Microcystin in aquatic food webs of the Baltic and Chesapeake Bay regions.

Paul A. Bukaveckas^{1*}, Jūratė Lesutienė², Zita R. Gasiūnaitė², Linas Ložys³, Irina Olenina⁴, Renata Pilkaitytė², Žilvinas Pūtys³, Spencer Tassone¹ and Joseph Wood⁵.

1. Virginia Commonwealth University, Center for Environmental Studies and Department of Biology, Richmond, VA.

2. Open Access Centre for Marine Research, Klaipėda University, Klaipėda, Lithuania

3. Laboratory of Marine Ecology, Nature Research Centre, Vilnius, Lithuania

4. Department of Marine Research, Environmental Protection Agency of Lithuania, Klaipėda, Lithuania.

5. Chesapeake Bay Foundation, Richmond, VA.

*Corresponding Author (pabukaveckas@vcu.edu)

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Abstract

We undertook a comparative study of the James River Estuary, a sub-estuary of Chesapeake Bay, and the Curonian Lagoon, a sub-estuary of the Baltic Sea, to better understand the factors that determine the presence and persistence of algal toxins in food webs. Over a 2-year period, we measured microcystin concentrations in water, sediment and biota (fish and shellfish) at both sites. Across both food webs we found highest levels of microcystin among consumers of suspended particulate matter, including planktivorous fishes and filter-feeding shellfish, and lower levels of toxin among piscivores, scavengers and benthic omnivores. Despite similar levels of microcystin in the water column at the two sites, we observed higher toxin levels in fish and sediments of the Curonian Lagoon. We attribute this difference to the legacy of prior toxic cyanobacteria blooms in the Curonian Lagoon and hydrologic factors that result in a predominance of autochthonously-derived organic matter in the sediments at this site. Our results suggest that a consideration of species-specific differences in feeding habits, and organic matter sources supporting food webs are important to understanding the accumulation and persistence of algal toxins in food webs and should therefore be considered in assessment of risks to aquatic biota and human health.

Introduction

Nutrient enrichment of coastal waters has resulted in an increase in the frequency, intensity and duration of harmful algal blooms (HABs; Paerl et al. 2009, Heisler et al. 2011, O'Neil et al. 2012, Michalak et al. 2013, Paerl and Otten 2013a). Harmful algae produce toxins which have deleterious effects on aquatic biota and pose a human health concern (Miller et al. 2010, Poste et al. 2011, Corbel et al. 2014, Ibelings et al. 2014). Human health threats arise from direct contact with waters containing harmful algae, and from ingestion of toxins via contaminated drinking water, crops (via irrigation), and fish-shellfish. Among the most widely occurring harmful algae are cyanobacteria, which are commonly found in fresh-brackish waters, including the Baltic Sea and tributaries of the Chesapeake Bay (Karjalainen et al. 2007, Anderson et al. 2008, Mazur-Mazec et al. 2013, Wood and Bukaveckas 2014). Cyanobacteria produce a variety of toxins, the most common of which is microcystin, a hepatotoxin known to cause damaging health effects on humans and biota (Corbel et al. 2014, Rastogi et al. 2014). Microcystin accumulates in the tissues of a wide range of aquatic consumers (Lehman et al. 2010, Wood and Bukaveckas 2014). Physiological responses of organisms may include liver damage, behavioral changes, reduced fecundity and mortality (Corbel et al. 2014, Rastogi et al. 2014). The toxin is thought to be a chemical defense against predators, though the environmental factors favoring its occurrence are poorly understood (Davis et al. 2009, Paerl and Otten 2013b, Boopathi and Ki 2014). The World Health Organization has established guidelines for consumption and exposure (WHO 2003); however, monitoring is often lacking, thus limiting our ability to assess environmental and human health risks. There is a need to better understand the factors governing the presence of algal toxins in aquatic biota, particularly among commercially and recreationally important fish and shellfish.

The presence and persistence of algal toxins in food webs is governed by a variety of factors including the frequency, timing and intensity of HAB events, food web dependence on autochthonous production, and factors governing the fate and storage of toxins (e.g., sedimentation, degradation, washout). Algal toxins are not known to biomagnify (Ibelings et al. 2005, Kozlowsky-Suzuki et al. 2012), therefore highest concentrations (per unit mass) are found in the algae themselves, with diminishing concentrations along the food chain. At higher trophic levels, toxin concentrations are related to the proportion of consumer diet derived from algal

biomass (Wood et al. 2014). Higher toxin concentrations would be expected among suspension feeders, including planktivorous fish and benthic filter-feeders, in comparison to predators and benthic omnivores, due to greater ingestion of toxic algae. Species-specific attributes such as depuration and ontogenetic shifts in feeding, are also important in determining variation in toxin levels among consumers. Few studies have characterized algal toxins at the food-web scale, thus limiting cross-system comparisons which are needed to disentangle these various factors and improve understanding of risks to aquatic biota and human exposure.

