2014). All tissue samples were run in duplicate and MC concentration expressed as the arithmetic mean of the two values. Microcystin values below the analytical detection limit were assigned a concentration of zero. All data were entered into an EXCEL spreadsheet and QA'd using a double-entry method prior to analysis. As MC concentrations in fish muscle tissue are typically a small fraction of liver values (Wood et al. 2014), only liver tissues were analyzed for MC; muscle tissue samples remain archived at VCU.

In order to evaluate possible sub-lethal effects from MC ingestion, a Stress Index (SI) was generated as the absolute value of individual somatic relative growth,  $W_2 - W_1 / W_1$ , where  $W_1$  is fish mass at the start of the trial and  $W_2$  is fish mass at the end of the trial, expressed as a daily rate (percentage). As all fish (dosed and control) were fed the same daily ration but lost somatic mass (i.e., negative growth) during the trial, this calculated SI should be a valid indicator of relative physiological stress among the treatment and control groups. Higher SI values correspond with greater loss of relative mass, and presumably greater physiological stress, during the MC feeding experiment. Fish somatic growth and condition indices are widely used to evaluate a range of stressors, including environmental contaminants and toxins (Brooks et al. 2012; Schindler et al. 2000).

## Results

### *Effects of Microcystin on Zooplankton Feeding, Survivorship and Reproduction*

Exposure to dissolved MC had no effect on development time, survivorship or longevity of *Bosmina* (Figure 5). Fecundity was lower at MC concentrations of  $0.8 \mu\text{g L}^{-1}$  ( $0.25 \pm 0.05$  neonates  $\text{ind}^{-1} \text{d}^{-1}$ ) and  $1.6 \mu\text{g L}^{-1}$  ( $0.22 \pm 0.02$  neonates  $\text{ind}^{-1} \text{d}^{-1}$ ) relative to the Control ( $0.47 \pm 0.11$  neonates  $\text{ind}^{-1} \text{d}^{-1}$ ) and  $0.4 \mu\text{g MC L}^{-1}$  treatments ( $0.54 \pm 0.18$  neonates  $\text{ind}^{-1} \text{d}^{-1}$ ). However, statistical analysis of these data (one-way ANOVA) did not reveal significant differences among the treatment groups ( $p = 0.21$ ). Similarly we did not observe effects of dissolved Microcystin on the feeding rates of *Bosmina* over the range of concentrations tested (Figure 6).

### *Effects of Microcystin on Rangia Grazing Rates*

Exposing Wedge Clams to dissolved MC resulted in a reduction in their grazing rates (Figure 7). The two replicate experiments performed in May and June yielded similar results and showed a curvilinear reduction in clearance rates with increasing MC concentrations. To compare results from the two experiments, we derived a parameter 'CR<sub>50</sub>' - the MC concentration at which clearance rates were reduced by 50%. For the May experiment, the curvilinear model provided strong fit to the data ( $R^2 = 0.94$ ) and yielded a CR<sub>50</sub> value of  $0.40 \mu\text{g}$

MC L<sup>-1</sup>. In June, the dose-response curve was somewhat less consistent ( $R^2 = 0.61$ ) and yielded a higher CR<sub>50</sub> value (1.15 µg MC L<sup>-1</sup>). Dietary experiments showed that in the absence of MC, clearance rates were higher among clams fed James River seston (April) or not significantly different between James and Pamunkey seston (May and June). During the September experiment, MC concentrations were higher in the James (0.20 µg L<sup>-1</sup>) relative to the Pamunkey (0.05 µg MC L<sup>-1</sup>). When elevated MC was present in the James (September), clearance rates were lower for clams fed James River seston (Figure 8). During the September experiment, CHLa concentrations were also higher in the James (36 µg L<sup>-1</sup>) relative to the Pamunkey (6.7 µg L<sup>-1</sup>) suggesting that the difference in clearance rates could be due to higher food availability in the James. However, higher CHLa concentrations in the James were also observed in the preceding two experiments (May and June) when no significant difference in clearance rates was detected.

### ***Effects of Microcystin on Fish Survivorship and Toxin Accumulation***

Exposure to dissolved MC had no measureable effect on the viability of eggs and larvae of American Shad and Blueback Herring (Table 1). The proportion of viable eggs and larvae was consistently high (mean = 97%) across all treatments at dosages up to 3.2 µg MC L<sup>-1</sup>. Exposure of YOY Gizzard Shad during sentinel experiments resulted in rapid accumulation of MC in fish tissues (Figure 9). By the end of the two-week exposure, MC in liver tissues of sentinel fish (mean = 0.30 ± 0.05 µg g<sup>-1</sup> dm) was higher than that of wild-caught fish from the James in both 2012 (mean = 0.10 ± 0.03 µg g<sup>-1</sup> dm) and 2013 (mean = 0.09 ± 0.02 µg g<sup>-1</sup> dm). The average MC concentration during the two-week experiment was 0.33 µg L<sup>-1</sup> (range = 0.24 to 0.41 µg L<sup>-1</sup>).

A single, 28-d MC exposure experiment was conducted with Atlantic Sturgeon during 18 March-15 April 2014. No control fish had detectable MC in liver samples. Mean total consumption of MC by non-control Atlantic Sturgeon fed dosed pellets ranged between 0.18 and 0.84 µg ind<sup>-1</sup> (Table 2). Percent mortality of experimental fish ranged between 22% and 50% but mortalities during the feeding trial were higher in dosed *versus* control treatments. Most dead and moribund fish showed evidence of a surficial fungal infection and it was not possible to determine if this pathogen was the primary cause of mortalities or merely an opportunistic agent. Liver tissue MC values were obtained for 48 juvenile Atlantic Sturgeon representing three treatment groups and a control group, and included both original (n=28) and replacement (n=20) fish. Almost half (43%) of non-control Atlantic Sturgeon—including some individual fish that consumed relatively high amounts of MC—had no detectable MC in livers (Figure 10). Dosed fish with no (below analytical detection limit) MC in tissue samples were distributed among all three treatment groups with no obvious pattern. Liver concentrations of MC in fish with detectable MC ranged up to 0.34 µg g<sup>-1</sup> DW and were weakly correlated with estimated intake of MC during the experiment (Figure 11). Concentrations of MC > 0.1 µg g<sup>-1</sup> DW in fish livers were comparable to values for wild benthic (e.g. Blue Catfish) and planktivorous fishes (e.g. Atlantic Menhaden) in reaches of the tidal James River that experience HAB events (Wood et al. 2014). Calculated stress index values for juvenile Atlantic Sturgeon were not significantly correlated with paired estimates of total MC ingested during the experimental trial (Figure 12). Stress index values among the four experimental treatments were not statistically different (Kruskall Wallis, p=0.49) but higher stress indices were weakly associated with dosed, rather than control, treatments (Figure 13).

## Conclusions

Results from these experiments suggest that dissolved MC is unlikely to cause mortality among living resources of the James at concentrations currently observed in this system. The lack of effects observed in the fish egg/larvae and *Bosmina* experiments may be due in part to the relatively low MC concentrations used in the exposure experiments. This range of exposures was selected to represent conditions in the James. Whereas concentrations in other waterbodies have been reported at much higher levels (100's  $\mu\text{g L}^{-1}$ ), concentrations in the James have not exceeded  $5 \mu\text{g L}^{-1}$ . Reported values represent total MC which is likely dominated by the particulate fraction. Assuming that 10-30% of MC is in the dissolved fraction, our range of dosages (up to  $1.6$  or  $3.2 \mu\text{g L}^{-1}$ ) may be representative of dissolved concentrations when total values are in the range of  $5$ - $30 \mu\text{g L}^{-1}$ . Empirical determination of dissolved and particulate fractions in the James would allow us to better relate the experimental range to environmental conditions.

