2015

The Export of an Algal Toxin into Terrestrial Predators via Emerging Aquatic Insects

Nicholas J. Moy
Virginia Commonwealth University

THE EXPORT OF AN ALGAL TOXIN INTO TERRESTRIAL PREDATORS VIA EMERGING AQUATIC INSECTS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University.

By

Nicholas J. Moy
Bachelor of Science, University of Illinois, 2012

Major Advisor: Lesley P. Bulluck, PhD [1,2]
Co-advisor: Paul A. Bukaveckas, PhD [1,2]

Committee Members:
James A. Vonesh [1]
Salvatore Agosta [1,2]

Graduate Director: Jennifer K. Stewart, PhD [1]

Department of Biology[1], Center for Environmental Studies[2]

Virginia Commonwealth University

Richmond, Virgina

August, 2015

Acknowledgement

The author wishes to thank several people. First and foremost, thank you to Lesley Bulluck for the generous help and invaluable time, one could not ask for a better adviser. As well, thanks to my co-adviser Paul Bukaveckas, my patient and guiding committee members, and Catherine Viverette for advice on this project. Jenna Dodson's tireless fieldwork, thoughtful suggestions and edits were irreplaceable; much of the effort around this project is accredited to her. Special thanks to Ashley Grupenhoff and Ethan Cox; their work in the field could not be appreciated enough. Thanks to Spencer Tassone for completing our microcystin lab work, and to Miranda Foster and Hannah Huddle for dissection and insect work. I offer a special thanks to our partners at U.S. Fish and Wildlife Service, specifically Cyrus Brame and Henry Wooley for their support and facilities at Presquile National Wildlife Refuge. Thank you to our sponsors the VCU Rice Rivers Center, and the American Field Ornithologists.

List of Tables

List of Figures

Abstract

THE EXPORT OF AN ALGAL TOXIN INTO TERRESTRIAL PREDATORS VIA EMERGING AQUATIC INSECTS

By Nicholas J. Moy, M.S. Candidate

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University.

Department of Biology, Virginia Commonwealth University 2015

Thesis adviser: Lesley P. Bulluck, PhD, Assistant professor, Department of Biology

Algal blooms are directly related to human-caused nutrient enrichment of water bodies. The cyanobacteria *Microcystis aeruginosa* produces microcystin (MC), a toxin that has been linked with mortalities and illness of many organisms. We show that MC is not constrained by the aquatic-terrestrial ecotone. MC was detected in a primary consumer and emerging aquatic invertebrate (*Hexagenia* Mayfly), a terrestrial insect and predator of emerging aquatic invertebrates (*Tetragnathidae* Spider), and a vertebrate consumer (Prothonotary Warbler). Mayfly and spider MC levels varied across the blooming period. MC levels in prothonotary warbler livers varied by age class; nestlings having the highest levels. MC levels decreased in fledglings over time. A more aquatic diet was related to higher MC levels in nestlings at one site and nestling fecal-sacs varied spatially, also indicating that aquatic diet is related to MC consumption. Warbler body condition and growth rate was not related to liver microcystin levels.

Introduction

Emerging aquatic insects are a substantial food source for terrestrial bats, reptiles, amphibians, spiders, and in riparian birds and can account for 50-90% of their monthly energy budget (Nakano & Murakami 2001; Vander Zanden & Sanzone 2004). As this energy flux of emerging aquatic insects crosses habitat boundaries, it can be shadowed by the movement of pollutants; this has been referred to as the "dark side of subsidies" and has been demonstrated with mercury and polychlorinated biphenyls (Menzie 1980; Walters et al. 2008). For example, a diet consisting mainly of emerged aquatic insects has been linked to higher mercury levels in several species of insectivorous birds (Tsipoura et al. 2008; Walters et al. 2008; Beck et al. 2013). This study expands on the concept of the "dark side of subsidies" to address the growing threat of toxins produced by harmful algal blooms (HAB's).

HAB's are algal overgrowths that have negative impacts on ecosystems causing structural damage, physicochemical disturbance, or by producing toxins (Paerl & Otten 2013). HAB's can occur naturally, however their extent has increased over the last several decades and has become a global concern due to increased eutrophication of watersheds, human-assisted transportation of algal species, and climate change (Heisler et al. 2008; Papadimitriou et al. 2009; O'Neil et al. 2012). Some cyanobacteria, largely of the genus *Microcystis*, can produce microcystins (MC), a class of monocyclic heptapeptide hepatotoxins (Rastogi et al. 2014). These toxins inhibit the activity of protein phosphatases 1 and 2A, which are important proteins in many cell cycles including apoptosis, and can induce osmoregulatory imbalance, reduction of antioxidant formation, and hepatocyte degradation (Ibelings et al. 2005; Huang et al. 2010; Paerl

& Otten 2013). It is also well documented that consuming MC-contaminated water or seafood causes illness in humans, and has been linked with human deaths and liver cancer (Ueno et al. 1996; Azevedo et al. 2002; World Health Organization 2003; Poste et al. 2011). The World Health Organization (WHO) has issued MC guidelines for human drinking water, recreational contact, and consumption (1 μg/L, 4 μg/L, and 0.04 μg/kg body weight/day, respectively)(World Health Organization 2003).

MC accumulates in a variety of aquatic invertebrates including zooplankton, bivalves, and crustaceans, as well as aquatic vertebrates such as wild and farmed fishes, sea otters, turtles, ducks and water birds (Wilson et al. 2008; Gérard et al. 2009; Garcia et al. 2010; Lance et al. 2010). In these organisms, MC consumption has been related to mass illness and mortalities – MC's acute and long-term toxicity make it a threat to public, economic, and environmental health. MC can be transported through food webs via consumption; however, there has been no evidence of biomagnification (Ibelings et al. 2005; Papadimitriou et al. 2012). Previous studies have shown aquatic-to-aquatic food web transfer of MC from prey to fish consumer (Ibelings et al. 2005) as well as aquatic invertebrate to mammal (Sea Otter; Miller et al. 2010). In a lab setting, sunfish fed MC-rich zooplankton pellets transported the toxin to a piscivorous fish predator (Smith & Haney 2006).

Previous studies have focused largely on aquatic ecosystems. The extent to which MC can move into terrestrial food webs through cross-habitat subsidies has only recently been documented and with limited scope. In a report for the reservoir of Isahaya Bay, Japan, Takahashi et al. (2014) found low levels of MC in midge-flies and dragonflies as aquatic-stage juveniles, as well as in a terrestrial predator; *tetragnathidae* spiders. These findings were ancillary to the main study and suggested further investigation. In a lab setting, MC has also been

found in the tissues of mayflies (in aquatic stages) and has been shown to affect juvenile mayfly development (Smith et al. 2008; Liarte et al. 2014).

In this study we assess whether MC can move from an aquatic foodweb into the surrounding riparian habitat. We studied three organisms in a cross-habitat food web: an emerging aquatic insect (*Hexagenia* mayfly), an invertebrate terrestrial predator (*Tetragnathidae* spider), and a vertebrate terrestrial predator (prothonotary warbler (*Protonotaria citrea*)) (Figure 1). In addition, this study aims to explain the variation in MC concentrations observed in these organisms (i.e. seasonal and spatial patterns related to diet and age class) and model the potential dietary MC input to nestling prothonotary warblers. Lastly, we assess whether MC is related to warbler body condition and nestling growth rates.

Methods

Site Descriptions

The James River Estuary is a freshwater-dominated sub-estuary of the Chesapeake Bay with low salinity zones comprising more than half of its surface area (tidal fresh and oligohaline; salinity <5ppt). The tidal fresh section of the James has many similarities with other systems where toxin accumulation in consumers has been reported including large anthropogenic nutrient loads, elevated chlorophyll *a* (CHL*a*; a measure of total phytoplankton concentrations) and presence of cyanobacteria (blue-green algae) (Tango & Butler 2008; Marshall et al. 2009; Wood & Bukaveckas 2014). During summer months (May-October) the James River experiences lower discharge conditions which coincide with elevated CHL*a* where the river transitions from deep and narrow to a broad, estuarine channel. In these shallower depths, cyanobacteria are better able to exploit available nutrient loads due to more favorable light conditions and less turbulent water (Bukaveckas et al. 2011; 2013). Cyanobacteria contribute a small proportion of phytoplankton biomass (~10%) but their presence results in persistent levels of Microcystin in the water column (typically 0.5 – 1.5 ug/L) and its widespread occurrence among consumers including fish and benthic macroinvertebrates (Wood et al. 2013).

This study was conducted at two sites in upper tidal freshwater creeks adjacent to the main channel of the lower James River – Four Mile Creek located in Deep Bottom Park (Henrico County) and an unnamed creek at Presquile National Wildlife Refuge (NWR) (Chesterfield County)(Figure 2). Deep Bottom Park is approximately 10 km upstream from Presquile NWR where the channel is narrower and 2005-2012 data showed lower average CHL*a* levels

(Bukaveckas et al. 2011). Two subsites were designated at each site, River Front and Back Creek, to account for intra-site variation specifically in aquatic insect emergence.

Study Species

We used burrowing mayflies, *Hexagenia spp.* (Ephemeroptera: Ephemeridae) as a primary consumer and model emerging aquatic insect. *Hexagenia* nymphs are benthic macroinvertebrates that build burrows through which they pump water and feed on suspended organic matter (Rasmussen 1988). These insects typically spend one- two years as aquatic nymphs and emerge simultaneously in large swarms. Emergence events occur in May through July where large numbers of nymphs swim to the surface of the water and molt into subimagos. Subimagos are terrestrial winged sub-adults that fly to land for 1-3 days before molting into reproductive adults. Adults mate, deposit fertilized eggs in the water, and die within 1-2 days (Edsall 2001). During this terrestrial life stage mayflies have atrophied mouthparts and do not feed in the terrestrial environment (Bauernfeind & Moog 2000). *Hexagenia* are often used to study bioaccumulation and have demonstrated to transfer mercury to higher trophic levels (Saouter et al. 1993; Bauernfeind & Moog 2000). Burrowing mayfly nymphs likely uptake MC through their gut wall from consumed MC in organic matter, or through filamentous gills from oxygenated water (Saouter et al. 1993; Smith et al. 2008; Liarte et al. 2014).