In this paper, we present a comparative analysis of microcystin in the food webs of the James River Estuary, a sub-estuary of Chesapeake Bay, and the Curonian Lagoon, a sub-estuary of the Baltic Sea. These sites were selected on the basis of having well-described food webs, a known history for the occurrence of toxic cyanobacteria and the presence of commercial and recreational fisheries (Wood et al. 2014, Lesutiene et al. 2014). We characterized microcystin concentrations in water, sediment, and fish-shellfish tissues to better understand how fish feeding habits, and the history of cyanobacteria blooms, effect toxin concentrations at higher trophic levels. Microcystin data for fish tissues were also used to assess potential human health risks.

Methods

This study was conducted in the tidal freshwater segment of the James (JTF) and the northern (Lithuanian) portion of the Curonian Lagoon (NCL) which are located in the mid-Atlantic region of the United States and northern Europe, respectively (Figure 1; Table 1). The two sites are similar in being eutrophic with summer chlorophyll-a (CHLa) typically exceeding $30 \mu\text{g L}^{-1}$ and maximum values over $100 \mu\text{g L}^{-1}$ (Bukaveckas et al. 2011, Bukaveckas and Isenberg 2013, Wood and Bukaveckas 2014, Zilius et al. 2014). The sites differ in their relative contributions of cyanobacteria to phytoplankton biomass. In the James, phytoplankton communities are dominated by diatoms and chlorophytes, with a small contribution from cyanobacteria (<10% of biomass) and low levels of cyanotoxins (Wood et al. 2014). Phytoplankton communities in the Curonian Lagoon are characterized by blooms of cyanobacteria during summer, low-wind conditions (Zilius et al. 2014). Stable isotope studies have shown that cyanobacteria biomass is rapidly exploited by a wide range of consumers in the Lagoon (Lesutiene et al. 2014).

95 *Sampling – Curonian Lagoon*

96 Water, sediment and fish-shellfish samples were collected from the Curonian Lagoon
 97 during 2013-2014. Near-surface (~1 m) water samples were obtained semi-monthly during
 98 August-October 2013 and May-October 2014 at 4 stations distributed throughout the northern
 99 half of the lagoon (total N = 105). Water samples were analyzed for CHLa and microcystin. In
 100 addition to data collected for this study, we provide historical data (2006-2014) on CHLa and
 101 phytoplankton community composition collected semi-monthly at a station located in the
 102 northern half of the lagoon by the Department of Marine Research of the Environmental
 103 Protection Agency of Lithuania. Sediment samples were collected in May, August and October
 104 2014 to represent pre-, peak- and post- bloom conditions, respectively. Three replicate cores
 105 were collected at each of two sites representing the dominant substrate types of the lagoon (silt
 106 vs. sand; Zilius et al. 2014). Cores were obtained using plexiglass tubes mounted in a hand corer.
 107 The cores were sectioned and the top (0-0.5 cm), middle (1-2 cm) and bottom (5-10 cm) sections
 108 were analyzed for dry mass (dm), CHLa and microcystin (total N = 62).

109 Fish and mollusks were sampled in September 2013, May 2014 and August 2014 to
 110 represent post-, pre- and peak- bloom conditions, respectively. Sampling was conducted along
 111 the eastern shore of the lagoon (near Ventė cape; Figure 1). Six fish species belonging to three
 112 ecologic groups were selected: predatory Pike-perch (*Sander lucioperca*), benthic omnivores
 113 European Perch (*Perca fluviatilis*), Roach (*Rutilus rutilus*) and Freshwater Bream (*Abramis*
 114 *brama*), and planktivorous Sabre Carp (aka Sichel; *Pelecus cultratus*) and Bleak (*Alburnus*
 115 *alburnus*). Bream, Roach, Perch and Pikeperch are the most important commercial fish in the
 116 Curonian Lagoon (comprising ~80 % of landings). For Perch and Roach we sampled both adults
 117 and juveniles to assess ontogenetic shifts in microcystin exposure. Adult fish were caught using
 118 gillnets deployed at 2-4 m depth. Sabre Carp were obtained from commercial fisherman.
 119 Juvenile fish and Bleaks were caught using a beach seine at up to 1 m depth. Microcystin
 120 analysis was performed on dorsal muscle and liver tissues. The dominant benthic filter-feeders,
 121 Zebra Mussels (*Dreissena polymorpha*) and Painter's mussel (*Unio* spp.), were collected from
 122 fish nets, washed and placed overnight for gut content evacuation. Whole soft tissues of small
 123 Zebra Mussels (1.0 ± 0.1 cm) and foot and mantle tissues of larger Zebra Mussels (~2 cm) and

Painter's mussel (6-8 cm) were dissected for microcystin analysis. A total of 566 fish and 40 mollusk samples were analyzed.

