Exposure to the Cyanotoxin Microcystin Arising from Interspecific Differences in Feeding Habits among Fish and Shellfish in the James River Estuary, Virginia.


Department of Biology and Center for Environmental Studies Virginia Commonwealth University, Richmond, Virginia 23284, United States

Supporting Information

ABSTRACT: The cyanotoxin, microcystin (MC), is known to accumulate in the tissues of diverse aquatic biota although factors influencing exposure, such as feeding habits and seasonal patterns in toxin production, are poorly known. We analyzed seasonal variation in the MC content of primary and secondary consumers, and used dietary analysis (gut contents and stable isotopes) to improve understanding of cyanotoxin transport in food webs. Periods of elevated toxin concentration were associated with peaks in the abundance of genes specific to *Microcystis* and MC toxin production (*mcyD*). Peak toxin levels in consumer tissues coincided with peak MC concentrations in seston. However, toxins in tissues persisted in overwintering populations suggesting that potential health impacts may not be limited to bloom periods. Interspecific differences in tissue MC concentrations were related to feeding habits and organic matter sources as pelagic fishes ingested a greater proportion of algae in their diet, which resulted in greater MC content in liver and muscle tissues. Sediments contained a greater proportion of allochthonous (terrestrial) organic matter and lower concentrations of MC, resulting in lower toxin concentrations among benthic detritivores. Among shellfish, the benthic suspension feeder *Rangia cuneata* (wedge clam) showed seasonal avoidance of toxin ingestion due to low feeding rates during periods of elevated MC. Among predators, adult Blue Catfish had low MC concentrations, whereas Blue Crabs exhibited high levels of MC in both muscle and viscera.

**INTRODUCTION**

Harmful algal blooms are a growing worldwide concern. Some harmful algae produce secondary metabolites that act as toxins and therefore pose threats to human health and aquatic biota. Microcystin (MC), a hepatotoxin, has received considerable attention due to its widespread occurrence in freshwaters and deleterious effects on humans and aquatic biota. Human exposure occurs through drinking water, recreational contact, or fish consumption. MC is water stable and resistant to boiling, thus posing a threat to drinking water supplies and fish consumption. Human exposure to MC raises concerns regarding impairment of designated uses (e.g., swimmable and fishable). The World Health Organization (WHO) has issued guidelines for drinking water (1 μg L⁻¹), recreational contact (low and moderate risk = 4 and 20 μg L⁻¹, respectively) and consumption (0.04 μg kg⁻¹ body weight d⁻¹). MC concentrations are typically highest in liver and viscera; shellfish may therefore pose a greater threat for human exposure because consumable portions include non-muscle tissues.

Cyanotoxins most frequently occur in warm, shallow, algal-rich waters that receive large anthropogenic nutrient loads. Genes for production of MC are found in *Microcystis* as well as others forms of cyanobacteria. MC accumulates in the tissues of a diverse group of organisms including fish, insects, crustaceans, bivalves, amphibians, birds, and mammals. Exposure is thought to occur primarily through consumption though little is known about the factors that contribute to variable exposure and toxin contamination among consumers. Field studies have demonstrated that long-lived species exposed to recurrent blooms accumulate the toxin and thus present a contamination source to higher-order consumers. Prior studies have been unable to establish direct relationships between diet variables and MC concentrations in consumers. This has been attributed to complex
processes regulating toxin exposure, elimination, and accumulation.25

Information regarding the spread of cyanotoxins into food webs is needed in order to assess implications for human health and aquatic resources. A trophic perspective that considers sources of organic matter (autochthonous vs allochthonous) and feeding habitats (pelagic vs benthic) may provide a useful framework for identifying risks to aquatic biota and human exposure. Dietary information based on gut content analysis and C isotopic signatures of consumer tissues provides a basis estimating algal contributions to consumer diets and their exposure to cyanotoxins. As part of a broader effort to assess harmful algal blooms and associated impairments in the James River Estuary, we (1) characterized seasonal variation in the MC content of suspended and sedimeted organic matter, and in the tissues of pelagic and benthic fishes, (2) tracked variation in toxin producers by monitoring a cyanobacteria-specific pigment (phycocyanin) and the abundance of genes specific to Microcystis and MC toxin production (mcyD), and (3) established linkages between toxin production and consumer exposure using information on dietary habits obtained by gut content and stable isotope analysis.