We used long-jawed spiders (Araneae: Tetragnathidae) as a model invertebrate terrestrial predator with a predominantly aquatic diet. This taxa builds webs on vegetated river banks and preys on emerging aquatic insects (Nakano & Murakami 2001; Marczak & Richardson 2007). In other systems this species has shown an aquatic isotopic-signature (Walters et al. 2008; Takahashi & Kaya 1993).

Prothonotary warblers (*Protonotaria citrea*) are a riparian songbird that breed in bottomland hardwood forests throughout the southeastern United States and over-winter in South America (Petit 1989). They are secondary cavity-nesters, and one of only two cavity-nesting wood warbler species. Females lay 4-6 eggs per clutch, and can raise two broods per season, typically one "early" and one "late" clutch (Blem et al. 1999; Bulluck et al. 2013). Our study population breeds in man-made nest boxes along the lower James River and is part of a long term study of their breeding ecology initiated in 1987 (Blem & Blem 1991). They are an ideal vertebrate predator in which to assess the movement of MC into the riparian habitat because mayflies and other emerging aquatic insects make up a significant portion of their diet. Further, because they nest in artificial boxes they are accessible for quantifying nestling diet (see *Diet Analysis* section below).

Sample Collection

We sampled the water column and riparian food web for microcystin and stable isotopes at the two sites. All sampling efforts coincided with the prothonotary warbler breeding season starting in May and continuing through October 2014. Water samples were collected from the water's surface near the mouth of the creek at each site, every other week.

Mayflies and other emerging aquatic insects were sampled using Pennsylvania-style light traps (Frost 1957; Kovats & Ciborowski 1989) on the shore and emergence traps (Davies 1984) on the water's surface 10 meters from the shore at each site. This included one light trap and four evenly spaced emergence traps at each subsite (riverfront and back of creek) for both Deep Bottom Park and Presquile NWR. However, at Presquile traps did not produce mayflies on the creek near the boxes. Mayflies were sampled and used for analysis from a third trap that was

placed on the channel-side of the island, about one kilometer from the back creek boxes. A sample of ~20 pooled mayflies collected from the middle of each month at each site from May through October were tested for the presence of MC. Entire bodies of mayflies were used for MC and stable isotope analysis.

The non-mayfly portion of the light trap sample was also tested for MC. Two types of aquatic insects dominated our samples – Chironomid midges (Diptera; Chironomidae) and caddisflies (Trichoptera), making up 33-98% of each sample. We removed other, larger insects (>1cm) from the sample, which generally included dobsonflies, beetles, and moths. The sample was then spread out evenly in a gridded tray and 25% of the cells were randomly chosen. The subsample of insects in these cells were identified to order or suborder and counted. Proportions of each taxa were assumed to be representative of the entire sample in the tray. The dry mass was recorded as the total of the insects on the tray. Across 6 monthly samples (May-Aug. at Deep bottom, and May-June at Presquile NWR), the estimated number of individuals per sample ranged from 475 – 4785 with an average of 1773 individuals per sample.

We hand-collected *Tetragnathidae* spiders opportunistically from structures and vegetation adjacent to the water. A sample of ~30 pooled spiders collected from the middle of each month at each site from May to October was tested for MC. Entire bodies of spiders were used for MC analysis.

MC has been shown to accumulate in a variety of tissues in vertebrates including stomach, spleen, and intestine, although highest levels have been found regularly in the liver (Chen et al. 2009; Huang et al. 2010). We collected Prothonotary warbler samples by sacrificing individuals and extracting liver tissue post-mortem. All birds were collected and sacrificed using thoracic compression as suggested by the Ornithological Council (Fair et al. 2010) and IACUC protocols

(VCU IACUC #AM10230, Federal Scientific Collection Permit #MB29235B, State Scientific Collection Permit #050784, USGS Federal Bird Banding Lab Permit # 23486). Nestlings were taken from the nest, and hatch-year fledglings and adults were captured using target mist-netting techniques and playback. Nestlings were sacrificed when 9-10 days old (fledging typically occurs on day 10-12) and mist-netted birds were aged as fledglings or adults using skull pneumatization techniques (Pyle 1997). Three nestlings and two adults were opportunistically found dead in nest boxes due to unknown causes (probable Tree Swallow competitive interaction for nest boxes) were tested for MC and included in the analyses. Fecal-sac samples were also collected opportunistically from nestlings in the hand during banding activities at 7-8 days old and pooled by nest (1-5 fecal-sacs per sample).

Lastly, in order to account for another large portion of Prothonotary Warbler nestling diet, we analyzed caterpillars for MC content. Caterpillars, largely of the family geometridae (Lepidoptera; geometridae) were collected from leaves opportunistically and pooled across site and time for MC analysis.

Microcystin Analysis

MC levels in all samples were analyzed using a commercial ELISA kit (Abraxis; Warminster, PA). The assay measures numerous forms of 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 as per Silva-Stenico et al. (2009). To extract MC from tissues, we used methods described by Wilson et al. (2008) and Garcia et al. (2010). Samples were dried at 80^{o}C for 48 hours, ground with a mortar and pestle, and extracted in 75%

aqueous methanol for 24 hours. 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. Samples were run in duplicate and 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.

Diet Analysis

We used two methods of diet analysis- video monitoring, and natural abundance stable-isotopes. Video monitoring of nestling birds is a common practice for determining diet (Goodbred & Holmes 1996; Burger et al. 2012). However, this method only samples a 1-3 hour snapshot of nestling diet, therefore, as a compliment, stable isotope data can be valuable. Nestling diet was quantified using video observations at 104 nests for a total of 263.3 hours, most of which were used for quantification of overall diet across sites (See figure 6., full analysis Dodson and Bulluck, Unpublished). A portion of videos were of nests with nestlings that were analyzed for MC (23 nests monitored for 57.19 hours total), fourteen of these were nests from which a nestling was sacrificed and nine were determined to be the nests from which fledglings were subsequently captured (based on identification from leg bands). Another set of videos were used for nestlings where fecal-sacs were collected (19 nests monitored for 42.79 hours) A Canon FS400 hand-held standard-definition camera was placed outside of the nest with a clear view of the nest box for 1.62 – 3.28 hrs. All video observations were conducted in the morning (6:40-9:41 am) when the nestlings were between 6 and 9 days old when provisioning is greatest (Dodson and Bulluck, unpublished).

Videos were reviewed to identify food items brought to the nest. For each visit, we recorded the type of food item, the number of food items brought, and the size of items. Size was ranked as a “1”, “2”, or “3” for smaller than the bill, same size as the bill, or larger than the bill,

respectively. These values were multiplied by the number of items to estimate the biomass being provisioned – this new value was labeled "foodscore". Mayflies were easily identified by their color (yellow for subimagos and darker for adults), large size in relation to the bird's bill, thin wings and long tails. Caterpillars were identified as legless, relatively long and hanging limp from the bill. "Total mayfly items", "total caterpillar items", and "other items" (items that were neither mayfly nor caterpillar) for each nest was divided by the number of nestlings and hours the nest was monitored (e.g., number of mayflies per chick per hour). The rate of fecal-sac removal by parents was identified in 2013 videos only at Deep Bottom (Tucker and Bulluck, Unpublished; 48 nests for 118.39 hrs.) and calculated as a measure of fecal-sacs per chick per hour. 2013 videos were watched by many of the same observers and using the same methods and we do not expect the rate of fecal-sac removal to change across year or site. These values were used in multiple regression models and predictive equations (see below).

Natural abundance of carbon and nitrogen stable-isotope ratios, $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$), respectively, are often used to determine the energy base of food webs as consisting of terrestrial and/or aquatic organic matter. This method has been extended to the study of cross-habitat diet: the progressive depletion of $\delta^{15}N$ and enrichment of $\delta^{13}C$ indicates a shift from terrestrial to aquatic diet (Finlay 2001). Further, PCB concentrations and mercury contamination have been shown to increase along these isotopic gradients tracking the movement of aquatic contaminants through aquatic insect prey (Walters et al. 2008; Jardine et al. 2012).

Individual samples of prothonotary warbler (74 total), and pooled samples of spiders (22 total), mayflies (4 months, 12 total samples), and caterpillars (three samples of 25 individuals) were analyzed for $\delta^{15}N$ and $\delta^{13}C$ (UC Davis Stable Isotope Analysis Lab, Davis CA). Breast muscle tissue was used for each prothonotary warbler while entire body was used for pooled invertebrate samples. With a relatively fast tissue turn-over, muscle tissue stable isotope

signature in adults is representative of diet from the preceding weeks (likely post breeding), and in nestlings is representative of diet from first week of life (Bauchinger & McWilliams 2010).

Body Condition and Growth Rate Analysis

Because nestlings of the same clutch are likely receiving similar amounts and types of food, we assume that the amount of MC found in the sacrificed individual to have also accumulated in the sibling nestlings of the same brood. Sacrificed chicks were chosen randomly from the nest in order to control for the presence of dominant or subordinate individuals. Measured individually using a digital balance and a caliper, nestling mass (g) and tarsus length (mm) was measured at 5/6 days and again at 7/8 days old. Growth rate was calculated as the change in body mass per day between these two measurements and averaged within a nest. A body condition index was calculated for nestlings and adults as the residuals from a least squares regression of mass (g) by tarsus length (mm). This index has been demonstrated as a good estimation of an individual's relative condition (Schulte-Hostedde et al. 2005) and is correlated with a stronger immune and better survival (Alonso-Alvarez & Tella 2001). For nestlings, these residuals were calculated separately by age (days). To check for sampling bias, a two-tailed t-test was used to confirm that growth rate and body condition of sacrificed nestlings was not different from that of nestlings that were not sacrificed ($P = 0.5799$ and $P = 0.2253$, respectively).

Statistical Analysis

Differences in MC values across sample type were compared using one-way analysis of variance. Backwards stepwise multiple linear regression analysis was used to determine what factors best predicted MC content in mayflies, spiders, all birds, adult birds, fledglings, nestlings,

and fecal-sacs. Factors thought to contribute to MC variation included in these models were site, sub-site, date, nestling diet, and age. A significance threshold of $P > 0.05$ was used as the criteria for exclusion from the model. Due to the inherent non-normal distribution of microcystin concentration in organisms (many low values and fewer high values), MC values were Logit transformed for regression analyses. All analyses were completed using JMP 11.0 statistical package (SAS Institute Inc. 2013).