Sampling – James River

Sampling of water, sediment and biota from the James River Estuary was conducted in 2012-2013 as previously described in Wood et al. (2014). Near-surface (1 m) water samples were collected ~weekly from May through November 2012 at three sites (total N = 72). Sediment samples were collected monthly from May to October 2012 at each of three near-shore sites (depth < 2 m). Sediments from these sites were typical for the area being dominated by silty deposits. Triplicate samples of surficial sediments (0-2 cm) were analyzed for dry mass, CHLa and microcystin (total N = 54). Fish sampling targeted abundant and ecologically important taxa including planktivores (juvenile American Gizzard Shad (*Dorosoma cepedianum*), Threadfin Shad (*D. petenense*), and Atlantic Menhaden (*Brevoortia tyrannus*)), benthivores (juvenile Blue Catfish (*Ictalurus furcatus*) and adult American Gizzard Shad) and piscivores (Blue Catfish > 40 cm). We also sampled the common wedge clam (*Rangia cuneata*), which is the dominant benthic filter-feeder, and Blue Crabs (*Callinectes sapidus*) as the latter are a pathway for human exposure. Approximately 10-15 individuals of each were obtained monthly during May-October 2012 with additional samples collected in March 2013 to assess toxin levels in over-wintering populations. Fish were obtained by electrofishing (low and high frequency) along multiple transects located in proximity to the water monitoring locations. Blue Crabs were obtained from multiple crab pots deployed in proximity to fish sampling areas. *Rangia* were collected from a near-shore site using oyster tongs and held in deionized water for 48 h 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 microcystin analysis. Microcystin analysis was performed on a total of 268 fish and 126 shellfish samples.

Analytical Methods

CHLa (water and sediments) was measured by extraction in 90% buffered acetone and fluorometric analysis (VCU) and by extraction in ethanol and spectrophotometric analysis (Department of Marine Research, DMR). Phytoplankton samples from the DMR long-term

monitoring station were analyzed by the inverted microscopy counting technique (Olenina et al. 2006, Jaanus et al. 2011). All microcystin samples were analyzed at Virginia Commonwealth University (VCU). Our analysis of water, sediment and tissue samples was based on ELISA, a method which offers several advantages for studies with large sample sizes, such as food web assessments. Commercially available kits allow for efficient sample processing while providing high sensitivity at low levels of microcystin relevant to ecotoxicological studies (ADDA ELISA Kit; detection limit $0.05 \mu\text{g L}^{-1}$; Abraxis; Warminster, PA). The assay measures numerous forms of free microcystin using polyclonal antibodies, which allows for quantification of all reactive microcystin variants in a single assay (reported as microcystin-LR equivalents). The assay measures only free microcystin and does not account for the presence of protein (covalently)-bound microcystin. Protein-bound microcystin has lower bioavailability, and therefore the extractable fraction (as measured by ELISA) may be a better indicator for human health concerns, though this has not been established experimentally (Ibelings and Chorus 2007, Neffling et al. 2010). It has been reported that for tissue samples, ELISA results in a higher proportion of positive detections at low concentrations ($< 10 \text{ ng g}^{-1} \text{ dm}$) compared to mass spectrometry-based methods (Geis-Asteggianti et al. 2011). It is unclear whether this discrepancy is due to false positives from cross-reaction with non-microcystin metabolites during ELISA, or if these represent findings below the detection limit for mass spectrometry. In our dataset, non-zero values in this range ($< 10 \text{ ng g}^{-1} \text{ dm}$) represented 7% (James) and 5% (Curonian Lagoon) of the samples analyzed.

To measure total microcystin in water, samples were thawed and refrozen two times (as recommended by the manufacturer), and then microwaved and sonicated to release microcystin from cells (Silva-Stenico et al. 2009). All water samples exceeding 0.15 ppt were pre-treated by passing through a resin column to remove interference from salinity (as per manufacturer's recommendations). Samples of tissue were dissected immediately upon collection and dried in preparation for extraction (see below). We determined both the wet and dry weight of each sample. Microcystin concentrations were expressed per unit of dry mass; for Estimated Daily Intake, we used concentrations per unit of wet weight (see below). For extraction, we used 0.5 to 1 g dm of liver or muscle tissue, with the exception of small fishes (bleak, juvenile Yellow Perch and juvenile roach), for which liver tissues samples were 0.1 to 0.3 g dm.