Benthic suspension feeders provide a valuable ecosystem service by filtering suspended particulate matter from the water column thereby enhancing water clarity and suppressing algal abundance (Cercó and Noel 2010, zu Ermgassen et al. 2013). Though we did not observe mortality effects, our experiments with *Rangia* showed that low concentrations of dissolved MC reduced their filtration rate. It is notable that even at the lowest concentration tested ( $0.40 \mu\text{g MC L}^{-1}$ ) clearance rates were significantly lower than Controls in both experiments. The presence of the toxin may cause *Rangia* to avoid exposure through decreased feeding and in turn lead to long-term declines in their abundance and diminished top-down control on algal blooms. Laboratory growth experiments have shown that bivalves are capable of removing colonial and filamentous cyanobacteria from the water column, but feeding on cyanobacteria results in low ingestion rates even in the absence of cyanotoxins (Pires et al. 2004; Bontes et al. 2007). Low ingestion rates associated with cyanobacteria in diet resulted in reduced growth and reproduction rates among various species of bivalves (Wacker and von Elert 2003; Basen et al. 2012), though we know of no studies specific to *Rangia*. To assess the potential occurrence of toxin inhibition of *Rangia* grazing rates, we determined the probability of exceeding a given Microcystin level at varying CHLa concentrations (Figure 14). Our results show that when CHLa concentrations are less than  $20 \mu\text{g L}^{-1}$ , the likelihood of Microcystin concentrations exceeding the minimum threshold where feeding inhibition was observed is low (<25%). When CHLa levels in the James are in the range of  $20$ - $40 \mu\text{g L}^{-1}$ , the likelihood of exceeding  $0.4 \mu\text{g MC L}^{-1}$  rises to ~50%. CHLa concentrations in excess of  $40 \mu\text{g L}^{-1}$  are associated with a high probability (75-100%) of MC exceeding the  $0.4 \mu\text{g MC L}^{-1}$  threshold, though it should be noted that there are relatively few observations in this range of values due to the relatively low CHLa and MC concentrations occurring in 2 of the 3 years of monitoring (2012-2013).

Our experiments to date have largely focused on the effects of dissolved MC. Microcystin exposure among consumers is thought to primarily occur through dietary ingestion (Prepas et al. 1997, Lürting and Van der Grinten 2003), though deleterious effects from dissolved MC have also been reported (Lance et al. 2010). Interpreting the effects of dietary exposure to MC is complicated by other factors that influence food quality such as algal abundance, the relative proportions of algal groups, and presence of non-algal particulate matter.

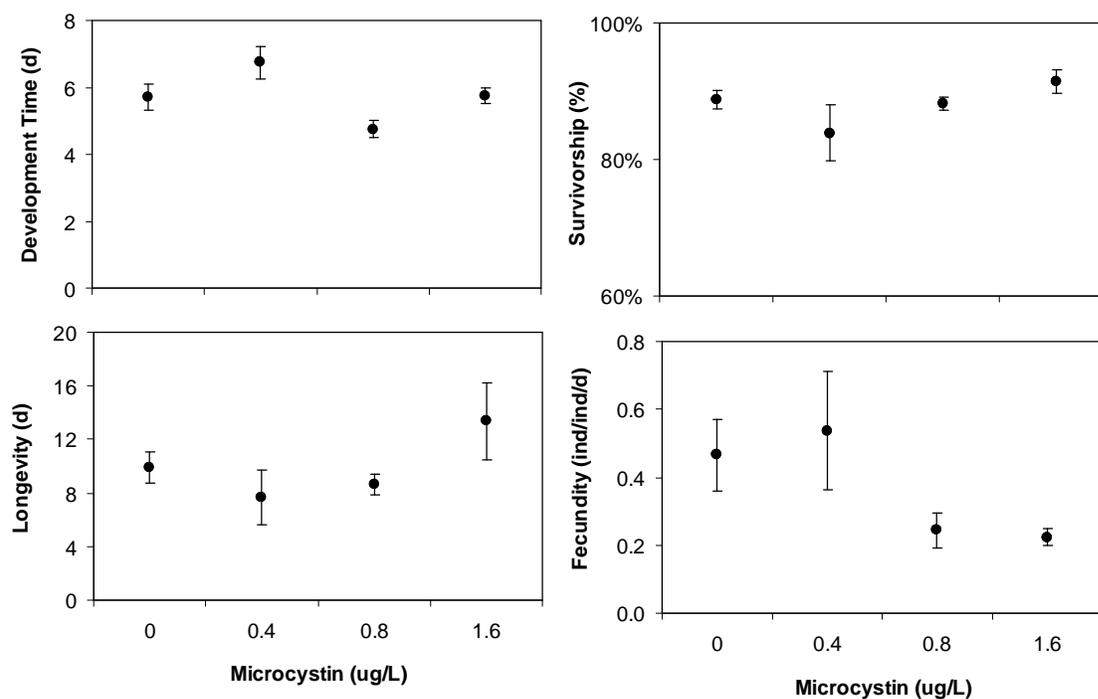
Furthermore, the use of natural food sources (seston) is dependent upon the occurrence of MC-producing algae in the environment, which is dependent upon a variety of factors influencing their abundance (water residence time, etc.). During the field exposure (sentinel) experiments performed with YOY Gizzard Shad, MC concentrations in the James were low ( $<0.5 \mu\text{g L}^{-1}$ ). Despite this, we observed rapid accumulation of the toxin to tissue concentrations that exceeded those observed in wild-caught fish of similar age. Higher tissue concentrations among sentinel fish may be because they are restricted to feeding in the water column by the enclosures, thereby increasing their MC consumption. Free-living Gizzard Shad gradually transition from a pelagic- to benthic-based diet during their first year and, as the sediments contain less MC (see prior report), a mixed diet of suspended and sedimented particulate matter would reduce exposure to MC. Dietary effects could potentially be addressed in a more controlled fashion using artificial food sources amended with MC. We are not aware of any published studies that would allow us to link the observed levels of toxin accumulation in Gizzard Shad with effects on mortality, growth or fecundity. However, Acuna et al. (2012) showed that dietary exposure to Microcystin resulted in adverse health effects and impaired reproductive potential in a closely related species (Threadfin Shad). Their study did not measure MC in tissues that would allow us to directly relate measured tissue concentrations to corresponding values from the James. However, their study tested two levels of dietary exposure ( $4.4$  and  $10 \mu\text{g g}^{-1}$  DM) which were lower than the Microcystin concentrations found in suspended particulate matter in the James ( $16 \mu\text{g g}^{-1}$  DM; Wood et al. 2014). Results of our laboratory studies with egg and larval-stage alosine fishes (dissolved MC exposure) and early juvenile Atlantic Sturgeon (dietary MC exposure) do not support the hypothesis that these species of concern are likely to be impacted by MC-producing events in the James River, Virginia.

**Table 1.** Effects of dissolved Microcystin on the viability of eggs and larvae from American Shad (ASA; *Alosa sapidissima*) and Blueback Herring (AAE, *Alosa aestivalis*).

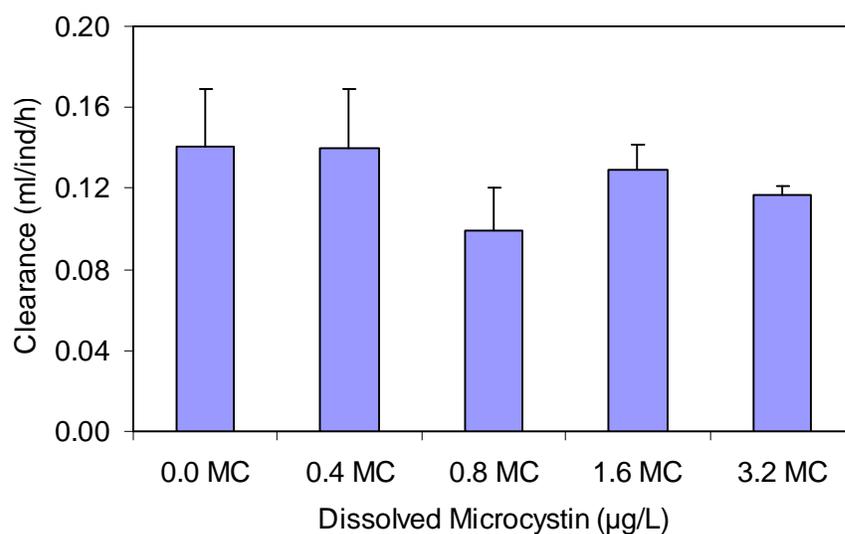
Life Stage	MC	Viable Proportion			
		ASA 1	AAE 1	AAE 2	AAE 3
Eggs	Control	0.98	0.91	0.98	1.00
	0.4 µg/L	0.98	0.93	0.98	0.99
	0.8 µg/L	1.00	0.91	0.98	0.99
	1.6 µg/L	0.99	0.91	0.97	1.00
Larvae	Control	0.96	1.00	0.99	1.00
	0.4 µg/L	0.94	0.91	0.99	1.00
	0.8 µg/L	0.95	nd	0.97	0.99
	1.6 µg/L	0.90	nd	0.94	1.00
	3.2 µg/L	nd	nd	nd	1.00

**Table 2.** Experimental treatments, concentration of MC in pellets, estimated total MC consumed, and percent mortality for captive age-0 Atlantic Sturgeon.

