Grand Valley State University

From the SelectedWorks of Amy L. Russell

2015

Microcystin detected in little brown bats (Myotis lucifugus)

Megan Woller-Skar, *Grand Valley State University*
Devin N. Jones, *Grand Valley State University*
Mark R. Luttenton, *Grand Valley State University*
Amy L. Russell

Available at: https://works.bepress.com/amy_russell/23/

Am. Midl. Nat. (2015) 174:331–334

Notes and Discussion Piece

Microcystin Detected in Little Brown Bats (Myotis lucifugus)

Abstract.—Recent studies documented the potential transfer of microcystin, a hepatotoxin produced by some cyanobacteria, from aquatic to terrestrial ecosystems. Using enzyme linked immunosorbent assay, we measured microcystins in emergent *Hexagenia limbata* mayflies and fecal samples collected from a maternity colony of little brown bats (*Myotis lucifugus*) located adjacent to a Michigan (U.S.A.) lake that experiences seasonal blooms of toxicogenic *Microcystis aeruginosa.* All *H. limbata* and *M. lucifugus* fecal samples contained microcystin (*H. limbata*: mean = 293.88 ng/g dw ± 35.99 se, n = 39; *M. lucifugus*: mean = 262.10 ng/g dw ± 31.08 se, n = 20). Ingestion of this toxin may represent a previously unrecognized stressor on bat populations in this region.

Introduction

Most North American bats are insectivorous (Hutson *et al.*, 2001), and many are generalist and opportunistic feeders. For example Lee and McCracken (2005) documented significant differences in insect composition in the diet of Brazilian free-tailed bats (*Tadarida brasiliensis*) that reflected the relative abundance of local insects through the dusk-to-dawn hours. In addition to temporal variation in opportunistic feeding, bats have also shown spatial variation, exploiting streetlamps (Rydell, 1992) and eavesdropping on conspecific echolocation calls (Gillam, 2007) to locate patches of increased insect concentrations.

Common bats in Michigan (U.S.A.) include the big brown bat (*Eptesicus fuscus*) and the little brown bat (*Myotis lucifugus*), both of which have been shown to feed opportunistically on large aquatic insect emergences (Hamilton and Barclay, 1998; Clare *et al.*, 2011) including those of Ephemeroptera (mayflies).

Microcystin (MC) is a hepatotoxin produced by some species of cyanobacteria such as *Microcystis aeruginosa*. Research has documented the potential accumulation of MC in aquatic nymphs of *Hexagenia* mayflies (Smith *et al.*, 2008). *Hexagenia* spend a portion of their lifecycle as aquatic nymphs and a portion in terrestrial environments as subimagos and imagos. Therefore, if nymphs are exposed to *M. aeruginosa* and accumulate MC, they may transfer MC from aquatic to terrestrial habitats via emergence of adult insects, the following spring.

Little Traverse Lake (LTL, Leelanau County, Michigan, U.S.A.) is a low nutrient lake basin that has experienced seasonal blooms of *M. aeruginosa* from mid-July to August since establishment of zebra mussel (*Dreissena polymorpha*) populations in approximately 2000. In addition MC was detected in LTL macroinvertebrate nymphs, including *Ephemera*, *Hexagenia*, and Heptagenaiidae in June, prior to the seasonal bloom (M. Woller-Skar, pers. obs.). The purpose of this project was to determine if a *M. lucifugus* colony adjacent to LTL was exposed to MC, possibly through feeding on *Hexagenia limbata* subimagos or imagos containing the toxin.

Methods

Samples were collected 13 June 2013 to coincide with the emergence of subimago *H. limbata* in and around Little Traverse Lake (LTL) in Leelanau County, Michigan, U.S.A. prior to seasonal *M. aeruginosa* blooms. We collected subimago and imago *H. limbata* using a black light, from the north shore of LTL (44.924681, −85.859879). Fecal samples for MC analysis were collected from under a *M. lucifugus* maternity roost, located in a barn approximately 350 m from the shore of LTL (44.923580, −85.821265). One adult male *M. lucifugus* was captured and humanely euthanized using isoflurane (IACUC # 09-07-A, 5/15/09); fresh feces and liver tissue were collected from this individual.