Estimating Microcystin Inputs and Outputs

With the information observed, predictive values of Microcystin accumulating in a nestling's body per hour were calculated. These predicted values were compared to the amount of MC in the livers of nestlings in order to assess how the amount of MC concentrating in the liver relates to consumption and excretion rates. Predictive values were calculated using the following set of equations:

I. $$MC_{TI} = Microcystin\ Total\ Input\left(\frac{MC}{\frac{chick}{t}}\right) =$$

$$\left(\frac{MF_{items}}{\frac{chick}{t}} \times \frac{MC}{MF_{items}}\right) + \left(\frac{Caterpillar_{items}}{\frac{chick}{t}} \times \frac{MC}{Caterpillar_{item}}\right) + \left(\frac{Other_{items}}{\frac{chick}{t}} \times \frac{MC}{Other_{item}}\right)$$

a. $$\frac{MF_{items}}{\frac{chick}{t}} \sim Date + Site + Subsite$$

b. $$\frac{Caterpillar_{items}}{\frac{chick}{t}} \sim Date + Site + Subsite$$

c. $$\frac{Other_{items}}{\frac{chick}{t}} \sim Date + Site + Subsite$$

In equation 1, "$\frac{MF_{items}}{\frac{chick}{t}}$" is the number of individual mayflies brought to a nest per chick per hour (t) as a function of (Ia) the date the nest was monitored, the site (Deep Bottom or Presquile) and the subsite (River Front or Back Creek). Since MC in mayflies did not

significantly vary over space and time "$\frac{MC}{MF_{items}}$" is the median amount of MC in an individual mayfly from seven pooled samples of 289 mayflies (0.002051 µg/ individual). Median used since MC values are inherently non-normal (right-skewed). "$\left(\frac{Caterpillar_{items}}{\frac{chick}{t}} \times \frac{MC}{Caterpillar_{item}}\right)$" and "$\left(\frac{Other_{items}}{\frac{chick}{t}} \times \frac{MC}{Other_{item}}\right)$" are the equivalent measure for caterpillars and other invertebrate items brought to the nest respectively (A function of Ib and Ic, respectively) (caterpillar median, 0.001212 µg/individual; "other insect" median, 0.00003340 µg/individual).

II. $MC_{TO} = Microcystin\ Total\ Output\left(\frac{MC}{\frac{chick}{t}}\right) = \frac{FS_{indv.}}{\frac{chick}{t}} \times \frac{MC}{FS_{indv.}}$

a. $\frac{MC}{FS_{indv.}} \sim Site$

In equation II, "$\frac{FS_{indv.}}{\frac{chick}{t}}$" is the number of individual fecal-sacs removed from nests per chick in the nest per hour (0.618992 fecal-sacs/hr.). "$\frac{MC}{FS_{indv.}}$" Is the median amount of MC in fecal-sacs by site, since there was a significant difference in MC in fecal-sacs between sites (Iia) (Deep Bottom fecal-sac median, 0.002670 µg/individual; Presquile fecal-sac median, 0.001750 µg/individual).

III. $MC_B = Microcystin\ Accumulating\left(\frac{MC}{\frac{chick}{t}}\right) = MC_{TI} - MC_{TO}$

In equation III, "MC_B" is the difference between total input from mayflies and total output from fecal-sacs, this was used as to predict the amount of MC accumulating in an individual chick per hour, and compared against observed MC levels in nestlings and fecal-sacs.

Results

Microcytin levels in water samples peaked during the first two weeks of July and at the upstream site, Deep Bottom Park, levels reached a maximum of 1.34 ppb on 7/15/14, a value that is above the WHO standard for drinking water (Table 1). Microcystin was detected in all species we tested in the riparian food web, and in comparable levels to aquatic organisms of the same system (Figure 3). Nestling warblers had significantly higher liver MC levels than adult warblers ($p=0.0053$), fledgling birds ($p<0.0001$), nestling fecal sacs ($p=0.0377$) and caterpillars ($p=0.0234$) and comparable levels with spiders, other aquatic insects and mayflies (ANOVA, Pairwise comparisons Tukey's HSD, see actual MC values in Table 2).

MC levels in mayflies and spiders could not be significantly predicted by site or date collected. As stated in the above analysis, age was the only significant predictor of MC level in prothonotary warblers ($p <0.0001$). Site and date collected were also not predictors of adult prothonotary warbler liver MC. Date collected was a significant predictor of liver MC in fledglings – fledglings caught later in the season were more likely to have lower liver MC than those caught earlier ($p = 0.0123$, Figure 4). This relationship held true when considering logit transformed total MC load (not per gram dry weight of liver) ($p=0.0061$) indicating that changes in liver size during this post-fledging time was not related to MC concentration. Diet and site were significant predictors of MC levels in nestlings – nestlings raised at Deep Bottom Park in nests that were fed more mayflies/chick/hour had higher liver MC ($p = 0.0063$, Figure 5) However, because so few nests at Presquile were fed mayflies at all (Table 4), this site was excluded from the analysis. When considered as univariate MC levels in nestling livers were

higher at deep bottom but not significantly (Figure 6c). Lastly, site was the only predictor of microcystin levels in fecal sac samples – fecal sacs collected from nestlings at Deep Bottom Park had higher MC levels than those collected at Presquile NWR ($p = 0.0502$, Figure 6b). MC levels were not predicted by diet, however, the proportion of aquatic foodscore in the diet was greater at Deep Bottom across all monitored nests (n=104 for a total of 263.3 hours)(Figure 6a).

Body condition was not correlated with microcystin levels in prothonotary warbler livers across all classes ($n = 65$, $p = 0.8558$), in adults (n=8, $p = 0.8551$), fledglings (n=42, $p = 0.9766$), nor in nestlings (n=16, $p = 0.6566$) (Figure 7a). Likewise, nestling growth rate was not correlated with liver MC ($n = 19$, $p = 0.8479$) (7b). However, overall growth rate for all nests at both sites, was significantly higher at Deep Bottom (Figure 6d).

Natural abundance $\delta 15N$ ($^0/_{00}$) by $\delta 13C$ ($^0/_{00}$) stable isotope data was plotted as averages by organism and site (Figure 8a), and with all records (Figure 8b). Spiders and prothonotary warblers are trophically enriched compared with caterpillars and Deep Bottom mayflies indicating the expected isotopic relationship between predator and prey. Adult and fledgling prothonotary warblers have more enriched $\delta 13C$ values compared with nestlings (ANOVA, Tukey's Pairwise HSD, Adult-Nestling p-value < 0.0001, Fledgling-Nestling p-value < 0.0001) indicating a potential shift in diet with age.

The predicted MC consumed and accumulated per chick per hour across site, subsite, and Julian date based on the above equations was low and decreased across the season at three of the four subsites (Figure 9a). Observed MC content in nestling livers and nestling fecal sacs were not significantly related to predicted MC accumulation per hour based on site, subsite, and date (Livers, p-value= 0.1789, r2= 0.0981; Fecal Sacs, p-value=0.7381, r2= 0.00544) (Figure 9b).

Discussion

We found widespread presence of MC in all portions of the riparian food web assessed in this study. MC levels varied as much as 1000-fold among individuals but the toxin was present in every sample analyzed, including caterpillars. Overall levels in these terrestrial organisms were at comparable levels to aquatic organisms of the same system as determined by Wood et al. (2014) (Figure 3a). The high levels of MC observed in terrestrial prothonotary nestlings, spiders and adult mayflies is of particular importance and our study is the first to describe the lateral transport of MC in this way. Our results are from a system with relatively low cyanobacteria content in the water (<10% of the algal biomass) and moderate levels of MC production (1 ug/L) and indicate that terrestrial ecosystems adjacent to more productive aquatic systems may also be at risk of MC exposure.

The pathway for potential MC-exposure to caterpillars is unclear. It has been shown that agricultural plants including rice and lettuce, can take up MC via irrigation with water from MC-contaminated sources (Corbel et al. 2014; Bittencourt-Oliveira, MC. et al., 2014; Rastogi et al. 2014). In these systems, hydroponic plants with roots directly in contact with contaminated water absorbed MC at considerable levels. At both Presquile NWR and Deep Bottom Park the riparian zone is flooded up to 50 meters from the river's edge during high tide, and high water level conditions. Because all caterpillars were sampled from this zone, it is possible that caterpillars are consuming the toxin from riparian tree leaves. These results include only three subsamples of 75 caterpillars pooled across sites and time – further samples and more work is underway to elucidate this mechanism.

In emerged mayfly adults neither site nor date predicted MC levels, however, it is important to note that MC was detected in mayflies before MC was detected at elevated levels in the water column. This may indicate that mayflies as aquatic nymphs can carry over MC from previous algal blooms. MC has been shown to persist in aquatic organisms of the James River during non-bloom conditions (Wood et al. 2014) and, in another system, in snails during fall and winter (Ozawa et al. 2003). MC levels in spiders appeared to track MC in mayflies- a common prey item, but were not significantly predicted by site or date. MC was present in mayflies at the first detection in spiders, but again, before MC was measureable in the water – a further illustration that MC can be transported to the terrestrial ecosystem during non-bloom periods.