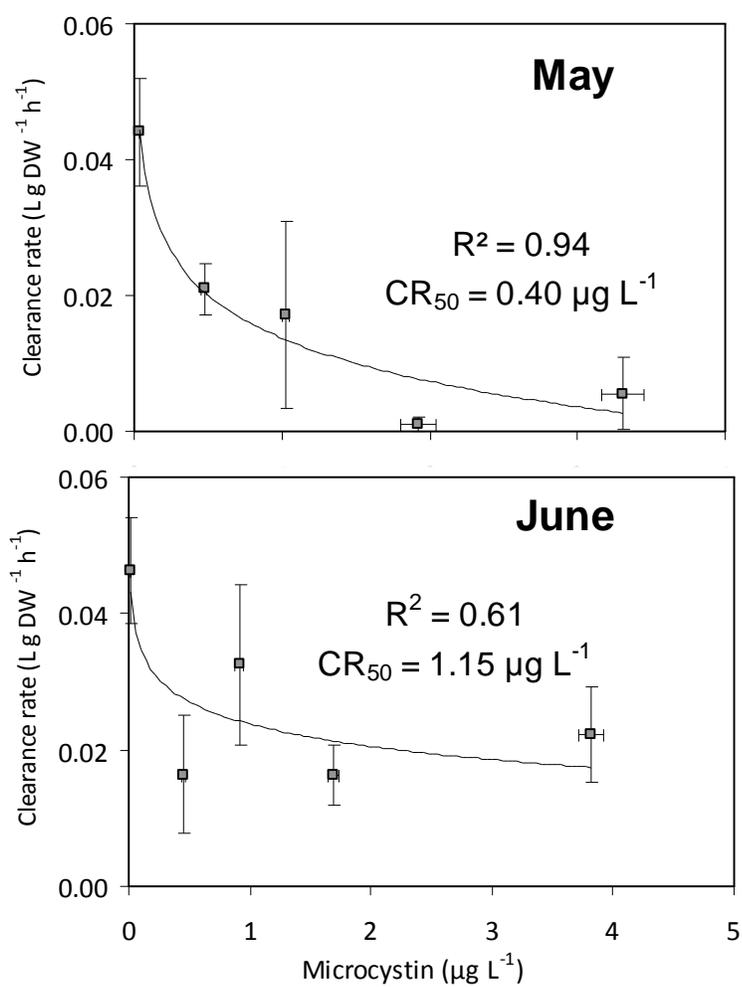
Treatment	Concentration of MC (µg/g WW) in diet	Mean total consumption of MC (µg ind <sup>-1</sup> )	Percent fish mortality during experimental trial
C (control)	0.000	0.00	22
T1 (dosed)	0.025	0.18	46
T2	0.050	0.35	50
T3	0.100	0.84	42



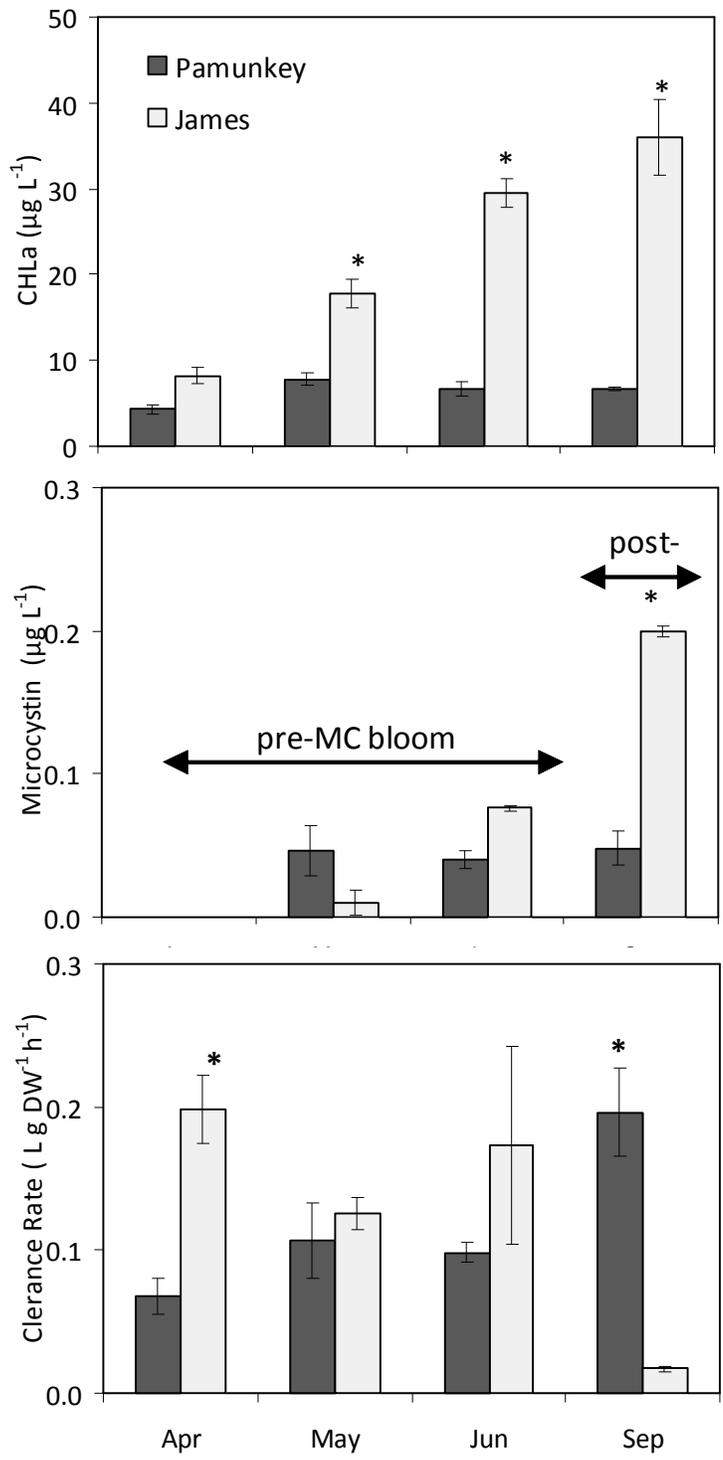
**Figure 5.** Effects of exposure to dissolved Microcystin on development time, survivorship, longevity and fecundity of a common James River zooplankter *Bosmina longirostris*.



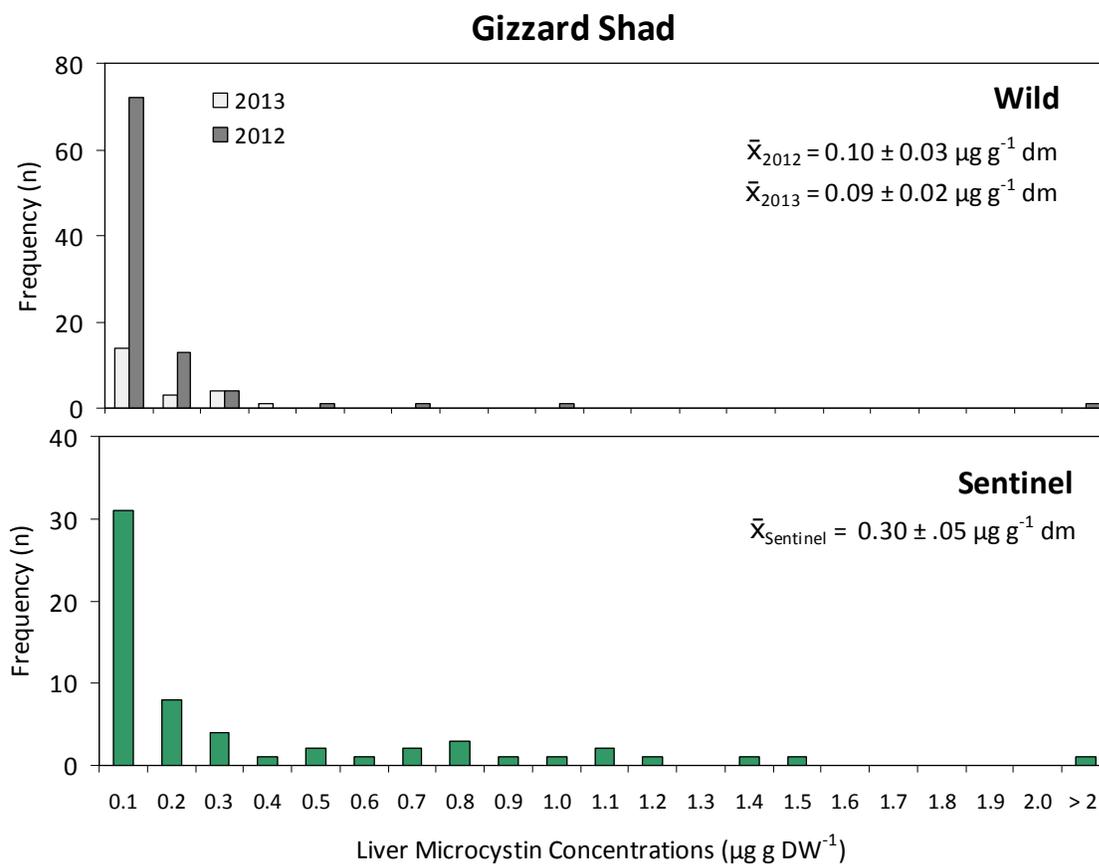
**Figure 6.** Clearance rates of *Bosmina* feeding on *Chlamydomonas* and exposed to varying concentrations of dissolved Microcystin.