■ MATERIALS AND METHODS

Site Description. The James River Estuary is located in the U.S. Mid-Atlantic region and a subestuary of Chesapeake Bay (see SI Figure S1). The tidal fresh segment (salinity <0.5 ppt) extends 115 km from the Fall Line (at Richmond, VA) to the confluence with the Chickahominy River. This segment is well-mixed (vertically and laterally) owing to fluvial inputs (freshwater replacement time = 5–25 days) and a large tidal prism (60 cm) relative to depth (mean ~3 m).28 It shares a number of features in common with systems where cyanotoxins have been reported including large anthropogenic nutrient loads, elevated chlorophyll-a (CHLa) and presence of cyanobacteria.29–31 During low discharge conditions (May–October), elevated CHLa is observed in the region where the James transitions from a deep, riverine channel to a broad, estuarine channel. Shallow depths provide favorable light conditions allowing phytoplankton to exploit proximal nutrient inputs from riverine and point sources.28,31 Prior work has documented the presence of MC in other subestuaries of Chesapeake Bay;52 this study presents the first regular monitoring of MC in water and the first comprehensive assessment of MC in biota for the Chesapeake region.

Sample Collection. Near-surface (1 m) water samples were collected ~weekly from May through November 2012 at three sites, all located within the region of elevated CHLa (see SI Figure S1). Two of the locations were main channel sites, which are also long-term monitoring stations for the Chesapeake Bay Program (CBP; JMS69, JMS75); one was a near-shore site located at the VCU Rice Center Research Pier. Water samples were analyzed for MC, CHLa and genetic markers. Total suspended solids (TSS) were also measured so that MC and CHLa could be normalized to dry weight (DW) when comparing suspended and sedimented material. 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 2 weeks. Triplicate surficial sediment samples (0–2 cm) were collected monthly from each of three near-shore sites (depth <2 m) located near JMS75. Sediments from these sites were typical for the area being dominated by silty-sandy deposits. Surficial sediments were analyzed for MC and CHLa.

Our sampling of the food web 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).33 Their feeding habits include pelagic filter-feeding (Atlantic Menhaden, Threadfin Shad and young Gizzard Shad), benthic detritivory (juvenile Blue Catfish, adult Gizzard Shad) and piscivory (adult Blue Catfish). The common wedge clam (Rangia cuneata) is the dominant benthic filter-feeder based on annual surveys during 2001–2010 (CBP). It is considered to be a generalist suspension feeder ingesting a mixture of algal and nonalgal suspended matter.34 We also measured MC in Blue Crabs as they represent the most likely pathway for human exposure. Blue Crabs (Callinectes sapidus) are predators and scavengers found in tidal fresh waters from May (as juveniles) through October.

Fish were collected for analysis of tissues (MC and stable C isotope ratios) and gut contents (CHLa and organic matter content). Approximately 10–15 individuals were obtained per month (May–October 2012) for each of six size-taxa groups: Gizzard Shad (adult and YOY), Threadfin Shad, Atlantic Menhaden and Blue Catfish (<20 and 20–40 cm total length). Size classes were used to assess potential effects of ongoenetic shifts in feeding on toxin content. Additional samples of water and fish were collected in March 2013 to assess toxin levels in overwintering populations. Fish were obtained by electrofishing (low and high frequency) along multiple transects located in proximity to the water monitoring locations (JMS75, Rice Pier). Fish were euthanized according to institutional animal care protocols (VCU AD#20042). Blue Crabs were obtained from multiple crab pots deployed in proximity to the Rice Pier. Rangia were collected from a near-shore site in proximity to JMS75 using oyster tongs. Clams were held in deionized water for 48 h prior to dissection to allow for clearance of gut contents. Muscle and liver (fish) or viscera (crabs, clams) were surgically removed. For juvenile fishes, individuals were occasionally pooled (2–3 per sample) to obtain sufficient material for MC analysis. A total of 395 paired measurements (muscle and liver/viscera) of tissue MC concentrations were made among all taxa during the study.