To extract microcystin 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 (Babica et al. 2006, Wilson et al. 2008, Garcia et al. 2010). 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, 8 standards and all samples were run in duplicate. The mean standard error among duplicate water samples was 0.02 $\mu\text{g L}^{-1}$; the mean standard error for duplicate tissue samples was 0.021 $\mu\text{g g dm}^{-1}$. Average recovery from positive internal controls was $98 \pm 4\%$. Average recoveries from spiked samples were as follows: water-sediment = $94 \pm 6\%$, sediment = $90 \pm 11\%$, shellfish viscera = $67 \pm 7\%$, fish liver = $74 \pm 15\%$ and fish muscle = $89 \pm 12\%$ (Wood et al. 2014). The method used in our study (ADDA ELISA with methanol extraction) was shown to have the best recovery among four sample processing methods, with low matrix effects even at concentrations less than 10 $\text{ng g}^{-1} \text{dm}$ (Preece et al. 2015).

Statistical Analysis

Microcystin concentrations in tissues are not normally distributed due to the occurrence of rare, high values. Therefore data for individual species are presented as both means and medians, the former reflecting the influence of the tail of the distribution, and the latter providing a better indicator of central tendency. For statistical analysis, the data were log-transformed. A 2-way ANOVA was used to determine what proportion of the variation in tissue microcystin concentrations among individuals could be accounted for by site (James River, Curonian Lagoon) and feeding group (planktivores, benthic omnivores and predators). One-way ANOVA of log-transformed data was used to test for significant differences in tissues microcystin concentrations across months (seasonal effects) and between juveniles and adults of the same species (ontogenic effects).

Estimated Daily Intake

To assess human health risks associated with consumption of fish and shellfish, we compared Estimated Daily Intake (EDI) with the Tolerable Daily Intake (TDI) proposed by the World Health Organization (2003). The TDI value ($0.04 \mu\text{g MC kg}^{-1} \text{body weight d}^{-1}$) is an allowable daily rate of toxin ingestion based on the consumer body mass. For a body mass of 60

kg, the ingestion threshold is $2.4 \mu\text{g MC d}^{-1}$. For comparison, we derived EDI values based on measured toxin concentrations in fish-shellfish and an assumed serving size of 300 g wet mass (wm; Garcia et al. 2010). EDIs were derived for a single (daily) serving and, for long-term exposure, based on one serving per week. Tissue concentrations were for muscle, except in Blue Crabs for which consumption was based on 80% muscle tissue and 20% viscera.

Results

The Curonian Lagoon experienced recurring algal blooms in 2006-2014 during periods of low discharge and warm water temperature (Figure 2). A large *Microcystis* bloom occurred in 2006 when biomass reached 5.3 mg L^{-1} and comprised 60% of total cyanobacteria biomass. A prior study reported that microcystin concentrations reached $134 \mu\text{g L}^{-1}$ in that year (Paldaviciene et al. 2015). The long-term data presented here shows that in subsequent years, cyanobacteria blooms were dominated by *Anabaena* (now *Dolichospermium*) and *Planktothrix*, with *Microcystis* accounting for ~5% of biomass. Over the 2-years when the food-web study was conducted, average CHLa concentrations were higher in the Curonian Lagoon ($73 \pm 18 \mu\text{g L}^{-1}$) relative to the James ($27 \pm 2 \mu\text{g L}^{-1}$), whereas microcystin concentrations were similar (0.30 ± 0.07 and $0.32 \pm 0.04 \mu\text{g L}^{-1}$, respectively) at the two sites (Figure 3). An exception was the bloom event occurring in the Curonian Lagoon on September 5 2014 when CHLa concentrations exceeded $100 \mu\text{g L}^{-1}$ and microcystin concentrations reached $4.34 \mu\text{g L}^{-1}$ at one of the 4 stations. Microcystin concentrations were otherwise less than $1 \mu\text{g L}^{-1}$ in both the James River Estuary and Curonian Lagoon.