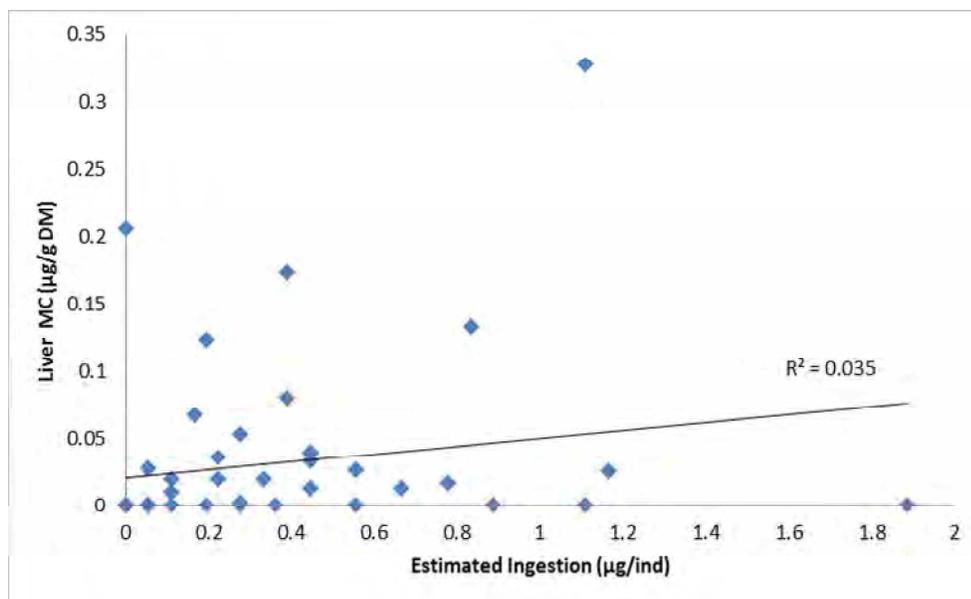
**Figure 7.** Effects of exposure to dissolved Microcystin on clearance rates of the benthic filter-feeder *Rangia cuneata*.



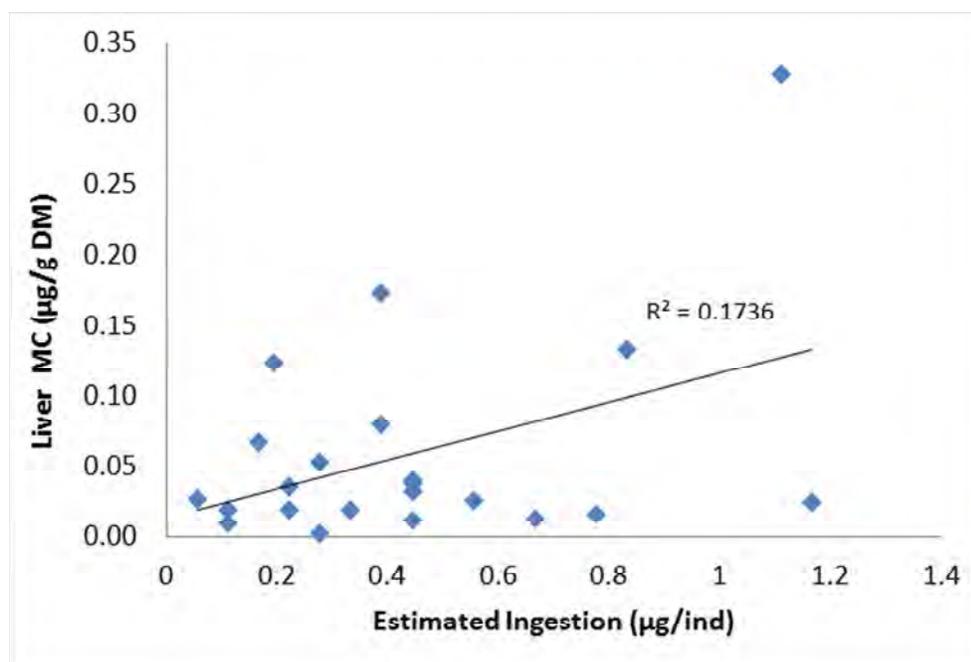
**Figure 8.** Clearance rates of James River clams fed seston from the James vs. Pamunkey Rivers with varying chlorophyll-a and Microcystin content.



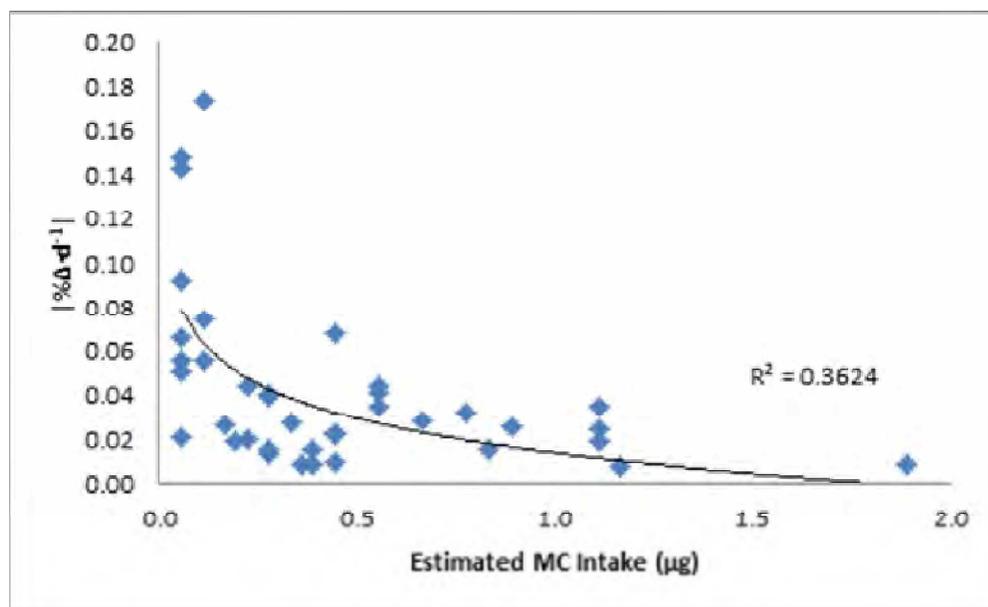
**Figure 9.** Comparison of Microcystin concentrations in liver tissues of wild-caught and sentinel fish from the tidal fresh segment of the James River Estuary. Data shown are for young-of-the-year Gizzard Shad (N = 60 for sentinels, N = 115 for wild-caught).