Sample Analysis. Filters for CHLa analyses (Whatman GF/A 0.5 μm nominal pore size) were extracted for 18 h in buffered acetone and analyzed on a Turner Design TD-700 Fluorometer.35 TSS was determined gravimetrically using preweighed, precombusted filters. MC samples were analyzed using the high sensitivity ADDA ELISA Kit (detection limit 0.05 μg L−1; Abraxis; Warminster, PA). The assay measures numerous forms of free MC using polyclonal antibodies with concentrations reported in MC-LR equivalents. To release MC from cells, water samples were thawed and refrozen two times (as recommended by the manufacturer), and then microwaved and sonicated to improve extraction efficiency.36 Salinity was <0.15 ppt for all water samples analyzed for MC. To extract MC from tissues and sediment, samples were dried at 60 °C for 48 h, ground with a mortar and pestle, and extracted in 75% aqueous methanol for 24 h.6,21 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. Average recovery from spiked samples was similar to previously published values (See SI Table S1).

Quantitative PCR (qPCR) was performed on weekly samples collected at JMS75 and targeted conserved regions of the 16S rRNA gene associated with the genus Microcystis and the mcyD gene for toxin production. Filters (0.45 μm polycarbonate) were stored frozen (−20 °C) until whole-community DNA could be extracted using the Mo Bio PowerWater DNA Isolation Kit (Carlsbad, CA). qPCR was conducted on a Bio-Rad CFX96 Real-Time system using SsoAdvanced SYBR Green qPCR Supermix (BioRad, Hercules, CA). Extracted DNA was amplified using the following primer sets: CYAN 108F38 and 377R39 for cyanobacterial 16S rRNA; MICR 184F and 431R40 for Microcystis-specific 16S rRNA; mcyD F2 and R241 for the MC-LR producing strains. All reactions were carried out in a total volume of 20 μL, which contained 10 μL of Bio-Rad Sso Advanced SYBR Greener Supermix, 40 μM of each primer, DNA suspension, and molecular-grade water. q-PCR programs for all genes included an initial 4 min at 95 °C. Microcystis reactions utilized 55 cycles consisting of 95 °C for 30 s, 56 °C for 60 s and 72 °C for 20 s while the mcyD assays were run for 50 cycles of 95 °C for 20 s, 55.5 °C for 30 s, and 72 °C for 30 s. A melt curve was analyzed for each assay to ensure appropriate gene amplification and checked on a 1.5% agarose gel using ethidium bromide staining. Standards curves were created in duplicate using 10-fold serial dilutions of genomic DNA obtained from cultures of M. aeruginosa (UTEX LB 2388). A nontemplate control was run in duplicate for each assay.

Consumer Diets and Feeding. As our interest was in fish exposure to algal toxins, we did not perform a detailed gut contents analysis, but rather measured the CHLa content of gut materials as an indicator of algal contributions to their diet. CHLa is known to degrade during passage through the digestive system and therefore our estimates are likely to be conservative with respect to the contribution of phytoplankton and phytodetritus to fish diets. Contents from the entire gut were removed for determination of wet weight, dry weight and CHLa content. Material for CHLa analysis was extracted overnight in 90% buffered acetone and analyzed on a TD-700 fluorometer. Feeding habits of fishes were also evaluated through stable C isotope analyses of muscle tissues. Autochthonous and allochthonous sources of organic matter differ in their C isotopic signatures providing a basis for inferring their proportional contributions to consumer diets.