In the Curonian Lagoon, substrate type (silty vs. sandy) was an important determinant of sediment microcystin and CHLa content (Figure 4). In silty sediments (southern lagoon, Figure 1), microcystin concentrations averaged $26 \pm 7 \text{ ng g}^{-1} \text{ dm}$ (mean across months), whereas at the sandy site (northern lagoon), sediment microcystin concentrations were $\sim 1 \text{ ng g}^{-1} \text{ dm}$. Higher microcystin concentrations in silty sediments corresponded to elevated CHLa content (mean = $192 \pm 13 \mu\text{g g}^{-1} \text{ dm}$) relative to sandy sediments (mean = $14 \pm 2 \mu\text{g g}^{-1} \text{ dm}$). In silty sediments, stratigraphic analysis revealed higher microcystin levels at 1-2 cm depth ($39 \pm 18 \text{ ng g}^{-1} \text{ dm}$) relative to surficial (0-0.5 cm mean = $14 \pm 9 \text{ ng g}^{-1} \text{ dm}$) and deeper (5-10 cm mean = $10 \pm 2 \text{ ng g}^{-1} \text{ dm}$)

¹ dm) sediments (see Supplemental Information). Areal values for total microcystin (top 10 cm of sediment) were 323 and 122 $\mu\text{g m}^{-2}$ (silty and sandy, respectively) as the lower mass density of silty (12 kg dm m^{-2}) vs. sandy (125 kg dm m^{-2}) sediments partially offset higher microcystin concentrations (per unit mass) in silty sediments. In the James, sediment microcystin concentrations ranged from below detection limits (0.1 ng g^{-1} dm; May, June and October) to 2.6 ± 0.1 ng g^{-1} dm (September). Seasonal patterns of microcystin concentrations corresponded to patterns in sediment CHLa concentrations, which ranged from 1 to 21 $\mu\text{g g}^{-1}$ dm, with peak values in September. Overall, the mean microcystin concentration of Curonian Lagoon sediments (17 ± 4 ng g^{-1} dm based on silty and sandy sites) was 40-fold higher than the mean value for the James Estuary (0.4 ± 0.2 ng g^{-1} dm).

Higher microcystin content of Curonian Lagoon sediments corresponded to higher microcystin concentrations among fish and shellfish relative to those observed in the James River (Figures 5 and 6). Across all feeding types, the average liver microcystin concentration among Curonian fishes (mean = 193 ± 9 ng g^{-1} dm; median = 144 ng g^{-1} dm) was significantly greater than the mean concentration among James River fishes (mean = 112 ± 14 ng g^{-1} dm; median = 33 ng g^{-1} dm). A two-way ANOVA revealed that both Site (James, Curonian) and Feeding Group (Benthic, Predator and Planktivore) were significant factors accounting for variation in microcystin concentrations ($R^2 = 0.27$, $F = 45.1$; $p < 0.0001$; $N = 635$). Benthivores of the Curonian Lagoon (Freshwater Bream, juvenile European Perch, and juvenile and adult Roach; range of medians = 143-197 ng g^{-1} dm) exhibited liver microcystin concentrations that were 5- to 10- fold higher than benthivores of the James Estuary (juvenile Blue Catfish and adult American Gizzard Shad; medians = 35 and 10 ng g^{-1} dm, respectively). Among piscivores, fishes of the Curonian Lagoon (Zander median = 175 ng g^{-1} dm, adult European Perch median = 113 ng g^{-1} dm) had 6-fold greater liver microcystin concentrations than adult Blue Catfish (James River; median = 25 ng g^{-1} dm). Blue Crabs (James River) were included in this predatory group and showed higher levels of microcystin (viscera) relative to Blue Catfish (median = 80 ng g^{-1} dm). Among planktivores, highest liver microcystin concentrations were found in Bleak (Curonian Lagoon; median = 312 ng g^{-1} dm). Concentrations were otherwise similar among juvenile American Gizzard Shad (James; median = 103 ng g^{-1} dm), Atlantic Menhaden (James; median = 95 ng g^{-1} dm), Threadfin Shad (James; median = 130 ng g^{-1} dm) and Sabre Carp

(Curonian Lagoon; median = 135 ng g⁻¹ dm). Among benthic filter-feeders, Painter's and Zebra Mussels (Curonian Lagoon; median = 401 and 224 ng g⁻¹ dm, respectively) had 10-fold higher microcystin levels (whole-body) relative to Wedge Clams found in the James (25 ng g⁻¹ dm).