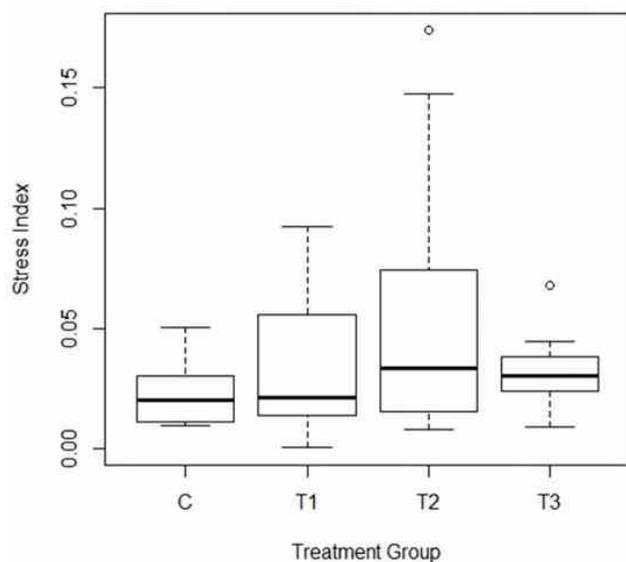
**Figure 10.** Relationship between the concentration of MC in liver tissues and estimated ingestion of MC in food for 39 captive, juvenile Atlantic sturgeon.



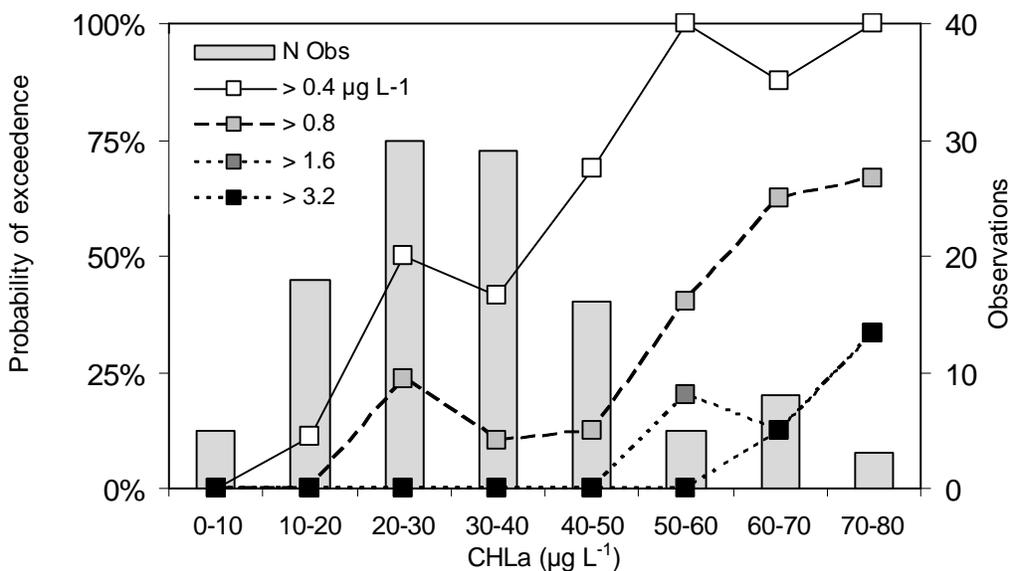
**Figure 11.** Relationship between the concentration of MC in liver tissues and estimated ingestion of MC in food for 22 captive, juvenile Atlantic sturgeon in non-control treatments that had detectable MC values (i.e., non-zero MC values).



**Figure 12.** Relationship between the stress index (standardized change in somatic mass) and estimated ingestion of MC in food for 39 captive, juvenile Atlantic Sturgeon.



**Figure 13.** Median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, and ranges of stress index values among the four experimental treatments for 48 captive, juvenile Atlantic Sturgeon fed pellets dosed with Microcystin. Among-treatment differences were not significant at a decision level of  $\alpha=0.05$  (Kruskal-Wallis,  $p=0.49$ ). Refer to Table 1 for treatment group attributes and to the text for a description of the stress index.



**Figure 14.** Probability of exceeding various levels of Microcystin concentrations as a function of CHLa based on paired measurements of Microcystin and CHLa obtained from the tidal-fresh segment of the James River during 2011-2013. Microcystin thresholds correspond to levels used in fish, zooplankton and *Rangia* exposure experiments.

## Part Three: Monitoring Results

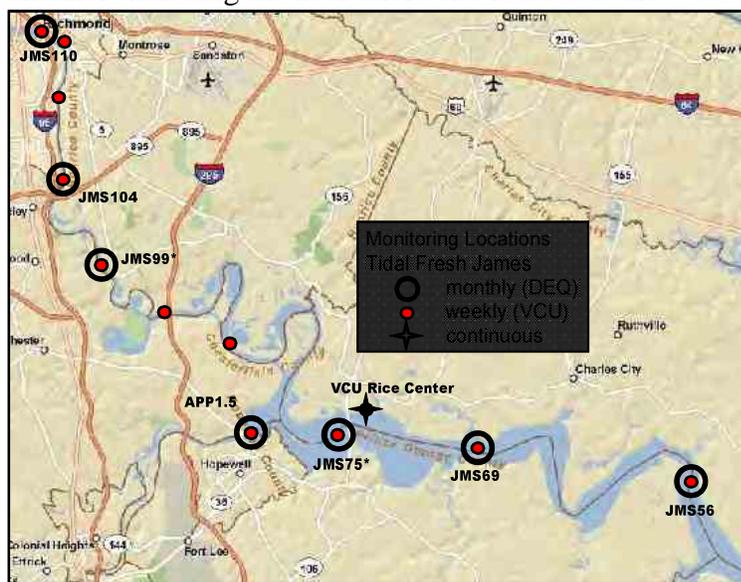
### Introduction

This segment of the report addresses Subtask 1.1 of the SAP Workplan which is to characterize spatial and temporal dynamics of algal blooms in the James. Prior studies of the tidal freshwater James River have documented the occurrence of persistent, elevated CHLa in the vicinity of river mile 75 (JMS75). Summertime CHLa concentrations at this site are among the highest annual average values observed throughout Chesapeake Bay and its tributaries. Elevated CHLa in this segment of the James River is attributed to natural and anthropogenic factors that favor high phytoplankton growth and nutrient utilization (Bukaveckas et al. 2011). The CHLa maximum occurs where channel geomorphometry transitions from a constricted, riverine channel to a broader, estuarine channel with extensive shallow areas. The decrease in average depth releases phytoplankton from light limitation and allows greater utilization of nutrient inputs from watershed and local point sources (Wood and Bukaveckas 2014). High CHLa is associated with elevated levels of the cyanotoxin Microcystin in water as well as tissues of fish and shellfish (see Part 2). Monitoring activities initiated in 2012 were continued in 2013 to characterize CHLa concentrations, the occurrence of harmful algal blooms and the presence of Microcystin in fish and shellfish. These data support the development of empirical relationships between CHLa and indicators of harmful algal blooms (e.g., MC concentrations) which are needed to determine whether existing CHLa criteria are protective against impairment arising from algal blooms. These data may also be used for modeling harmful algal blooms in the James.

### Methods

#### Sample Collection

Near-surface (1 m) water samples were collected ~weekly from May through November 2013 at 12 sites spanning the tidal fresh segment (see map). All of the sites were in the main channel excluding the near-shore site located at the VCU Rice Center Research Pier and one



located in the Appomattox River sub-estuary. Seven of the sampling locations are also long-term monitoring stations for the Chesapeake Bay Program (CBP). Water samples from all sites were analyzed for CHLa and nutrient concentrations; MC was measured at 6 sites (JMS56, JMS69, JMS75, JMS99, APP1.3, and Rice Pier). Phycocyanin was monitored continuously (15 min) at the Rice Research Pier using a YSI 6600 multiparameter data sonde (0.5 m depth) equipped with an *in vivo* fluorescence sensor (Yellow Springs,

OH) calibrated every two weeks. In addition to the weekly monitoring in the tidal-fresh segment, we also analyzed CHLa and MC from CBP stations throughout the James during scheduled sampling events in August and September (11 sites: JMS110 to JMS5.7).