The only significant predictor of MC across all prothonotary warbler samples was age category, where nestlings had significantly higher levels of MC compared to older birds. Although there is no one clear explanation for this difference, our results provide some evidence to hypothesize a toxin reduction in individual birds after they leave the nest and prepare for fall migration. For example, fledglings caught later in the season had lower MC levels than those caught earlier. Although unequal variance across sampling period may influence this finding (Figure 4), we pose four, non-mutually exclusive, hypotheses of biological factors that may drive this inverse relationship: 1] Fledglings with high MC levels have decreased survival rates. 2] Fledglings shift diet to terrestrial invertebrates because of decreasing emerging aquatic food availability or inherent differences in food preference with age that lead to less MC consumption. 3] As the season progresses fledglings acclimate to MC levels and are able to process consumed toxin more efficiently. 4] Changing liver-to-body-size ratios, as individuals mature, change an individual's ability to handle MC diet loads. This in concordance with the relatively high feeding rates of nestlings compared to other life stages could lead to inflated MC levels in nestlings. A

change in diet with age has shown to change MC accumulation in Gizzard Shad, as mature shad feed differentially on algae (Wood et al. 2013)

Stable isotope results can help to assess these hypotheses as they indicate the potential for diet shifts among age classes. Mayflies at Deep Bottom, had relatively depleted δ13C, and enriched δ15N values compared to caterpillars. Qualitatively, this is reflected in predator stable isotope values (after trophic enrichment) with spiders having the most enriched δ15N and most depleted δ13C indicating a greater reliance on food isotopically more similar to mayflies from deep bottom than caterpillars. These results also show an enrichment of δ13C from nestlings to fledglings to adults. This indicates a potential change in diet in prothonotary warblers as they age - from food sources isotopically more similar to mayflies from deep bottom to sources more isotopically similar to caterpillars. This movement suggests a more terrestrial diet, however a greater variety of prey species need to be analyzed to determine exact diet composition. Since adults and fledglings are necessarily caught later in the season it is difficult to determine the driving influence of this change. δ13C could be enriched from a diet shift as birds mature or from less aquatic prey availability later in the season. However, adults captured earlier in the season also had a more enriched 13C values (Figure 8b) suggesting that a diet shift with age is more probable. More mayfly samples also need to be considered to address the great disparity in isotope signatures in this species between sites.

Provisioned mayfly items per chick per hour, was positively correlated with liver MC in nestlings at Deep Bottom (Figure 5). This site had much higher mayfly abundance that Prequile NWR where very few nests were provisioned mayflies at all. Interestingly, the stable isotope values for nestlings from both sites do not indicate a more terrestrial diet at Presquile NWR compared with Deep Bottom. This may be because there were unknown aquatic prey (small

chironomids/midges, etc.) that were not detectable in the provisioning videos. The mayflies at Presquile NWR show unexpectedly enriched dC values. Further samples for SI analysis collected with more resolution across time and subsite may clarify this difference in mayfly SI values across site. Regardless, the increase in nestling liver MC with an increase in mayfly provisioning at one of our sites indicates an important connection between a terrestrial predator and an aquatic toxin via an emerging aquatic food source.

Microcystin level in prothonotary warbler fecal sacs was best predicted by site with Deep Bottom showing higher levels. Even though the levels were not related to the quantity of mayflies fed per chick per hour for the nests sampled, the proportion of aquatic foodscore in the diet was greater at Deep Bottom across all monitored nests. This overall difference in nestling diet between sites has been attributed to differences in food availability (Dodson and Bulluck, unpublished data) and may explain differences in the amount of MC being passed in fecal sacs by nestlings. It is also noteworthy that while the amount of MC in fecal sacs is greater at Deep Bottom, MC levels in nestling livers do not mirror this trend. This may suggest that while input of MC is higher at Deep Bottom output is also higher, and at fine scales this input/output may only be coarsely related to MC levels in the liver. Further, overall growth rate for all nests at both sites, is significantly greater at Deep Bottom where nestlings may be eating more aquatic food and have more MC in their fecal sacs.

MC levels were not correlated with body condition of prothonotary warblers or growth rate of nestlings, providing no evidence of major health affects to nestlings or adult prothonotary warblers at the levels detected. However, because both of these measures of health are relatively coarse, few conclusions can be drawn about more subtle health affects to individuals. Some livers appeared to have portions yellow in color indicating jaundice or other liver damage.

However, since the birds were kept frozen for a period of time after collection and the livers with discoloration were not related to exceedingly high MC the cause of the deformities is unclear. The liver has important metabolic function and aids in fat deposition – a critical process prior to and during migration. Because birds are most susceptible to high mortality rates during migration (Alerstam 1991), MC accumulation, even at very low levels, could lead to decreased survival. However, it has been shown that migratory insectivores may have greater tolerance to environmental toxins than other passerine species due to their evolutionary history of exposure to a more diverse array of toxins (Rainio et al. 2012). This suggests that this study, of a migratory bird, may be conservative in estimating effects of MC on other insectivorous songbirds. Further studies on long-term sub-lethal exposure of MC is needed in both wild and aviary settings to fully understand the processing and accumulation of MC in birds of varying age and prior and during periods of migration/fat deposition.

Overall, the predictive equations calculate very little MC input for nestlings in one hour. These very low to negative values indicate that MC must accumulate over time for MC levels in nestling livers to be relatively high. It is likely that over larger timescales the vast difference in MC input/output in comparison to the amount retained in the liver tissue as well as the variation in input/output coarsen the models. The inability of these models to predict MC values in nestlings gives further insight into the unpredictability of MC levels using observational variables. In future studies, experimental settings may be conducive for analyzing variation in physiological uptake of MC in riparian species.

This study provides evidence that MC is not constrained at the aquatic-terrestrial ecotone, and that MC may not have major or immediate effects in sub-lethal doses on riparian birds, but more research is needed. The presence of MC in emerged *Hexagenia* mayflies, before and during

the seasonal bloom, has implications for insectivorous organisms in the terrestrial ecosystems including bats, reptiles, amphibians and birds, some of which are species of management concern or provide important ecosystem functions. This study provides evidence that human- induced algal blooms may have broader environmental impacts than previously considered.

Tables and Figures

Table 1. MC content of water at Deep Bottom Park and Presquile NWR during 2014.

DATE (day/mo./2014)	Deep Bottom (µg/L) ± SE	Presquile (µg/L) ± SE
05/20	0.017 ± 0.017	0.000 ± 0.000
06/03	0.013 ± .0132	0.035 ± 0.022
06/17	0.006 ± 0.004	0.000 ± 0.004
07/01	0.862 ± 0.329	0.381 ± 0.026
07/15	1.335 ± 0.063	0.497 ± 0.070
07/29	0.076 ± 0.004	0.215 ± 0.017
08/12	0.162 ± 0.002	0.493 ± 0.127
08/26	0.342 ± 0.016	0.151 ± 0.027
09/09	0.232 ± 0.003	0.303 ± 0.038
09/23	0.725 ± 0.139	0.560 ± 0.052
10/14	0.254 ± 0.007	0.308 ± 0.022
10/28	0.106 ± 0.039	0.086 ± 0.025

Table 2. Summary data of MC content by sample type. All values are reported as whole body concentrations except for prothonotary warbler samples, reported as liver concentrations.

Sample Type	(n)	MC (µg/g DW) (avg. ± SE)	(Median)	(Max.)	Significant Differences
Prothonotary Warbler Nestlings	20	0.190 ± 0.035	0.142	0.616	A
Mayflies	7	0.107 ± 0.035	0.171	0.433	AB
Tetragnathidae Spiders	21	0.186 ± 0.029	0.138	0.450	AB
Nestling Prothonotary Warbler Fecal-Sacs	23	0.091 ± 0.022	0.042	0.386	BC
Other Light Trap Insects	6	0.048 ± 0.014	0.076	0.087	C
Caterpillars	3	0.045 ± 0.003	0.046	0.049	C
Fledgling Prothonotary Warbler	45	0.038 ± 0.006	0.025	0.172	C
Adult Prothonotary Warbler	9	0.033 ± 0.012	0.023	0.115	C

Table 3. Variables and predictors in multiple regression models

Response Variable	(n)	Input Predictor Variables	Significant or Near-Significant Predictors	F-statistic	p-value
MC in Mayflies	14	Site and Date	-	-	-
MC in Spiders	21	Site and Date	-	-	-
MC in Prothonotary Warblers in all age classes	73	Site, Date, and Age Category (Adult, Fledgling, or Nestling)	Age Category	71.52	<0.0001
MC in Prothonotary Warbler Adults	9	Site and Date	-	-	-
MC content in Prothonotary Warbler Fledglings	44	Site and Date	Date	5.92	0.0193
MC content in Prothonotary Warbler Nestlings	14	Site, Date, Age of Nestling (days since hatch), Subsite, and Diet (aquatic food score fed chick^{-1} hour^{-1})	Site and Diet Site Diet	5.55 7.09 11.10	0.0168 0.0186 0.0050
MC content in Prothonotary Warbler nestling fecal-sacs	23	Site , Date, Age of Nestling (days since hatch), Subsite and Diet (mayfly items fed chick^{-1} hour^{-1})	Site	4.32	0.0502

Table 4. Summary of Nestling and Fecal-sac Samples by site, date taken, and age of nestling

Samples Type	Site	(n)	Average Age (days)	Average Date Collected	Average Mayfly items fed (chick^{-1} hour^{-1})
Prothonotary Warbler Nestlings	Deep Bottom	7	8	6/4/14	2.87
	Presquile NWR	13	7.1	6/26/14	0.04
Prothonotary Warbler Nestling Fecal-sacs	Deep Bottom	13	6.8	6/11/14	2.72
	Presquile NWR	10	6.9	6/27/14	0.04

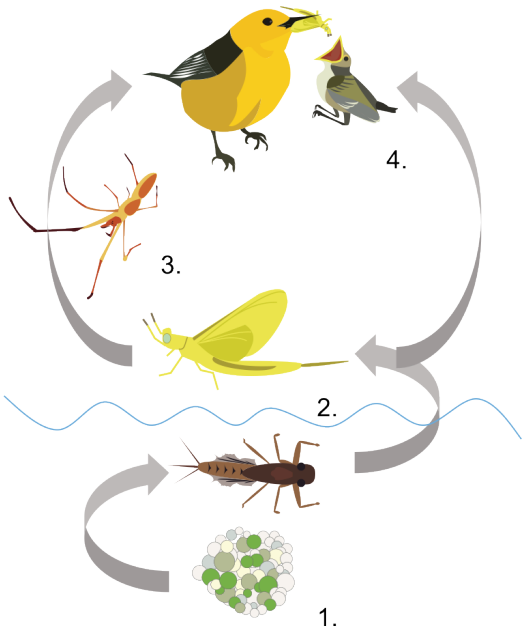

Figure 1. Predicted food chain in the lower James River, VA that is the focus of this study: 1] Producer and MC source (*Microcystis aeruginosa*), 2] an emerging aquatic insect (*Hexagenia* mayfly), 3] an invertebrate terrestrial predator (*Tetragnathidae* spider), and 4] a vertebrate terrestrial predator (prothonotary warbler (*Protonotaria citrea*).