Seasonal variation in tissue microcystin concentrations was observed in both systems, with highest concentrations generally occurring in Summer or Fall, and lowest levels observed in Spring (Figure 7). Statistically significant seasonal differences were observed for Perch (juveniles), Roach, Bream, Gizzard Shad (juveniles), Blue Catfish and Threadfin Shad. Greatest seasonal variation occurred among planktivores in the James (Threadfin Shad and juvenile American Gizzard Shad) which exhibited 5-10 fold higher liver concentrations in Summer. Microcystin levels in juvenile European Perch were found to be significantly higher than in adults (mean = 192 ± 21 ng g⁻¹ dm and 132 ± 10 ng g⁻¹ dm, respectively; F = 5.05, p = 0.027, N = 94). This likely reflects ontogenic shifts in diet from benthic omnivory as juveniles to piscivory as adults. A similar pattern was observed among American Gizzard Shad in the James with a shift from high microcystin in planktivorous juveniles (mean = 247 ± 101 ng g⁻¹ dm) to lower microcystin in detritivorous adults (mean = 38 ± 9 ng g⁻¹ dm; Wood et al. 2014, Schaus et al. 2002). No significant differences in microcystin content were found between juvenile and adult Roach (F = 0.01, p = 0.9, N = 68).

Results of the EDI analysis for muscle tissue showed that while single servings of some fish exceeded the TDI threshold, the long-term exposure, based on one serving per week, did not (Table 2). Among fishes of commercial importance in the Curonian Lagoon, EDI for single servings of Freshwater Bream (3.4 µg MC d⁻¹), Roach (3.0 µg MC d⁻¹) and Sabre Carp (3.7 µg MC d⁻¹) exceeded the TDI threshold (2.4 µg MC d⁻¹). In the James Estuary, single servings were below EDI values (<0.4 µg MC d⁻¹) with the exception of Blue Crabs (2.3 µg MC d⁻¹). The higher value for Blue Crabs was due to the assumption that 20% non-muscle tissue was ingested, and because viscera contained high levels of microcystin (24 ± 4 ng g⁻¹ wm) relative to muscle (4 ± 2 ng g⁻¹ wm). The long-term exposure EDI values were <0.8 µg MC d⁻¹ for fish and shellfish from both systems, with the exception of Bleak (a non-commercial species). Bleak exhibited the highest liver microcystin concentrations and its muscle concentrations were 6-fold higher than the next closest fish (juvenile Roach).

Discussion

Our prior work showed that variable levels of algal toxins among fishes of the James River were linked to their dietary habits in that planktivorous fishes were found to have higher concentrations of microcystin relative to benthic omnivores and piscivores (Wood et al. 2014). These differences were correlated with higher CHLa in planktivore gut contents due to feeding on suspended particulate matter. Results from the present study comparing food webs of the James Estuary and Curonian Lagoon support this finding as we found statistically significant differences among feeding groups, with highest microcystin concentrations among suspension feeders such as the Painter's Mussel (Curonian Lagoon), Bleak (Curonian Lagoon) and juvenile American Gizzard Shad (James River). Lowest toxin concentrations were observed in piscivorous (adult) Blue Catfish, benthivorous (adult) American Gizzard Shad and Wedge Clams. These findings along with related studies (Wilson et al. 2008) suggest that a trophic perspective based on consumer feeding habits provides a basis for assessing risk to fisheries and human health posed by algal toxins.

Our comparative study revealed that the two systems exhibited similar and low levels of microcystin in the water column during the period of study, whereas microcystin levels were higher in sediments of the Curonian Lagoon. A prior study of the Curonian Lagoon had shown elevated levels of microcystin in Zebra Mussels over several years following the large *Microcystis* bloom in 2006 (Paldaviciene et al. 2015). Our findings expand on these results showing that elevated microcystin levels have persisted 7-8 years after the bloom event and extend to a wide range of consumers including benthivorous, planktivorous and piscivorous fishes. The persistence of lipophilic toxicants in sediments and food webs is well documented (e.g., mercury, PCBs). The low lipophilicity of microcystin and its depuration from biota would be expected to result in transient occurrence in biota during toxin-producing blooms. Our results for the Curonian Lagoon suggest that this is not the case and raise concerns for chronic exposure for biota and long-term human health risks. The low levels of microcystin in the water column ($<5 \mu\text{g L}^{-1}$ during the period of this study) are unlikely to cause mortality effects on biota, which are typically observed at much higher concentrations (100's – 1000's $\mu\text{g L}^{-1}$; Smith et al. 2008). However, little is known regarding sub-lethal effects and chronic, low-level exposure to microcystin in biota or humans.