Fish sampling targeted abundant and ecologically important taxa in the James. The tidal fresh James River has large resident fish populations of Gizzard Shad (*Dorosoma cepedianum*), Threadfin Shad (*D. petenense*), and Blue Catfish (*Ictalurus furcatus*), as well as transient populations of Atlantic Menhaden (*Brevoortia tyrannus*). Fish were collected on August 15-16 from 3 sites: two located in the tidal fresh (JMS99, JMS75) and one located in the oligohaline (JMS42). Approximately 10-15 individuals were obtained at each site for each taxa. Fish were obtained by electrofishing (low and high frequency) along multiple transects located in proximity to the water monitoring locations. Water samples were collected and analyzed for MC concurrent with fish sampling. Fish were euthanized according to IACUC protocols (VCU AD#100000441). We also measured MC in Blue Crabs as they represent the most likely pathway for human exposure. Blue Crabs were obtained monthly (June to September) from multiple crab pots deployed at two sites: the VCU Rice Pier (near JMS75) and at Jamestown (near JMS42).

### *Sample Analysis*

Filters for CHLa analyses (Whatman GF/A 0.5  $\mu\text{m}$  nominal pore size) were extracted for 18 h in buffered acetone and analyzed on a Turner Design TD-700 Fluorometer. MC samples were analyzed using the high sensitivity ADDA ELISA Kit (detection limit 0.05  $\mu\text{g L}^{-1}$ ; Abraxis; Warminster, PA). The assay measures numerous forms of MC using polyclonal antibodies with concentrations reported in MC-LR equivalents. For water, samples were thawed and refrozen two times (as recommended by the manufacturer), and then microwaved and sonicated to lyse the cells and release MC. Salinity was  $< 0.15$  ppt for all water samples analyzed for MC. To extract MC from tissues, muscle and liver (fish) or viscera (crabs) were surgically removed. For juvenile fishes, individuals were occasionally pooled (2-3 per sample) to obtain sufficient material for MC analysis. Samples were dried at 60°C for 48 h, ground with a mortar and pestle, and extracted in 75% aqueous methanol for 24 h. Extracts were centrifuged and supernatant collected. Subsamples were diluted with deionized water such that samples to be run on the ELISA plate contained  $< 5\%$  methanol. Plates were read on an ELISA plate reader at 450 nm. For each 96-well plate, six standards were run in duplicate to derive plate-specific standard curves.

## *Results*

### *CHLa & MC*

CHLa concentrations in the tidal fresh segment of the James were low in comparison to previous years (Figure 15). For the July-August index period, average concentrations in 2013 (29  $\mu\text{g L}^{-1}$ ) were lower compared to 2010, 2011 and 2012 (91, 59 and 39  $\mu\text{g L}^{-1}$ , respectively). Peak CHLa concentrations in 2013 (49  $\mu\text{g L}^{-1}$ ) were also lower relative to previous years (141, 80 and 55  $\mu\text{g L}^{-1}$ ). Lower CHLa in 2013 can be attributed to higher-than-normal discharge resulting in faster flushing of the estuary. High discharge events typical of winter-spring persisted through the summer months of 2013 (Figure 16). Freshwater Replacement Time during July-August 2013 was 6 d, whereas corresponding values for 2010-2012 were 27, 22 and 22 d (respectively). Inter-annual variation in CHLa corresponded to trends in MC with lower

average values observed in 2013 ( $0.24 \mu\text{g L}^{-1}$ ) relative to 2011-2012 (1.38 and  $0.61 \mu\text{g L}^{-1}$ , respectively; no MC data for 2010). Lower MC concentrations in 2013 coincided with lower cyanobacteria abundance as indicated by measurements of phycocyanin (Figure 17). Phycocyanin exceeded 8 RFU during peaks in July, August and September of 2012 which were associated with highest MC concentrations. In 2013, phycocyanin exceeded 8 RFU on only one day and average values (mean = 3.8 RFU) were half of those measured in the preceding year (mean = 7.8 RFU). Results from the longitudinal sampling conducted in August and September showed well-defined CHLa and MC maxima centered on stations JMS75 and JMS69 (Figure 18). Both parameters were rapidly attenuated seaward with low concentrations observed at JMS56 and throughout the lower James. CHLa was found to be a strong predictor of MC within this dataset ( $R^2 = 0.68$ ,  $P < 0.0001$ ).

### *MC in Fish and Shellfish*

The MC content of fish tissues varied longitudinally (Figure 19). Among planktivores, highest average fish tissue concentrations were measured at JMS75 ( $0.28 \pm 0.07 \mu\text{g g}^{-1} \text{ dm}$ ) with lower average values at JMS42 ( $0.12 \pm 0.03 \mu\text{g g}^{-1} \text{ dm}$ ) and JMS99 ( $0.09 \pm 0.02 \mu\text{g g}^{-1} \text{ dm}$ ). Inter-site differences were statistically significant based on a one-way ANOVA ( $p = 0.006$ ). For all species combined, similar trends were observed among the three sites, but differences were not statistically significant ( $p = 0.065$ ). Pooling across taxa resulted in lower and more similar average values across sites due to near-zero MC concentrations for benthivores at all sites. Spatial variation in the MC content of fish tissues followed longitudinal patterns in water MC concentrations with highest values measured at JMS 75 ( $0.55 \mu\text{g L}^{-1}$ ) and lower concentrations at JMS42 ( $0.03 \mu\text{g L}^{-1}$ ) and JMS99 ( $0.10 \mu\text{g L}^{-1}$ ). To assess inter-annual variation we used a subset of our data for fish collected in August near JMS75 (Table 3). There were sufficient data for three taxa to draw comparisons between years; however, two of these (juvenile Blue Catfish and adult Gizzard Shad) are benthivores which showed near-zero concentrations in both years. MC concentrations in Threadfin Shad were higher and similar in both years.

The MC content of Blue Crab tissues varied seasonally and spatially (Table 4). As in 2012, we found that the MC content of viscera and hepatopancreas tissues was greater than that of muscle tissue. In 2013, we analyzed hepatopancreas tissues separately from viscera (previously analyzed as a single sample) and found that the former contained higher MC concentrations. For crabs collected at the Rice Center during July-September, hepatopancreas MC concentrations ( $0.156 \mu\text{g g}^{-1} \text{ dm}$ ) were 2-fold higher relative to viscera ( $0.074 \mu\text{g g}^{-1} \text{ dm}$ ) and 5-fold higher than in muscle ( $0.033 \mu\text{g g}^{-1} \text{ dm}$ ). The proportion of crabs with measureable MC concentrations was also higher for viscera, hepatopancreas and viscera-hepatopancreas samples (mean = 79% for all samples) than for muscle (mean = 56%). To assess inter-site and inter-annual variation, we performed a one-way ANOVA to partition variance among three groups: Rice-2012, Rice-2013 and Jamestown-2013. We used muscle data from all crabs collected during July-September as these yielded similar sample size among the groups ( $N = 44$ , 46 and 50, respectively). Highest average concentrations were measured at the Rice Pier in 2013 ( $0.031 \pm 0.005 \mu\text{g g}^{-1} \text{ dm}$ ) with lower average values at Jamestown ( $0.005 \pm 0.001 \mu\text{g g}^{-1} \text{ dm}$ ) and the Rice Pier during 2012 ( $0.017 \pm 0.003 \mu\text{g g}^{-1} \text{ dm}$ ). Between-group differences were statistically significant ( $p = 0.048$ ). Inter-site differences in muscle concentrations followed trends in water MC concentrations with higher concentrations measured at the Rice Pier ( $0.20 \mu\text{g L}^{-1}$ ) relative to Jamestown ( $0.10 \mu\text{g L}^{-1}$ ). However, when we analyzed hepatopancreas data (2013 only) these were not significantly different between sites. Inter-annual differences in

tissue concentrations did not follow trends in water concentrations as muscle MC content at the Rice Pier was higher in 2013 despite lower water-MC concentrations ( $0.20 \mu\text{g L}^{-1}$ ) than in 2012 ( $0.58 \mu\text{g L}^{-1}$ ). We had previously reported that seasonal variation in crab MC content followed seasonal patterns in water concentrations. In 2012, we observed the highest water, muscle and hepatopancreas-viscera concentrations in August. A similar pattern was observed at the Rice Pier in 2013 with highest viscera and hepatopancreas concentrations occurring in August and peak water concentrations measured on August 13<sup>th</sup>. Muscle tissue concentrations were higher in May than in August but the May average value was based on a small sample size ( $N=3$ ) and large uncertainty (mean =  $0.151 \pm 0.1003 \mu\text{g g}^{-1} \text{ dm}$ ). Seasonal patterns were less evident at the oligohaline site (Jamestown) though highest hepatopancreas concentrations were in August.