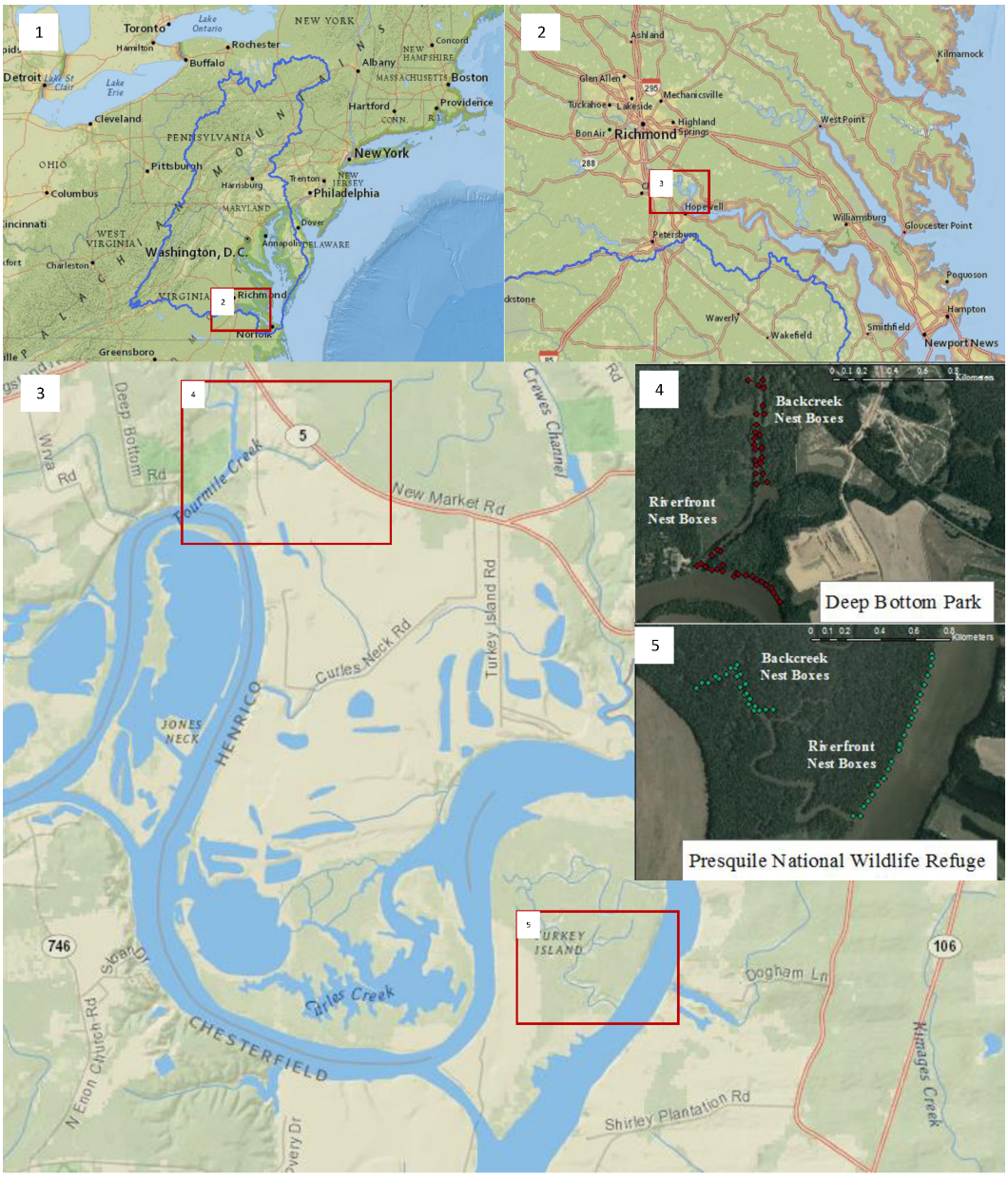

Figure 2. Locations of the two field sites (Deep Bottom Park and Presquile National Wildlife Refuge) showing designated subsites (River Front and Back Creek). Sites are both within the Chesapeake Bay watershed represented with the blue line in Map 1.

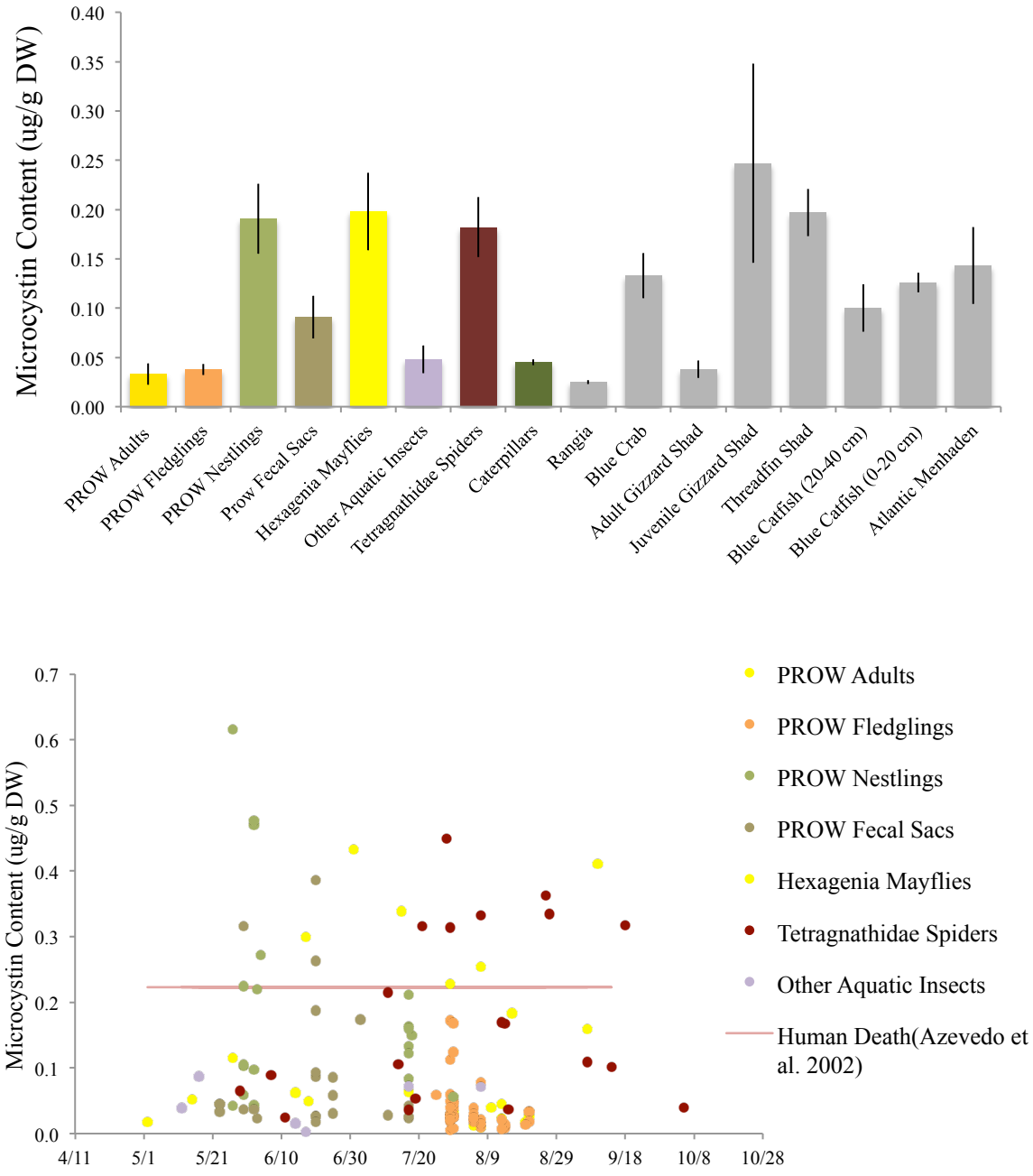

Figure 3. a. MC levels by organism in this study (in color) and in aquatic organisms of the same system (Wood et al. 2014 in gray) - error bars are one standard error of the mean. b. MC levels of all terrestrial samples by date. For reference, the red line indicates the average MC concentration in livers of humans post-mortem whose deaths were attributed to acute exposure to MC during contaminated renal dialysis treatment (Azevedo et al. 2002).

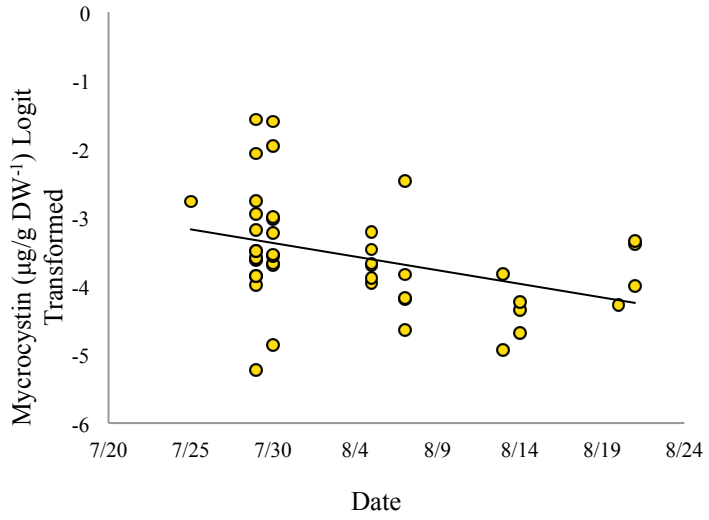

Figure 4. Prothonotary warbler fledglings liver MC by date. Levels significantly decline over sampling period from late July through late August ($p = 0.0123$, $R^2 = 0.1372$, $Y = -0.0397X + 1660.1$)