We hypothesize that elevated levels of microcystin in the food web of the Curonian Lagoon is due to the persistence of the toxin in sediments. Consumer exposure occurs as a result of detritivory (by benthic omnivores) and, for suspension feeders, following periodic re-suspension of sediments during wind-driven mixing events (Paldaviciene et al. 2015). The predominance of autochthonously-derived organic matter in the sediments of the Curonian Lagoon may be a contributing factor to the occurrence of high microcystin concentrations in sediments. Sources of particulate organic matter to the lagoon are dominated by internal production with phytoplankton accounting for 69% of total inputs (Galkus and Jokšas 1997, Daunys et al. 2006). Allochthonous contributions are small because the principal tributary (Nemunas River) is a regulated, lowland river which drains peaty-sandy soils and carries low suspended particulate matter (Mikhailova and Zaromskis 2013). In addition, the Curonian Lagoon has a low hydrological loading factor (ratio of contributing area to surface area = 63) which, combined with the low sediment delivery (Daunys et al. 2006) translates to a loading rate of $278 \text{ g m}^{-2} \text{ y}^{-1}$. By comparison, the annual areal load for the tidal fresh segment of the James (1501 $\text{g m}^{-2} \text{ y}^{-1}$) is approximately five times higher (average for 2012-2014 based on our weekly monitoring of TSS and daily discharge recorded by the US Geological Survey). This reflects the large and mountainous watershed of the James (hydrologic loading factor = 184) which has a high sediment yield ($\text{TSS} > 200 \text{ mg L}^{-1}$ during high discharge events; Smock et al. 2005, Wood et al. 2016).

Our contention is that in coastal waters experiencing large sediment loads, inputs of allochthonous particulate matter will dilute autochthonously-derived organic matter thereby reducing long-term exposure to algal toxins. In systems dominated by autochthonous sources of particulate matter, such as the Curonian Lagoon, there is less dilution of algal toxins resulting in long-term exposure for consumers and persistence in food webs. Stratigraphic data from our silty site showed that sediments enriched with microcystin have been partially buried since the 2006 bloom event, with peak microcystin concentrations observed 1-2 cm below the sediment-water interface. Other studies have also documented the presence of microcystin in sediments (Misson et al. 2012, Takahashi et al. 2014), though questions about persistence and bioavailability remain. Based on the relative contributions of silty (44%) and sandy (55%) areas in the Lithuanian portion of the Curonian Lagoon (Zilius et al. 2014) we estimate a total mass of

84 kg of microcystin in the 0-10 cm sediment layer. By comparison, the mass of microcystin in the water column during the 2006 *Microcystis* bloom event was 5380 kg (based on a mean concentration of $3.43 \mu\text{g L}^{-1}$ measured at 7 stations; Paldaviciene et al. 2009). Prior research has demonstrated rapid degradation of cyanotoxins associated with sediment deposition in the Baltic Sea (Kankaanpaa et al. 2009). Our data from the Curonian Lagoon support this finding suggesting that the bulk of microcystin degraded or became covalently bonded within the sediment, and therefore not susceptible to conventional extraction techniques (Schmidt et al. 2014). Despite this, the small proportion of microcystin residing in the sediments (~2% relative to the mass in the water column in 2006) has resulted in persistent elevated levels of toxin in biota.

Our estimates of daily intake suggest that microcystin in fish-shellfish from the James River Estuary and Curonian Lagoon does not pose a human health risk based on current WHO guidelines and an assumed consumption rate of one serving per week. Observed microcystin levels also fell below proposed seasonal and acute tolerable intake values (Ibelings and Chorus 2007). Our analysis is based on arithmetic mean values for tissue concentrations; these are more protective of health risks than other indicators of central tendency (e.g., median or geometric mean) because the arithmetic mean is sensitive to the tail of the distribution (i.e., rare, high values). However it is important to note that there is a wide range of toxin concentrations within a population, and therefore individual fish can exceed the recommended guidelines, even for species in which mean concentrations fall below the guidelines.

In conclusion, our comparative study of microcystin in food webs of two coastal, eutrophic waterbodies suggests that a trophic perspective which considers consumer feeding habits and sources of organic matter to waterbodies may be useful in predicting the levels and persistence of algal toxins in food webs. Despite similar levels of microcystin in the water column, we found that microcystin concentrations in the food web and sediments of the Curonian Lagoon were higher than in the James River Estuary. We attribute this difference to a legacy effect of algal blooms occurring prior to this study, coupled with hydrologic factors that result in a predominance of autochthonous organic matter in the sediments of the Curonian Lagoon.

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394

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Table 1. Site characteristics of the James River Estuary and the Curonian Lagoon (JTF = James Tidal-Fresh segment; NCL = Northern Curonian Lagoon; Bukaveckas et al. 2011, Bukaveckas and Isenberg 2013, Ferrarin et al. 2008, Bresciani et al. 2012, Zemlys et al. 2013).