## Conclusions

Monitoring results show that CHLa and MC concentrations in the tidal fresh segment were lower in 2013 compared to previous years. Lower concentrations were attributed to above-average discharge resulting in shorter water residence time. Despite lower MC concentrations in water, tissue concentrations in fish were similar to the prior year. We have previously (2012) demonstrated that seasonal variation in fish MC tracks water MC, and results from 2013 generally support this finding. However, our dataset is not well-suited to assessing inter-annual variation in MC concentrations in biota, in part due to changes in sampling design. In 2012, our focus was characterizing seasonal patterns and therefore we collected monthly samples at a single site (near JMS75). In 2013, the objective was to assess longitudinal variation and therefore we sampled at three sites in a single month. Although a large number of fish tissue samples have been analyzed over the two years, the subset of these data that can be used to assess inter-annual variation (August samples collected near JMS75) is relatively small ( $N=95$ ). Furthermore, numerically-dominant benthivores (juvenile Blue Catfish and adult Gizzard Shad) are included among these samples, but have limited value for tracking inter-annual variation of MC in living resources as their diet is largely comprised of allochthonous organic matter. Targeted sampling of planktivorous fishes (YOY Gizzard Shad, Threadfin Shad and Atlantic Menhaden) would be required to obtain sufficient sample sizes each for developing empirical relationships with water MC concentrations. Blue Crab data supporting inter-annual comparisons are more extensive ( $N>40$  per year) as similar sampling designs were used in both years (except for change in tissue sampling for hepatopancreas vs. viscera). These results show that muscle MC concentrations were higher in 2013 despite lower water concentrations. Results from inter-site comparisons are somewhat equivocal in that fish tissue concentrations were lower at sites above (JMS99) and below (JMS42) the region where MC concentrations in water are highest (JMS75). For Blue Crabs, MC concentrations in muscle were lower at the oligohaline site (JMS42) relative to the tidal fresh site (Rice Pier), whereas hepatopancreas concentrations were not significantly different.

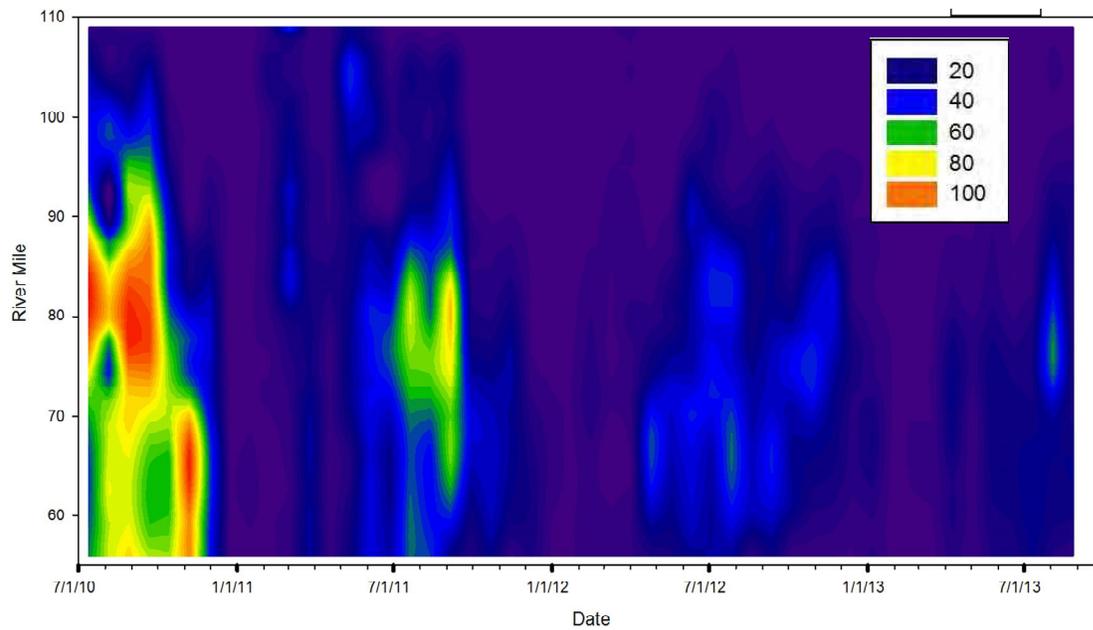
In summary, our monitoring data for 2013 confirm the earlier (2012) findings that there is widespread occurrence of MC in water and biota from the tidal fresh segment of the James. The 2013 data also support prior findings showing that MC concentrations are higher in planktivorous (vs. benthivorous) fish and that highest concentrations are found in liver-viscera tissues for both fish and shellfish. The 2013 data shed new light on longitudinal patterns of the occurrence of MC in living resources. Microcystin was commonly detected in fish collected 24 miles above (JMS99) the site where maximal water concentrations were measured (JMS75) and

in fish and shellfish collected 33 miles below (JMS42). These data documenting seasonal and spatial variation may be used to develop empirical relationships relating tissue MC to water MC, and water MC to CHLa. These relationships may be useful for assessing risks to living resources and human exposure.

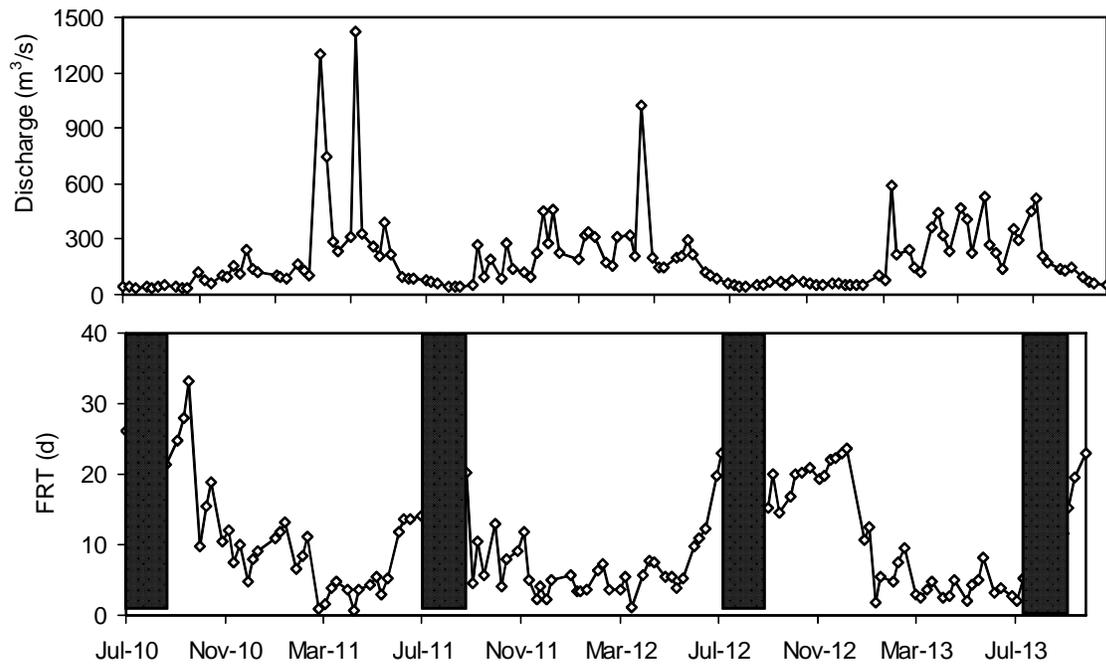
**Table 3.** Microcystin concentrations in liver tissues of common fish species of the tidal fresh James during 2012 and 2013. Fish were collected during August in proximity to JMS75.