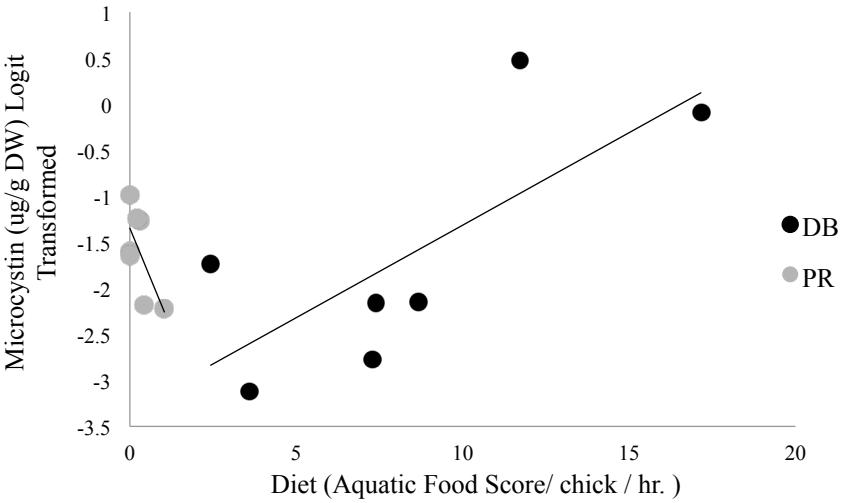

Figure 5. Prothonotary warbler nestling liver MC at Deep Bottom Park and Presquile NWR compared with Aquatic Food Score/ Chick/ Hour that were brought to the nest as determined by video observation. Site differences were attributed to very low aquatic food score values, however diet ($p = 0.0063$) was a significant predictor of MC levels in nestlings at Deep Botom Park ($n = 7$).

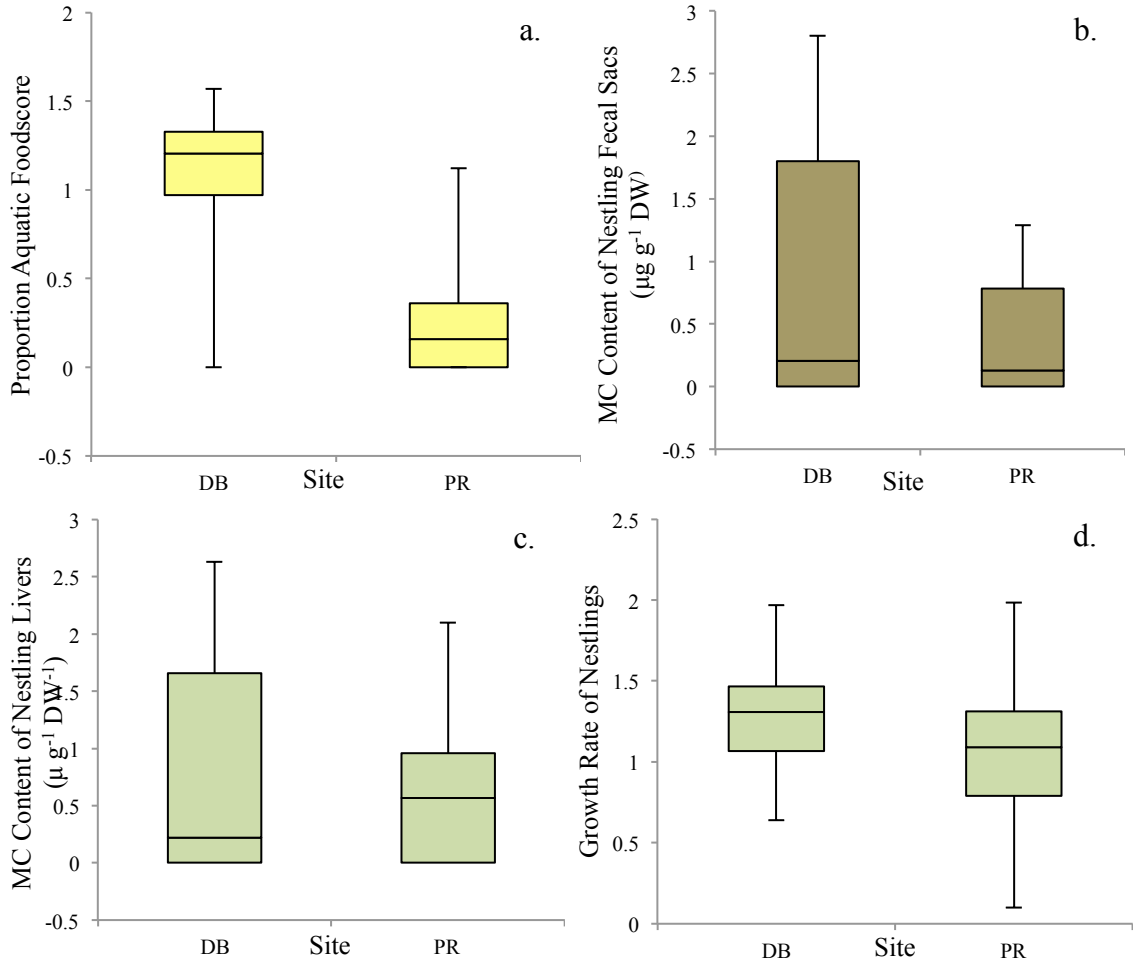

Figure 6. a. Proportion of aquatic prey in the nestling diet as determined with provisioning videos by site and arcsine square root transformed (t-test, n = 104, DB mean = 1.112, PR mean = 0.214, p-value < 0.0001). b. MC levels in nestling fecal-sacs by site. Fecal-sacs collected at Deep Bottom Park (DB) had higher levels of microcystin than those collected at Presquile NWR (PR) (n = 23, DB mean = 0.1278, PR mean = 0.0427, p = 0.0359*). c. Liver MC levels of nestlings by site (n=20, DB mean = 0.2217, PR mean = 0.1736, p= 0.6631). d. Growth rate of all nestlings by site (grams/ day averaged by nest) (n = 104, DB mean= 1.284, PR mean= 1.070, p-value = 0.0079*) by site. Boxes represent means, quartiles, min and max. All MC values were logit transformed for analysis, however, are reported here as raw values.

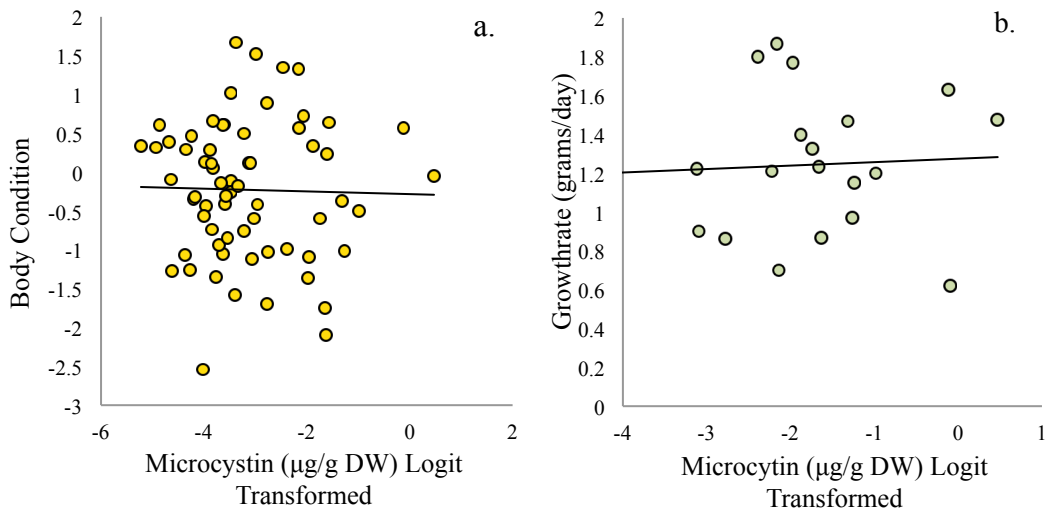

Figure 7a. Body condition of warblers (all age classes; residuals of mass by tarsus) by liver MC level (n = 65, p = 0.8558, R^2 = 0.0005, Y= -0.0175X – 0.826). b. Growth rate in grams per day of prothonotary warbler nestlings by liver MC level (n = 19, p = 0.8479, R^2 = 0.0022, Y= 0.0177X + 0.1275).