To aid in the interpretation of the fish stable isotope data, we characterized differences in δ13C of autochthonous and allochthonous organic matter using seston samples collected during our weekly monitoring. Samples (N = 32) collected during high-discharge, low CHLa conditions were used to represent allochthonous dominated C isotopic signatures and samples collected during low-discharge, high CHLa conditions were used to represent isotopic signatures with greater autochthonous contributions. For seston and fish tissue samples, we followed preparation protocols of, and submitted samples to, the UC-Davis Stable Isotope Lab. Tissue samples were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20−20 isotope ratio mass spectrometer. POM and sediment samples were analyzed using an Elementar Vario EL Cube or Micro Cube elemental analyzer interfaced to a PDZ Europa 20−20 isotope ratio mass spectrometer. The long-term standard deviation for 13C is 0.2‰. Relationships between dietary variables (gut content and stable isotope analysis) and MC concentrations in consumer tissues were analyzed using Model II regression analysis for data pooled by species across months.
As it was not practical to sample *Rangia* gut contents, we measured their grazing rates in order to relate toxin content of tissues to exposure. Experimental design followed Wong et al.\textsuperscript{34} whereby we monitored changes in CHLa (at 0, 2, and 4 h) in 20 L mesocosms with and without clams. In the presence of clams, concentrations of suspended particulates 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 particulate material based on the mass removed by consumers and average concentration in the water.\textsuperscript{49} Water with natural seston was collected from the Rice Pier for use in the grazing experiments. Clams were obtained in proximity to one of the water monitoring sites (near JMS69) for monthly trials performed during March-October 2012. Clams were kept overnight for acclimation to experimental conditions that included two temperature treatments: a standard reference of 20 °C and the ambient river temperature (which ranged from 14° to 32 °C). Each mesocosm contained a similar mass of clams (3−10 individuals depending on size; average body mass of 2.6 g ind\textsuperscript{-1}; range = 0.5−5.0 g ind\textsuperscript{-1}) and final results were normalized to soft-tissue body mass. Six mesocosms were used for each temperature treatment (three with, three without clams). 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.

**RESULTS**

**Cyanobacteria.** We characterized cyanobacteria abundance based on continuous measurement of phycocyanin and weekly measurement of MC concentrations and the abundance of genes specific to *Microcystis* and MC (*mcyD*). Phycocyanin monitoring revealed six peaks occurring at ~monthly intervals from May through October (Figure 1). The first peak (May) was associated with low toxin concentrations (<0.10 μg L\textsuperscript{-1}) and low abundance of *Microcystis* and *mcyD* genes. Subsequent peaks in phycocyanin were associated with higher toxin levels and increased *Microcystis* gene abundance. The July and August peaks were noteworthy for exhibiting peaks in both *Microcystis* and *mcyD* gene abundances and highest toxin concentrations. Peak MC concentrations occurred on July 17 (0.92 μg L\textsuperscript{-1}) and August 28 (0.78 μg L\textsuperscript{-1}; average values for three sampling locations). Intersite variability in MC concentrations was low (Figure 1; average SE = 0.03 μg/L). By November 27, MC was undetectable at all stations (data not shown). Phycocyanin
fluorescence (daily average values) was a useful predictor of MC concentrations in the James ($R^2 = 0.63, p < 0.01$).