	James River Estuary	Curonian Lagoon
Surface Area (km ²)	604	1,584
Mean Depth (m)	3.5	3.8
Salinity (ppt)	0.1 to 20 (<0.5 in JTF)	0.1 to 7
Principal Tributary	James River (92% of total runoff)	Nemunas River (96% of total runoff)
Riverine Inputs (m ³ s ⁻¹)	227	698
Freshwater Replacement Time (d)	15-25 (JTF in Summer)	up to 80 (NCL)
Summer CHLa (µg L ⁻¹)	30-60 (max ~100)	>40 (max >400)

Table 2. Estimated daily ingestion (EDI) of microcystin via consumption of fish and shellfish from the Curonian Lagoon and James River Estuary (*denotes commercially and recreationally important species). EDIs were derived for a single serving on one day (300 g ww) and for long-term consumption based on one serving per week. Microcystin (MC) concentrations are for muscle tissue, except in Blue Crabs, for which consumption was based on 80% muscle and 20% viscera.

Site	Common Name	Tissue MC (ng/g ww)			EDI (µg MC/d)	
		N	Mean	SE	1 serv/d	1 serv/wk
Curonian Lagoon	Bream*	40	11	10	3.4	0.49
	Bleak	27	82	62	24.5	3.49
	European Perch*	40	4	2	1.1	0.16
	European Perch (juv)	56	10	8	2.9	0.42
	Roach*	39	10	16	3.0	0.42
	Roach (juv)	35	14	8	4.3	0.61
	Sabre Carp*	29	12	8	3.7	0.53
	Pikeperch*	32	5	3	1.5	0.22
James River Estuary	Atlantic Menhaden*	23	0.7	0.6	0.2	0.03
	Blue Crab Muscle*	36	4	2	2.3	0.66
	Blue Crab Viscera*	36	24	4		
	Blue Catfish (0–20 cm)*	26	0.9	0.5	0.3	0.04
	Gizzard Shad (adult)	60	0.3	0.1	0.1	0.01
	Gizzard Shad (juv)	32	0.5	0.2	0.1	0.02
	Threadfin Shad	42	1.0	0.2	0.3	0.04

Figure 1. Sampling locations within the James River Estuary and the Curonian Lagoon. Circles denote water and sediment sampling sites; triangles are fish-shellfish sampling sites; the square is a long-term phytoplankton monitoring station for the Environmental Protection Agency of Lithuania.

Figure 2. Seasonal and interannual variation in chlorophyll-a, temperature and cyanobacteria biomass in the Curonian Lagoon during 2006-2014. Discharge data for the principal tributary (Nemunas River) were provided by the Lithuanian Hydrometeorological Service.

Figure 3. Chlorophyll (CHLa) and microcystin (MC) concentrations in the Curonian Lagoon (2013-14) and the James River Estuary (2012-13). Values represent means for 4 (CL) and 3 (JRE) sampling locations (with standard error).

Figure 4. Chlorophyll-a (CHLa) and microcystin concentrations of surficial sediments (0-2 cm) from the James River Estuary and the Curonian Lagoon (note difference in y-axis scales). Curonian Lagoon sediments were sampled in May, August and October only. Values represent means for three replicate cores (with standard error) from each sampling location.

Figure 5. Comparison of microcystin concentrations in water/seston, sediment and biota of the James River Estuary and the Curonian Lagoon. Fish data are based on concentrations found in the liver; data for invertebrates are for viscera (mean $\pm$ SE). Sediment and biological data are mass-specific (ng MCg⁻¹dm); water data are volumetric (ng MC L⁻¹).

Figure 6. Microcystin concentrations in liver tissues of fishes from the Curonian Lagoon and James River Estuary. Benthic omnivores: juvenile Blue Catfish (Cat), adult Gizzard Shad (Gz), Bream (Br), juvenile Perch (jPe), Roach (Ro), juvenile Roach (jRo). Predators: adult Blue Catfish (C40), Blue Crabs (Crb), Zander (Zan), adult Perch (Pe). Planktivores: juvenile Gizzard Shad (jGz), Atlantic Menhaden (Men), Threadfin Shad (Thr), Bleak (Blk), and Sabre Carp (Carp). The line within each box represents the median, box boundaries are 25th and 75th percentiles, whiskers are 10th and 90th percentiles, and points are outliers.

Figure 7. Seasonal variation in microcystin (MC) content of fish-shellfish from the Curonian Lagoon and James River Estuary. Fish data are for liver tissues; Blue Crab values are for viscera. Blue Catfish data are for juveniles (<40 cm). P values are for 1-way ANOVAs testing for seasonal differences.