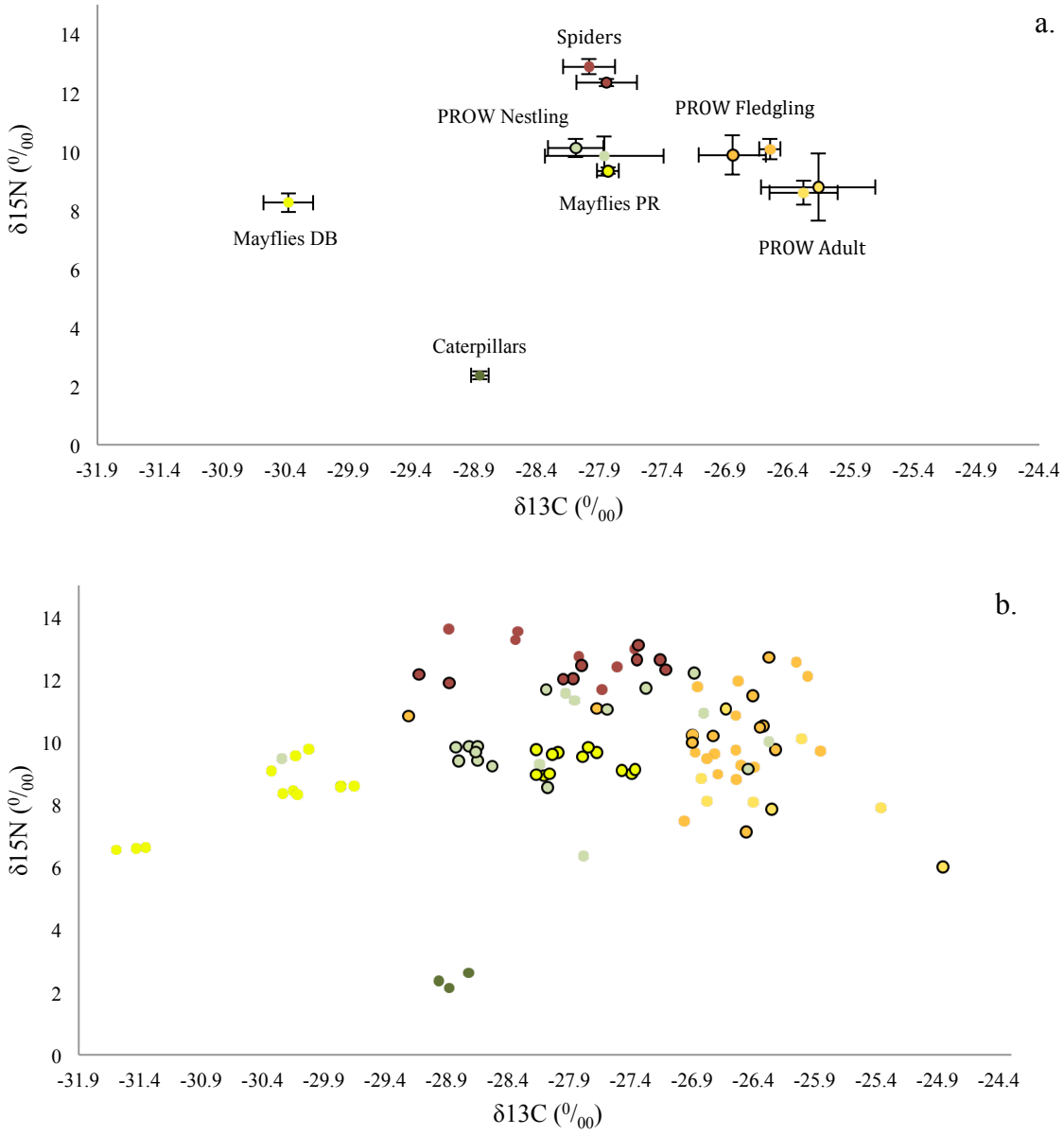

Figure 8: a. Bi-plot of natural abundance of δ15N (‰) by δ13C (‰) of food web organisms averages by site with one standard error of the mean. Outlined circles denote organisms collected at Presquile NWR, circles without outlines represent samples collected at Deep Bottom Park. b. All stable isotope records of food web organisms.

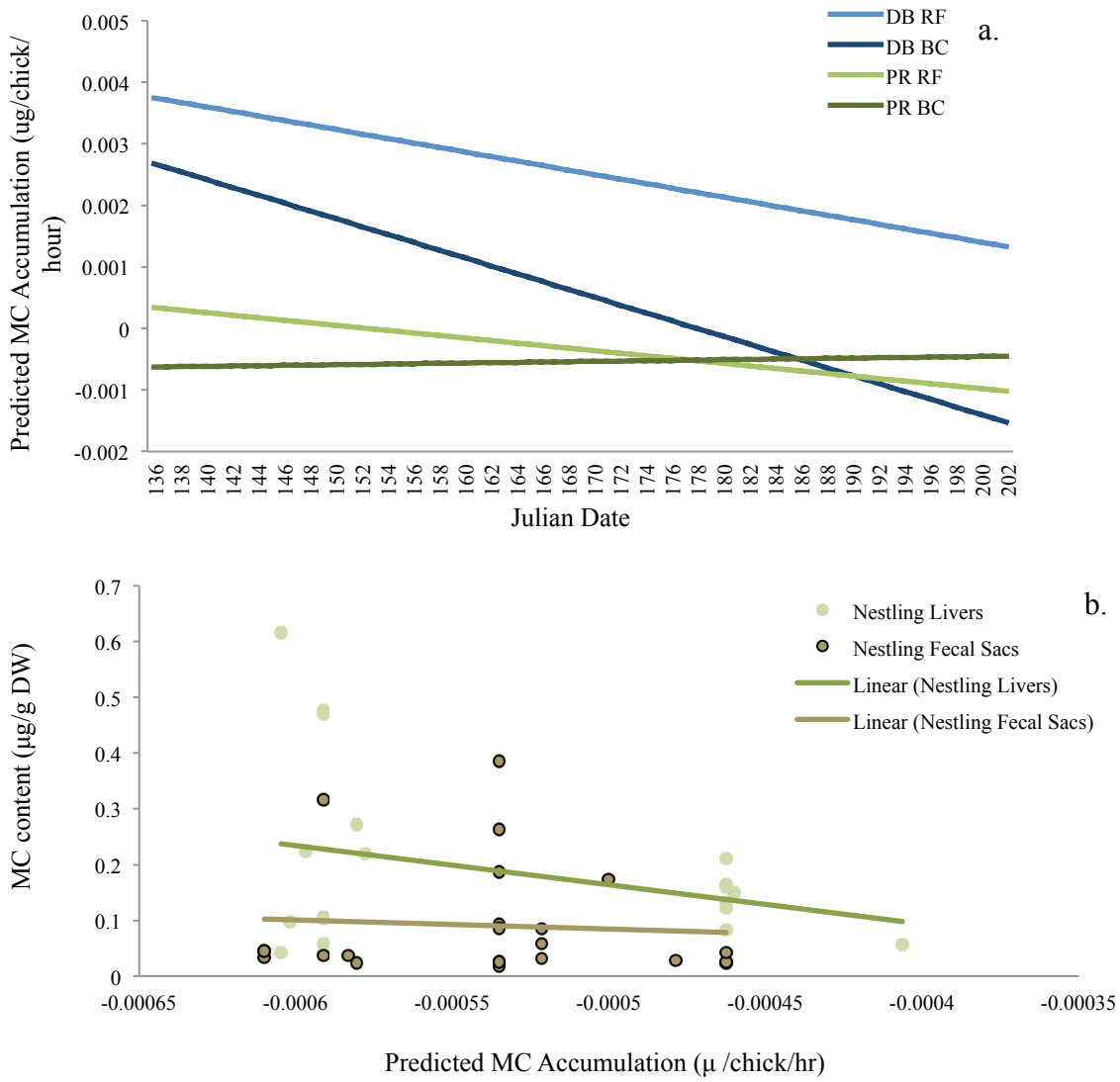

Figure 9. a. Predicted total accumulation of MC/ chick/ hour by site (Deep Bottom Park (DB) or Presquile NWR (PR)), subsite (River Front (RF) or Back Creek (BC)), and date as per equation III. b. Observed MC content in nestling livers and nestling fecal-sacs was not significantly related to predicted MC accumulation per hour based on site, subsite, and date (Livers, p-value= 0.1789, r2= 0.0981, y=-702.88x – 0.1876; Fecal-sacs, p-value=0.7381, r2= 0.00544, y= -163.34x – 0.0028).

Literature Cited

Literature Cited

Alerstam, T., 1991. Bird flight and optimal migration. *Trends in Ecology & Evolution*, 6(7), pp.210–215.

Alonso-Alvarez, C. & Tella, J.L., 2001. Effects of experimental food restriction and body-mass changes on the avian T-cell-mediated immune response. *Canadian Journal of Zoology*, 79(1), pp.101–105.

Bittencourt-Oliveira, MC. et al., 2014. Phytotoxicity associated to microcystins : a review. *Brazilian Journal of Biology* , 74(4), pp.753–760.

Azevedo, S.M.F.O. et al., 2002. Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. *Toxicology*, 181-182, pp.441–6.

Bauchinger, U. & McWilliams, S.R., 2010. Carbon turnover in tissues of a passerine bird: allometry, isotopic clocks, and phenotypic flexibility in organ size. *Physiological and Biochemical Zoology : PBZ*, 83(6), p.1032.

Bauernfeind, E. & Moog, O., 2000. Mayflies (Insecta : Ephemeroptera) and the assessment of ecological integrity : a methodological approach. *Hydrobiologia*, 422/423(1), pp.71–83.

Beck, M.L., Hopkins, W. a. & Jackson, B.P., 2013. Spatial and temporal variation in the diet of tree swallows: Implications for trace-element exposure after habitat remediation. *Archives of Environmental Contamination and Toxicology*, 65(3), pp.575–587.

Blem, C.R. & Blem, L.B., 1991. Nest-Box Selection by Prothonotary Warblers (Selección de Cajas para Anidar por Parte de Protonotaria citrea) Author (s): Charles R . Blem and Leann B . Blem Source : Journal of Field Ornithology , Vol . 62 , No . 3, pp . 299-307 Pub. *Journal of Field Ornithology*, 62(3), pp.299–307.

Blem, C.R., Blem, L.B. & Barrientos, C.I., 1999. Relationships of Clutch Size and Hatching Success to Age of Female Prothonotary Warblers Relationships of Clutch Size and Hatching Success to Age of Female Prothonotary Warblers. *The Wilson Bulletin*, 111(4), pp.577–581.

Bukaveckas, P. A. et al., 2011. Factors Determining the Location of the Chlorophyll Maximum and the Fate of Algal Production within the Tidal Freshwater James River. *Estuaries and Coasts*, 34(3), pp.569–582.

Bukaveckas, P. A. & Isenberg, W.N., 2013. Loading, Transformation, and Retention of Nitrogen and Phosphorus in the Tidal Freshwater James River (Virginia). *Estuaries and Coasts*, 36, pp.1219–1236.

Bulluck, L. et al., 2013. Age-specific responses to spring temperature in a migratory songbird: Older females attempt more broods in warmer springs. *Ecology and Evolution*, 3(10), pp.3298–3306.

Burger, C. et al., 2012. Climate change, breeding date and nestling diet: How temperature differentially affects seasonal changes in pied flycatcher diet depending on habitat variation. *Journal of Animal Ecology*, 81(4), pp.926–936.

Chen, J. et al., 2009. Simultaneous determination of microcystin contaminations in various vertebrates (fish, turtle, duck and water bird) from a large eutrophic Chinese lake, Lake Taihu, with toxic Microcystis blooms. *The Science of the Total Environment*, 407(10), pp.3317–22.

Corbel, S., Mougin, C. & Bouaïcha, N., 2014. Cyanobacterial toxins: Modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops. *Chemosphere*, 96, pp.1–15.

Davies, I.J., 1984. Methods for the Assessment of Secondary Productivity in Fresh Waters. In J. A. Downing & F. H. Rigler, eds. *Sampling Aquatic Insect Emergence*. Oxford, UK: Blackwell Scientific Publications, pp. 161–227.