**MC in Fish and Shellfish.** Seasonal variation in toxin content among fish and shellfish generally followed patterns of MC concentration in the water column (Figure 2; SI Table S2). Crab viscera contained on average 6-fold higher levels of MC than muscle (all months), with similar seasonal patterns observed for both. Tissue concentrations were low but detectable during the first sampling (May); concentrations in August were $\sim$3-fold greater and coincided with peak water column concentrations. Among fishes for which we were able to resolve seasonal patterns (juvenile and adult Gizzard Shad, Threadfin Shad), highest MC concentrations were observed in July–August. Young-of-the-year (YOY) Gizzard Shad exhibited an early and exceptionally high peak in liver MC concentrations ($\sim 1 \mu g \text{ g}^{-1} \text{ DW}$) in comparison to other fishes ($<0.3 \mu g \text{ g}^{-1} \text{ DW}$). Across all taxa, the proportion of fish with measurable MC concentrations in liver tissue generally increased during the succeeding peaks in cyanobacteria abundance with highest incidence observed in August (94%) and September (83%). Samples obtained in March 2013 showed no detectable concentrations in water, whereas tissue content was low but measurable (in livers, 67% of individuals among all taxa; mean $= 0.044 \pm 0.007 \mu g \text{ MC g DW}^{-1}$). Wedge clams (*Rangia*) exhibited seasonal patterns of toxin content similar to those observed in fish and Blue Crabs although tissue concentrations were $\sim$10-fold lower and peak values occurred later (August–September; Figure 3). The rise in tissue concentrations coincided with increasing water column concentrations of MC and a decline in feeding rates. Biomass-specific clearance rates were highest before the onset of elevated MC (March–May mean $= 0.19 \pm 0.03 \text{ L g}^{-1} \text{ h}^{-1}$) and lowest in Summer (June–September $= 0.07 \pm 0.01 \text{ L g}^{-1} \text{ h}^{-1}$). Partial recovery in clearance rates was observed in Fall (October–November $= 0.11 \pm 0.03 \text{ L g}^{-1} \text{ h}^{-1}$) after the decline of MC concentrations in water. Clams incubated at standardized (20 °C) and in situ (river) temperatures exhibited similar seasonal patterns in clearance rates (see SI Table S3). Clearance rates were significantly correlated with water MC concentrations ($R^2 = 0.66, p < 0.01$); a nonlinear model depicted rapid declines in clearance when MC exceeded 0.2 $\mu g \text{ L}^{-1}$.

Interspecific variation in MC content among primary consumers was related to dietary habits as inferred from gut contents and stable isotope analyses (Figure 4). CHL$\alpha$ concentrations in fish gut contents and $\delta^{13}$C values of muscle tissue were both significant predictors of interspecific differences in liver MC ($R^2 = 0.82, p = 0.012$ and $R^2 = 0.62, p = 0.038$, respectively). Intraspecific (month-to-month) variation in the two dietary metrics was generally low (SE < 25% of mean) with the exception of YOY Gizzard Shad, which also showed the greatest variability in liver MC concentrations. Highest liver MC concentrations and lowest $\delta^{13}$C values were observed among planktivores (Threadfin Shad, YOY Gizzard Shad, and Atlantic Menhaden). Two of these taxa (excluding Menhaden) also exhibited high concentrations of CHL$\alpha$ in their gut contents. By comparison, the benthic detritivores (Blue Catfish, adult Gizzard Shad) showed lower liver MC concentrations, less depleted $\delta^{13}$C values and lower CHL$\alpha$ in their gut contents. These findings were consistent with observed differences in CHL$\alpha$, MC, and $\delta^{13}$C signatures between suspended and sedimented materials. Suspended particulate matter collected during peak algal abundance was depleted in $\delta^{13}$C ($-28.5 \pm 0.2 \%$) compared to samples collected during high discharge ($-26.8 \pm 0.1 \%$) and from surficial sediments ($-26.7 \pm 0.1 \%$). Suspended particulate matter contained 200-fold greater CHL$\alpha$ (1,650 $\mu g \text{ g}^{-1} \text{ DW}$) than sedimented materials (7 $\mu g \text{ g}^{-1} \text{ DW}$) and was 4 orders of magnitude higher in MC (16 100 $\mu g \text{ kg}^{-1} \text{ DW}$ and 0.4 $\mu g \text{ kg}^{-1} \text{ DW}$, respectively). MC concentrations in sediment varied seasonally with peak values in September corresponding to highest sediment CHL$\alpha$ content (see SI Table S4).