All samples collected for MC analysis were wrapped in foil and frozen. Enzyme linked immunosorbent assays (ELISA) were conducted at the University of Michigan field station (Pellston, Michigan, U.S.A.) to quantify MC-equivalents (including the congeners LR, LA, RR, and YR) in the *H. limbata*, bat liver and feces. Relatively fresh (likely not more than 2 w old) fecal samples collected from the barn floor beneath the colony weighed approximately 50 mg, whereas the fecal sample collected from the euthanized bat weighed 1.9 mg. These samples, along with the entire bat liver (29 mg dw), were placed

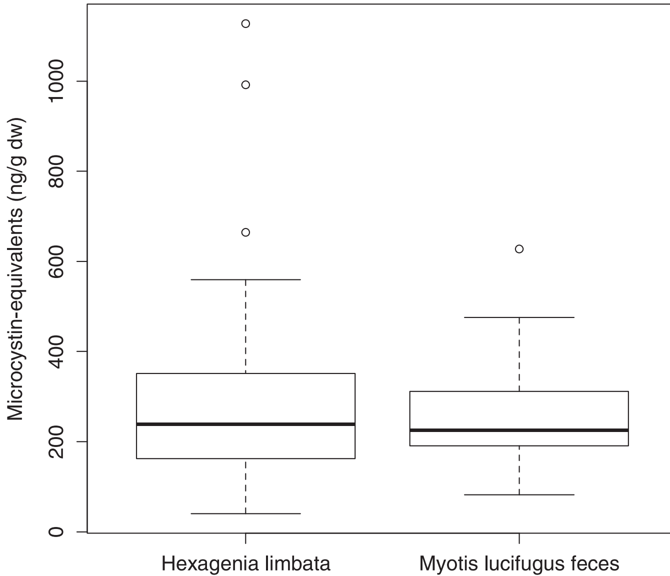

FIG. 1.—Boxplot of median MC-eq (ng/g dw) in subimago and imago *H. limbata* (n = 39) and fecal pellets (n = 20) collected from beneath a *M. lucifugus* colony, including interquartile range. Outliers denoted by open circles

in glass scintillation vials and frozen at −80 C for at least 1 h. The scintillation vials were covered in parafilm that had been punctured to allow moisture to escape during freeze-drying. Freeze-drying was completed in a lyophilizer with the samples left to dry overnight. Dried samples were ground in a mortar and pestle and weighed in 25 mL glass test tubes with Teflon-lined caps for extraction (extracted dried sample was stored in capped scintillation vials). To extract the microcystin, 2.0 mL of 80% aqueous methanol (v/v) was added to the sample test tube, and the sample was sonicated in an ice bath for 10 min. After sonication was complete, the samples were allowed to stand at room temperature overnight. All samples were then centrifuged at 3500 rpm for 10 min. Finally, 150 μL aliquot of the 80% methanol supernatant was removed and diluted (1:10) with deionized water in a microfuge tube before running on the ELISA plate. All ELISA assays were performed using plate kits obtained by EnviroLogix (Portland, Maine, U.S.A.). The procedure followed manufacturer's instructions with the optional orbital shaker being used at 200 rpm during each incubation period. Optical density, measured on a Stat Fax 2100 microplate reader in dual wavelength mode, was determined at 450 nm and 630 nm. The latter wavelength was used to correct for interferences from bubbles or scratches in the sample wells. All measurements were referenced against air.

RESULTS

Microcystin-eq was present in all *H. limbata* (n = 39) and environmental fecal samples from *M. lucifugus* (n = 20). The amount of MC-eq measured in *H. limbata* ranged from 40.5-1127.7 ng/g dw; the feces of *M. lucifugus* taken from the barn floor ranged from 81.8-627.5 ng/g dw (Fig. 1). In contrast to *H. limbata* (approximately 35 mg) and environmental fecal (approximately 50 mg) samples, those collected from the adult male *M. lucifigus* were small (fecal = 1.9 mg; liver = 29 mg dw) making their analyses for microcystins challenging. Microcystin-eq measured in the feces of the adult male *M. lucifugus* were 213.8 ng/g dw, but outside the range of the standard curve of the kit, making the accuracy questionable, whereas microcystin-eq in the liver was less than the limit of detection.

DISCUSSION

Microcystin found in the feces of *M. lucifugus* indicates individuals near LTL were exposed to the toxin. Miller *et al.* (2010) reported concentrations of MC in the livers of sea otters ranging from 1.36-348 ppb (ng/g) wet weight and a fecal sample of 16.4 ppb wet weight (quantified using high performance liquid chromatography tandem mass spectrometry, LC-MS/MS). This range in microcystin concentrations in the sea otter livers was similar to that found in *M. lucifugus* feces. However microcystin concentrations found in the *M. lucifugus* feces were almost twice as high as those measured in sea otter livers. This difference could be the result of the use of a wet weight by Miller *et al.* (2010) as opposed to a dry weight, the comparison of liver to fecal samples, or the chemical analyses ELISA and LC-MS/MS. Miller *et al.* (2010) were unable to determine the source of microcystin in the fecal samples; whether it was from ingesting contaminated prey items or the circulation of enterohepatic toxin. Here, the source of microcystin in *M. lucifugus* is likely due to trophic transfer from emergent aquatic insects.