Edsall, T.A., 2001. Burrowing mayflies (Hexagenia) as indicators of ecosystem health. *Aquatic Ecosystem Health and Management*, 4, pp.283–292.

Fair, J.M., Paul, E. & Jones, J., 2010. *Guidelines to the use of wild birds in research*, Washington, D.C.

Finlay, J.C., 2001. Stable-Carbon-Isotope Ratios of River BIota: Implications for Energy Flow in Lotic Food Webs. *Ecology*, 82(4), pp.1052–1064.

Frost, S.W., 1957. The Pennsylvania insect light trap. *Journal of Economic Entomology*, (50), pp.287–292.

Garcia, A.C. et al., 2010. Evaluating the potential risk of microcystins to blue crab (Callinectes sapidus) fisheries and human health in a eutrophic estuary. *Harmful Algae*, 9, pp.134–143.

Gérard, C. et al., 2009. Influence of toxic cyanobacteria on community structure and microcystin accumulation of freshwater molluscs. *Environmental Pollution*, 157(2), pp.609–17.

Goodbred, C.O. & Holmes, R.T., 1996. Factors affecting food provisioning of nestling Black-throated Blue Warblers. *Wilson Bulletin*, 108(3), pp.467–479.

Heisler, J. et al., 2008. Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae*, 8(1), pp.3–13.

Huang, P., Zheng, Q. & Xu, L., 2010. The Apoptotic Effect of Oral Administration of Microcystin-RR on Mice Liver. *Environmental Toxicology*, 26(5), pp.443–452.

Ibelings, B.W. et al., 2005. Distribution of microcystins in a lake foodweb: no evidence for biomagnification. *Microbial Ecology*, 49(4), pp.487–500.

JMP®, Version 11, 1989-2007, Cary, NC: SAS Institute Inc.

Jardine, T.D., Kidd, K. a & Rasmussen, J.B., 2012. Aquatic and terrestrial organic matter in the diet of stream consumers: implications for mercury bioaccumulation. *Ecological Applications*, 22(3), pp.843–55.

Kovats, Z.E. & Ciborowski, J.J.H., 1989. Aquatic Insect Adults as Indicators of Organochlorine Contamination. *Journal of Great Lakes Research*, 15(4), pp.623–634.

Lance, E. et al., 2010. Impact of toxic cyanobacteria on gastropods and microcystin accumulation in a eutrophic lake (Grand-Lieu, France) with special reference to Physa (= Physella) acuta. *The Science of the Total Environment*, 408(17), pp.3560–8.

Liarte, S. et al., 2014. Histological effects and localization of dissolved microcystins LR and LW in the mayfly Ecdyonurus angelieri Thomas (Insecta, Ephemeroptera). *Toxicon*, 92, pp.31–35.

Marczak, L.B. & Richardson, J.S., 2007. Spiders and subsidies: Results from the riparian zone of a coastal temperate rainforest. *Journal of Animal Ecology*, 76(4), pp.687–694.

Marshall, H.G. et al., 2009. Assessment and significance of phytoplankton species composition within Chesapeake Bay and Virginia tributaries through a long-term monitoring program. *Environmental Monitoring and Assessment*, 150(1-4), pp.143–155.

Menzie, C.A., 1980. Potential SIgnificance of Insects in the Removal of Contaminants from Aquatic Systems. *Water, Air, and Soil Pollution*, 13(2), pp.473–479.

Miller, M. a et al., 2010. Evidence for a novel marine harmful algal bloom: cyanotoxin (microcystin) transfer from land to sea otters. *PloS one*, 5(9).

Nakano, S. & Murakami, M., 2001. Reciprocal Subsidies: Dynamic Interdependence Between Terrestrial and Aquatic Food Webs. *Proceedings of the National Academy of Sciences of the United States of America*, 98(1), pp.166–170.

O'Neil, J.M. et al., 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*, 14, pp.313–334.

World Health Organization, 2003. Guidelines for safe recreational water.

Ozawa, K. et al., 2003. Accumulation and depuration of microcystin produced by the cyanobacterium Microcystis in a freshwater snail. *Limnology*, 4(3), pp.131–138.

Paerl, H.W. & Otten, T.G., 2013. Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microbial Ecology*, 65(4), pp.995–1010.

Papadimitriou, T. et al., 2009. Accumulation of Microcystins in Water and Fish Tissues : An Estimation of Risks Associated with Microcystins in Most of the Greek Lakes. *Environmental Toxicology*, 25(4), pp.418–427.

Papadimitriou, T. et al., 2012. Assessment of microcystin distribution and biomagnification in tissues of aquatic food web compartments from a shallow lake and evaluation of potential risks to public health. *Ecotoxicology*, 21(4), pp.1155–66.

Petit, L.J., 1989. Breeding Biology of Prothonotary Warblers in Riverine Habitat in Tennessee. *The Wilson Bulletin*, 101(1), pp.51–61.

Poste, A.E., Hecky, R.E. & Guildford, S.J., 2011. Evaluating microcystin exposure risk through fish consumption. *Environmental Science and Technology*, 45(13), pp.5806–5811.

Pyle, P., 1997. *Identification guide to North American birds*, Bolinas, CA: Slate Creek Press.

Rainio, M.J. et al., 2012. Variation of basal EROD activities in ten passerine bird species - relationships with diet and migration status. *PloS One*, 7(3).

Rasmussen, J.B., 1988. Habitat Requirements of Burrowing Mayflies (Ephemeridae : Hexagenia) in Lakes , with Special Reference to the Effects of Eutrophication. *Journal of the North American Benthological Society*, 7(1), pp.51–64.

Rastogi, R.P., Sinha, R.P. & Incharoensakdi, A., 2014. The cyanotoxin-microcystins: current overview. *Reviews in Environmental Science and Biotechnology*, pp.1–35.

Saouter, E. et al., 1993. Mercury Accumulation in the Burrowing Mayfly Hexagenia Rigida (Ephemeroptera) Exposed to CH3HgCl or HgCl2 in Water and Sediment. *Water Research*, 27(6), pp.1041–1048.

Schulte-Hostedde, A.I. et al., 2005. Restitution of mass-size residuals: Validating body condition indices. *Ecology*, 86(1), pp.155–163.

Silva-Stenico, M.E. et al., 2009. Hepatotoxin Microcystin-LR Extraction Optimization. *Journal of the Brazilian Chemical Society*, 20(3), pp.535–542.

Smith, J.L. et al., 2008. Toxicity of Microcystin-LR , a Cyanobacterial Toxin , to Multiple Life Stages of the Burrowing Mayfly , Hexagenia , and Possible Implications for Recruitment. *Environmental Toxicology*, 23(4), pp.499–506.

Smith, J.L. & Haney, J.F., 2006. Foodweb transfer, accumulation, and depuration of microcystins, a cyanobacterial toxin, in pumpkinseed sunfish (Lepomis gibbosus). *Toxicon*, 48(5), pp.580–9.

Takahashi, S. & Kaya, K., 1993. Quail Spleen Is Enlarged by Microcystin RR as a Blue-Green Algal Hepatotoxin. *Natural Toxins*, 285(I 993), pp.283–285.

Takahashi, T., Umehara, A. & Tsutsumi, H., 2014. Diffusion of microcystins (cyanobacteria hepatotoxins) from the reservoir of Isahaya Bay, Japan, into the marine and surrounding ecosystems as a result of large-scale drainage. *Marine Pollution Bulletin*, 89(1-2), pp.250–258.

Tango, P.J. & Butler, W., 2008. Cyanotoxins in Tidal Waters of Chesapeake Bay. *Northeastern Naturalist*, 15(3), pp.403–416.

Tsipoura, N. et al., 2008. Metal concentrations in three species of passerine birds breeding in the Hackensack Meadowlands of New Jersey. *Environmental Research*, 107(2), pp.218–28.

Ueno, Y. et al., 1996. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. *Carcinogenesis*, 17(6), pp.1317–1321.

Walters, D.M., Fritz, K.M. & Otter, R.R., 2008. The Dark Side of Subsidies: Adult Stream Insects Export Organic Contaminants to Riparian Predators. *Ecological Applications*, 18(8), pp.1835–1841.

Wilson, A.E. et al., 2008. Evaluation of the Human Health Threat Associated with the Hepatotoxin Microcystin in the Muscle and Liver Tissues of Yellow Perch (Perca flavescens). *Canadian Journal of Fisheries and Aquatic Sciences*, 65, pp.1487–1497.

Wood, J.D. et al., 2014. Exposure to the Cyanotoxin Microcystin Arising from Interspecific Differences in Feeding Habits among Fish and Shell fish in the James River Estuary, Virginia. *Enviromental Science & Technology*, pp.5194–5202.

Wood, J.D. & Bukaveckas, P. A., 2014. Increasing Severity of Phytoplankton Nutrient Limitation Following Reductions in Point Source Inputs to the Tidal Freshwater Segment of the James River Estuary. *Estuaries and Coasts*, 37(5), pp.1188–1201.

Vander Zanden, J.M. & Sanzone, D.M., 2004. Food Web Subsidies at the Land-Water Ecotone. In *Food Webs at the Landscape Level*. pp. 185–188.

Vita

Nicholas J. Moy was born in Gurnee, Illinois on November 26, 1990. He completed his bachelors of science at the University of Illinois at Urbana-Champaign in May 2012, here he studied Integrative Biology and was involved in entomological and ornithological lab and field research. After graduating Nicholas spent time working and volunteering as a field technician for the Bruce Peninsula Bird Observatory in Ontario, Canada, for the National Park Service in Tennessee, and for the U.S. Fish and Wildlife Service in Utah. Nicholas started the masters program at Virginia Commonwealth University in 2013 in the Department of Biology. During this time he taught courses including Panama Avian Field Ecology, Ornithology Lab, and Introductory Biology Lab; served as president of the Graduate Organization of Biology Students; worked as a field technician with an at-risk species the Golden-winged Warbler in Highland County, VA; as well as achieved his Post-baccalaureate Graduate Certificate in Geographic Information Systems from the L. Douglas Wilder School of Government and Public Affairs at VCU.