Species	Year	N	MC ( $\mu\text{g g}^{-1} \text{dm}$ )	
			Mean	SE
Blue Catfish	2012	14	0.00	0.00
	2013	25	0.03	0.00
Gizzard Shad (adult)	2012	9	0.01	0.01
	2013	6	0.03	0.01
Threadfin Shad	2012	12	0.29	0.12
	2013	10	0.33	0.03

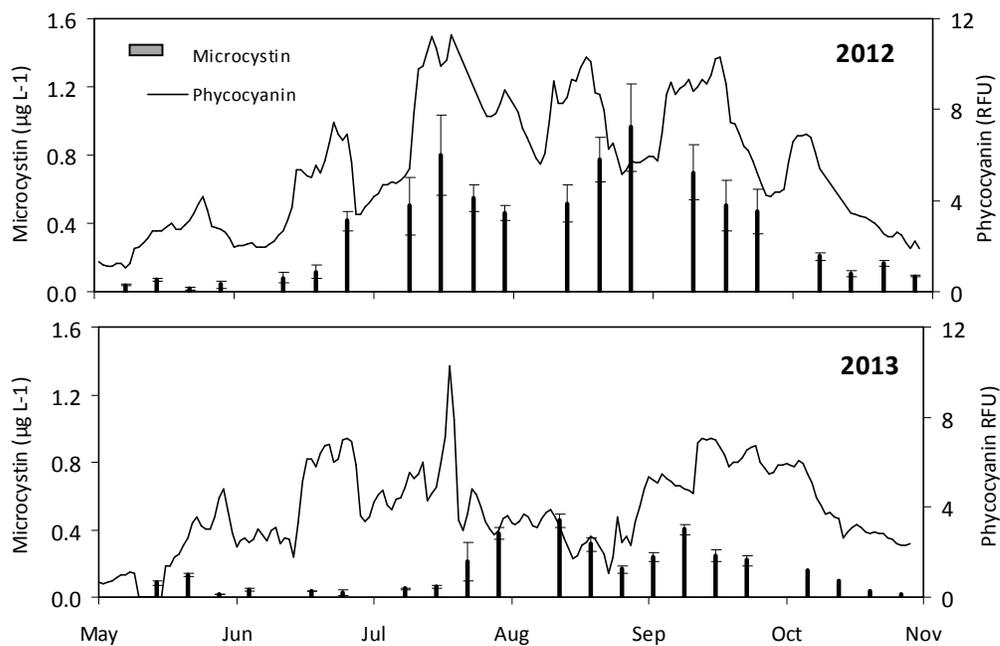




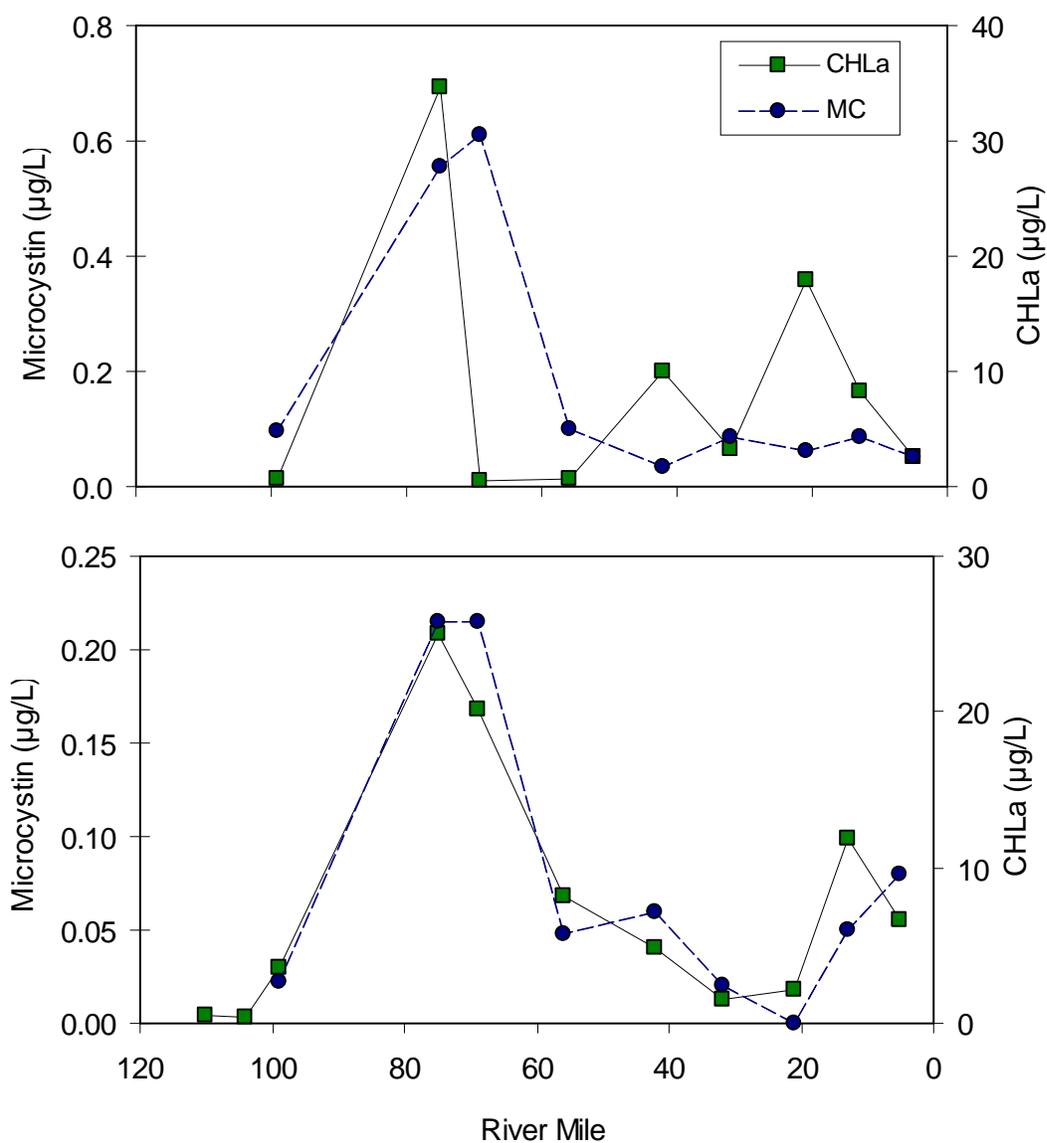
**Figure 15.** Seasonal and longitudinal variation in CHLa concentrations in the tidal fresh James River from July 2010 through September 2013. Data used for this figure are ~weekly measurements at 12 stations spanning river miles 56 (seaward) to 110 (landward).



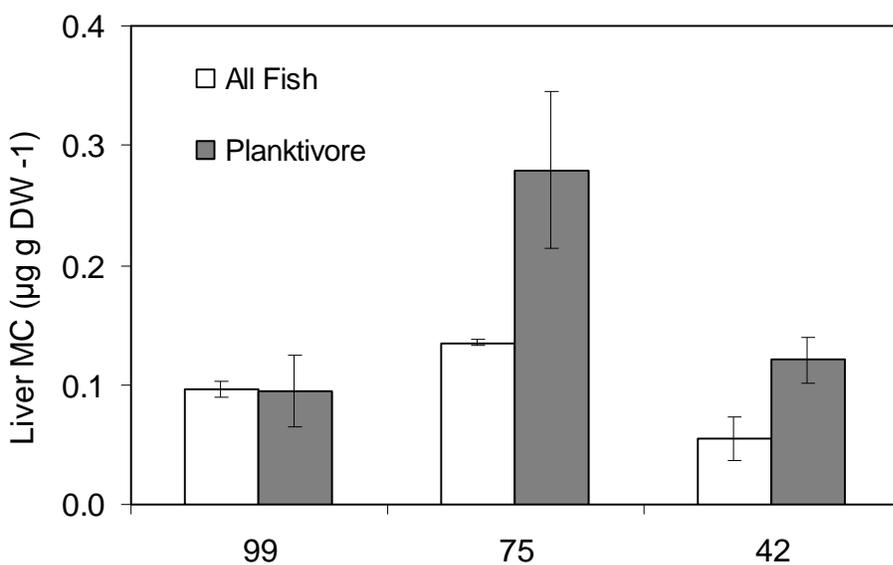
**Figure 16.** Variation in riverine inputs and freshwater replacement time of the tidal fresh segment of the James River Estuary. Riverine inputs represent the combined discharge of the James and Appomattox Rivers based on data from the USGS.



**Figure 17.** Seasonal variation in Phycocyanin and Microcystin in the tidal freshwater James River (JMS75) during 2012 and 2013. Phycocyanin measurements are daily values derived from continuous (15 min) monitoring.



**Figure 18.** Longitudinal variation in CHLa and Microcystin in the tidal freshwater James River during August (top panel) and September (bottom panel) 2013. Data are from samples collected at CBP long-term monitoring stations.



**Figure 19.** Microcystin concentrations in liver tissues of fishes collected at two sites in the tidal fresh (JMS99, JMS75) and one site in the oligohaline (JMS42) segment of the James River. Data shown are average values (with SE) for all fish (N=136) and for the subset that are planktivorous (N=70).

## References

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