While there are no known die-offs of bats attributed to microcystin, mass mortality events in bats have been attributed to *Anabaena* toxin (Pybus *et al.*, 1986) and anthropogenic environmental toxins (Thies and Thies, 1997; Clark, 2001). Microcystin is known to cause skin irritations, vomiting, cancer of the liver, and death in humans, livestock, pets, and many aquatic organisms (Kuiper-Goodman *et al.*, 1999; Sivonen and Jones, 1999), and it poses a potential health risk to bats. Because North American bats face significant conservation threats in the form of white-nose syndrome (Coleman and Reichard, 2014) and wind turbine mortality (Arnett and Baerwald, 2013), it is important that we understand the full context of environmental stressors faced by these populations. Future work should focus on the effect of MC on the liver of bats, possibly by pooling tissues from multiple individuals to provide sufficient material for reliable toxin quantification.

Acknowledgments.—This project was funded by GVSU's Center for Scholarly and Creative Excellence. We thank Ben Brown for allowing us access to the *Myotis lucifugus* roost on his property.

LITERATURE CITED

ARNETT, E. B. AND E. F. BAERWALD. 2013. Impacts of wind energy development on bats: implications for conservation. *In:* R. A. Adams and S. C. Pedersen (eds.). Bat evolution, ecology, and conservation. Springer, New York.

CLARE, E. L., B. R. BARBER, B. W. SWEENEY, P. D. N. HERBERT, AND M. B. FENTON. 2011. Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Mol. Ecol.*, **20**:1772–1780.

CLARK, D. R. 2001. DDT and the decline of free-tailed bats (*Tadarida brasiliensis*) at Carlsbad Cavern, New Mexico. *Arch. Environ. Contam. Toxicol.*, **40**:537–543.

COLEMAN, J. T. H. AND J. D. REICHARD. 2014. Bat white-nose syndrome in 2014: a brief assessment seven years after discovery of a virulent fungal pathogen in North America. *Outlooks Pest Manage.*, **25**:374–377.

GILLAM, E. H. 2007. Eavesdropping by bats on the feeding buzzes of conspecifics. *Can. J. Zool.*, **85**:795–801.

HAMILTON, I. M. AND R. M. R. BARCLAY. 1998. Diets of juvenile, yearling, and adult big brown bats (*Eptesicus fuscus*) in southeastern Alberta. *J. Mamm.*, **79**:764–771.

HUTSON, A. M., S. P. MICKLEBURGH, AND P. A. RACEY. 2001. Microchiropteran Bats: Global Status Survey and Conservation Action Plan, IUCN, Gland.

KUIPER-GOODMAN, T., I. FALCONER, AND J. FITZGERALD. 1999. Human health aspects, p. 113–153. *In*: I. Chorus and J. Bartram (eds.). Toxic Cyanobacteria in water, a guide to their public health consequences, monitoring, and management. Spon Press, New York.

LEE, Y. F. AND G. F. MCCRACKEN. 2005. Dietary variation of Brazilian free-tailed bats links to migratory populations of pest insects. *J. Mamm.*, **86**:67–76.

MILLER, M. A., R. M. KUDELA, A. MEKEBRI, D. CRANE, S. C. OATES, M. T. TINKER, M. STAEDLER, W. A. MILLER, S. TOY-CHOUTKA, C. DOMINIK, D. HARDIN, G. LANGLOIS, M. MURRAY, K. WARD, AND D. A. JESSUP. 2010. Evidence for a novel marine harmful algal bloom: Cyanotoxin (microcystin) transfer from land to sea otters. *PLoS One*, **5**(9):e12576.

PYBUS, M. J., D. P. HOBSON, AND D. K. ONDERKA. 1986. Mass mortality of bats due to probable blue-green algal toxicity. *J. Wildl. Dis.*, **22**:449–450.

RYDELL, J. 1992. Exploitation of insects around streetlamps by bats in Sweden. *Func. Ecol.*, **6**:744–750.

SIVONEN, K. AND G. JONES. 1999. Cyanobacterial toxins, p. 41–111. *In:* I. Chorus and J. Bartram (eds.). Toxic Cyanobacteria in water, a guide to their public health consequences, monitoring, and management. Spon Press, New York.

SMITH, J. L., G. L. BOYER, E. MILLS, AND K. L. SCHULZ. 2008. Toxicity of microcystin-LR, a cyanobacterial toxin, to multiple life stages of the burrowing mayfly, *Hexagenia,* and possible implications for recruitment. *Env. Toxicol.*, **23**:499–506.

THIES, M. L. AND K. M. THIES. 1997. Organochlorine residues in bats from Eckert James River Cave, Texas. *Bull. Environ. Contam. Toxicol.*, **58**:673–680.

M. MEGAN WOLLER-SKAR, DEVIN N. JONES, MARK R. LUTTENTON, AND AMY L. RUSSELL, Department of Biology, Grand Valley State University, Allendale, Michigan 49401. *Submitted 11 December 2014; accepted 12 June 2015.*

Copyright of American Midland Naturalist is the property of University of Notre Dame / American Midland Naturalist and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.