### DISCUSSION

We found widespread cyanotoxin contamination among the dominant fish and shellfish of the tidal freshwater segment in the James River Estuary. The ubiquitous presence of MC in water (>90% of samples) and biota (~67% of individuals) was surprising given that cyanobacteria are a minor component of the phytoplankton community. During the period of study, cyanobacteria accounted for 6% of biomass (peak = 9%) with diatoms (76%) and chlorophytes (16%) accounting for the bulk (mean for weekly samples; H. Marshall, ODU, Pers. comm.). Although the tidal-fresh James exhibits some of the common features of eutrophic systems (shallow depth, high CHL$\alpha$, elevated nutrient loads), it is actively mixed by tidal forces, which negate the advantages of buoyancy that favor some cyanobacteria (*e.g.*, *Microcystis*). Strong tidal mixing likely accounts for the low spatial variability observed in MC concentrations among the three sites. Our findings show that even in systems with low cyanobacteria abundance (<10% of algal biomass) and moderate levels of MC ($\sim$1 L$^{-1}$), toxin exposure may be widespread among consumers. Avoidance of cyanobacteria has been documented in a wide range of consumers, but these mechanisms may be inefficient or

![Figure 3. Seasonal variation in feeding rates and microcystin content in tissues of the common wedge clam (*Rangia cuneata*) in the tidal freshwater segment of the James River Estuary during May 2012 to March 2013 (mean $\pm$ SE; some error bars not visible).](image-url)
nonadvantageous when cyanobacteria constitute a minor component of food resources.

An important and novel finding from this study is that variable levels of MC in consumer tissues can be linked to feeding habits and dietary exposure. Pelagic-feeding, planktivorous fishes had higher levels of CHLa in their diet as well as higher concentrations of MC in their tissues compared to benthic-feeding detritivores. These differences reflect the orders of magnitude lower concentrations of CHLa and MC in sediments in comparison to suspended particulate matter. Lower sediment concentrations are likely due to dilution by watershed-derived particulate matter. Prior references re
ter-feeding clams may also re
dilution by watershed-derived particulate matter may also contribute to low MC concentrations in sediment. The ratio of CHLa to MC in suspended particulate matter was ∼80:1, whereas the corresponding value for surficial sediments was >1000, suggesting postdepositional biodegradation of MC.\(^55\) It has also been reported that MC can adsorb to clay particles and thereby become resistant to conventional extraction procedures. Rinta-Kanto et al.\(^57,58\) attributed the lack of microcystin in Lake Erie sediments to this mechanism, though other studies have reported high values in sediments using similar extraction techniques.\(^57,58\) ELISA-based results may be indicative of bioavailable (vs total) MC in sediment as this test detects free MCs and metabolized MCs that cross-react with antibodies by immuno-affinity.

Inferences regarding algal contributions to consumer diets based on CHLa analysis of gut contents were supported by stable isotope results. Carbon isotopic signatures of surficial sediments and tissues of benthic-feeding fishes were similar to seston samples collected during high discharge events when contributions from terrestrial sources are greatest. Isotopic signatures of planktivorous fishes were more similar to seston samples collected during periods when autochthonous contributions were greater (high CHLa:TSS). Thus, both lines of evidence (gut contents and stable isotopes) show that toxin exposure is linked to diet whereby autochthonous sources comprise a greater proportion of the diets of pelagic fishes and lead to greater MC content in tissues. The benefit of linking toxin content in tissues with dietary habits is in providing a framework for assessing threats to aquatic biota and pathways of human exposure. In the James, species at greatest risk for cyanotoxin exposure include Atlantic Menhaden, YOY Gizzard Shad, Threadfin Shad and anadromous shad (\(Alosa\) spp.; not sampled in this study). Benthic feeders such as juvenile Blue Catfish, adult Gizzard Shad and Atlantic Sturgeon experience lower exposure.

The benthic filter-feeder Rangia exhibited low tissue MC concentrations comparable to benthivorous fishes. \(Rangia\) is considered a generalist suspension feeder but \(\delta^{13}C\) values suggest that autochthonous organic matter comprises a small fraction of their diet. When feeding, \(Rangia\) extend their siphons above the sediment–water interface and may ingest sedimented and recently resuspended particulate matter. Prior work has shown that benthic sources of particulate matter can be important to bivalves such as oysters.\(^59\) Low toxin content of clams may also reflect avoidance of MC ingestion due to seasonally variable feeding rates. For the average body size of individuals used in our experiments, clearance rates were 12.0 L ind\(^{-1}\) d\(^{-1}\) during periods when MC concentrations were low (Spring and Fall) and 4.0 L ind\(^{-1}\) d\(^{-1}\) when MC concentrations were elevated (June–September). Taking into account their density in this segment of the James (mean = 30 ind m\(^{-2}\) based on CBP annual benthic surveys for 2001–2010) and the average depth (1.3 m), this seasonal decline in feeding corresponds to a drop in water-column filtration rates from 28% to 10% d\(^{-1}\). This drop does not appear to be a temperature effect as it was observed in clams incubated at both ambient (river) and standard (20 °C) temperatures (SI Table S3). Without controlled experiments, we can not infer whether reduced feeding rates are caused by the presence of MC, other aspects of food quality,\(^59\) or from endogenous factors such as reproductive cycles.\(^60\) This question merits further investigation as it has direct implications for assessing harmful bloom effects on valuable ecosystem services provided by filter-feeding bivalves.\(^61\)

Our data on toxins in secondary consumers is limited to Blue Crabs and adult (>40 cm) Blue Catfish. Adult, piscivorous catfish exhibited lower MC concentrations (0.026 μg g\(^{-1}\) DW) relative to smaller, detritivorous catfish (<20 cm = 0.086 μg g\(^{-1}\) DW; 20–40 cm = 0.093 μg g\(^{-1}\) DW) consistent with prior

Figure 4. Microcystin content in liver tissues of fish and shellfish in relation to algal contributions to their diet as indicated by CHLa in gut contents and stable C isotopic signatures of consumer tissues. Data points are mean and SE based on monthly collections during May–October 2012. Microcystin concentrations for the wedge clam (\(Rangia\)) are based on analysis of viscera. Regression lines are based on Model II regression analysis.
studies showing that MC does not bioaccumulate. In contrast, Blue Crabs exhibited the highest incidence of muscle contamination among all consumers (72% of individuals) and 4- to 10-fold higher toxin concentrations than their prey (Rangia). Our estimates of MC in Blue Crab muscle tissue (0.020 μg g⁻¹ DW) were similar to those previously reported by Garcia et al. for a eutrophic Louisiana estuary (0.021 μg g⁻¹ DW). Laboratory studies by Dewes et al. on an estuarine burrowing crab (Chasmagnathus) demonstrated that tissue concentrations exceeding 0.013 μg MC g⁻¹ induced physiological and biochemical imbalances. These findings suggest that toxins may have detrimental effects on James River Blue Crab populations. As reported in other studies, we observed lower toxin levels in muscle relative to liver and viscera. Our estimates of MC in Blue Crab muscle tissue (0.020 μg g⁻¹ DW) were similar to those previously reported by Garcia et al. and using our monthly crab MC concentrations, we found that for a serving size of 300 g (wet weight), MC ingestion corresponded to 31 to 150% of the TDI guideline (mean = 73% for all months). This serving size exceeded the TDI guideline in all months (mean = 114% of TDI) when we included a small proportion of viscera (10%) in the consumed portion due to the higher concentrations of MC in hepatopancreas tissues. However, it should be noted that the long-term average shellfish consumption would be substantially lower than the serving size used for this analysis and therefore unlikely to exceed TDI guidelines over a lifetime of exposure. At present, little is known regarding acute vs chronic exposure to MC in humans or other biota to allow an assessment of their relative risks.

Seasonal variation in tissue MC concentrations was apparent in all consumers; in some cases, these tracked seasonal patterns in water MC concentrations (SI Figure S2). Statistically significant correlations between water and tissue concentrations were observed in Threadfin Shad and Wedge Clams but not among Catfish, Gizzard Shad or Blue Crabs. Intraspecific variation in tissue MC concentrations was small in relation to interspecific differences with the exception of YOY gizzard shad. Gizzard Shad shift their feeding habits from planktivory as juveniles to greater reliance on detritivory as adults. We observed decreasing CHLa in gut contents and lower liver MC concentrations in succeeding months suggesting that ontogenic shifts in feeding reduced their exposure to MC. For all consumers, measurable toxin levels persisted in tissues during periods when MC was not detected in the water column (March 2013 sampling). Ozawa et al. similarly reported measurable levels of MC in freshwater snails during fall and winter following a spring cyanobacterial bloom. These findings suggest that although the toxin is known to be metabolized, health effects may occur outside of bloom periods when toxins are produced.

In addition to monitoring toxin levels, we used a variety of approaches to track the abundance of cyanobacteria. Prior work has shown that phycocyanin can predict the occurrence of elevated MC. We found that continuous monitoring of phycocyanin was useful for discerning bloom events at fine-scale (daily) resolution and in providing real-time information to trigger sampling activities. The genetics analysis allowed us to bridge the phycocyanin and toxin results by identifying bloom events associated with peaks in the number of gene copies specific to Microcystis and toxin production (mcyD). The July and August peaks (as delineated by phycocyanin) were associated with highest toxin levels as well as highest abundance of Microcystis and mcyD, whereas the May, June, and October peaks exhibited low toxin levels and low abundance of mcyD. An exception was the September peak during which MC concentrations were high, but Microcystis and mcyD abundance was low. Elevated toxin levels may indicate persistence of the toxin in the water column from preceding peaks. There was a strong correlation between gene copies of Microcystis and mcyD (R² = 0.83, p < 0.01) suggesting that either the former contributed directly to the latter, or that variation in Microcystis abundance was synchronized to that of other toxin-producers. On average, the number of copies of the toxin-coding gene was 20% of Microcystis gene copies. This proportion was higher (40%) during periods of elevated toxin concentration (July and August), and below 10% in other months. Thus, variation in toxin concentrations was principally driven by cyanobacteria abundance (which varied by orders of magnitude) and secondarily by variation in the proportion of toxin-producing strains (which varied by 3-fold).

In summary, we surveyed the dominant consumers comprising the James River food web to assess seasonal and interspecific variation in toxin exposure. Peak occurrence of toxin contamination (% of individuals) and peak tissue concentrations were observed in months with highest toxin levels in the water column. Low but measurable toxin levels persisted in overwintering populations suggesting that potential impacts on aquatic biota may not be confined to bloom periods. Tissue MC concentrations varied 10-fold among species and revealed variable levels of exposure associated with differences in feeding habitats (benthic vs pelagic) and dietary sources (autochthonous vs allochthonous). Measurements of CHLa in gut contents and stable C isotopes allowed us to characterize food sources supporting secondary production and to explicitly link feeding habits with cyanotoxin exposure. Highest MC levels were found among pelagic, planktivorous fishes with lower concentrations measured in benthic detritivores. Pelagic feeders were subject to greater toxin exposure due to a greater proportion of autochthonous organic matter in their diets. For the suspension-feeding wedge clam, exposure was mitigated by low feeding rates in summer when MC was present in the water column. Among secondary consumers, linkages between feeding and exposure were less clear as adult Blue Catfish exhibited lower toxin levels than their prey, whereas Blue Crabs had higher levels of toxin. Elevated toxin levels in Blue Crabs raise concern for detrimental effects on their populations and human health impacts.

### ASSOCIATED CONTENT

#### Supporting Information

Map of study site (Figure S1), plots of water column vs tissue MC concentrations for fish and shellfish (Figure S2), comparison of extraction efficiencies to previously published values (Table S1), body size and average microcystin concentrations in tissues of fish and shellfish (Table S2), clearance rates by the wedge clam at ambient and standard temperatures (Table S3), CHLa and microcystin concentrations in suspended and sedimented particulate matter (Table...
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REFERENCES


(33) Jenkins, R. E.; Burkhead, N. M. *Freshwater Fishes of Virginia; American Fisheries Society*, 1994.


