**Mercury concentrations in bat guano from caves and bat houses in Florida and Georgia, USA**

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ABSTRACT

Insectivorous bats are terrestrial animals that show increasing concentrations of mercury via bioaccumulation, as indicated in their guano. In this study, we measured mercury concentrations in bat guano collected from surface and Russian core samples from 10 caves and 2 bat houses in Florida and Georgia, USA. These results were used for comparison to dated guano in a previous study and to compare concentrations between the different predominant bat species utilizing bat houses versus caves. Accounting for differences among sampling locations, the mean concentration of mercury was estimated at 0.53 +/- 0.04 ppm. Average concentrations of mercury in bat guano between caves and bat houses did not differ significantly, with mean concentrations in caves estimated to be 0.04 +/- 0.07 ppm higher than those in bat houses. The mean concentrations between caves are found to be significantly different, as well as the concentrations between the two bat houses. This is similar to the mean for the modern/recent guano, and higher than ancient and historical guano in a previous study of mercury concentrations with age-dated guano at Mammoth Cave National Park. This indicates the modern/fresh guano in Florida and Georgia caves could be increasing from historical levels, likely due to bioaccumulation from the increasing concentrations of mercury in the environment. This has implications for both the health of bats and cave ecosystems, as guano is an important food source for other cave species and could lead to increased mercury levels throughout the cave food web.

*Key words*: Bat guano, mercury, insectivorous bats, bioaccumulation

INTRODUCTION

Increasing concentrations of mercury in the environment from anthropogenic activity is a health threat for both humans and wildlife. Elemental mercury can transform in aquatic systems by bacterial methylation to methylmercury, a neurotoxin which bioaccumulates in aquatic and terrestrial food webs (Selin 2009). Insectivorous bats are particularly susceptible to mercury bio- accumulation via trophic transfer through the food chain (Iskali and Zhang, 2015; Syaripuddin et al., 2014). Bats take up mercury when consuming large quantities of insects that accumulate mercury during their aquatic larval stages in mercury-contaminated waterbodies, as well as when feeding on terrestrial insects that bioaccumulate mercury (Brack and Whitaker, 2001). Insects with aquatic larval stages that insectivorous bats are known to ingest include Trichoptera, certain Diptera and Coleoptera, Megaloptera, Odonata, Neuroptera, and Ephemeroptera, as well as insects such as some Lepidoptera and Coleoptera that don’t have an aquatic larval stage (Bogdanowicz et al, 1999; Fukui et al. 2006).

Bats have unquestionably been affected by this heavy metal, since several studies have found the presence of mercury in bat muscles, kidneys, livers, brains and fur (Miura *et al*., 1978; Powell, 1983; Hickey *et al*., 2001; O’Shea *et al*., 2001; Syaripuddin et al., 2014; Yates *et al*., 2008; Wada *et al*., 2010; Yates *et al*., 2012). This heavy metal contamination is linked to bat population declines (Mickleburgh *et al*., 2002) and sub-lethal biological effects like impaired reproduction and chronic health issues, as well as death in bats exposed to high contaminant loads of heavy metals (Clark and Shore, 2001; Hickey *et al*., 2001). Syaripuddin *et al*. (2014) found levels of mercury in insectivorous bat fur above and below the 10 mg/kg threshold in which detrimental effects occur in bats.

Mercury concentrations in bats can also be measured in their waste, called guano. After ingesting a meal containing mercury, some of the metal is excreted in their guano, which primarily consists of bat hair, insect remains and bat mucus (Maher, 2006). Few studies have focused on heavy metal concentrations in bat guano. Petit and Altenbach (1973) dated a guano core from a cave in Colorado and found levels of mercury throughout the core were related to production at a local copper smelter and open pit mine. O’Shea *et al*. (2001) found higher concentrations of environmental contaminants, including mercury, in bat guano near a superfund site than at a reference site in Colorado. Petit (1975) investigated mercury concentrations in a 1100 year-old guano core from an Arizona cave and suggested that mercury concentrations had been higher than expected in pre-industrial times, possibly due to geological processes such as volcanic activity. Clark *et al*. (1986) found elevated concentrations of the metals cadmium, chromium and zinc in guano from a cave in Florida, but did not analyze for mercury. Cuculić *et al*. (2011) found bat guano was responsible for high concentrations of metals, particularly cadmium, in anchialine caves in Croatia (mercury was not analyzed). A recent study by Hagan (2014) analyzed three dated groups of bat guano from Mammoth Cave National Park in Kentucky and found that modern/fresh guano had higher concentrations of mercury than historical guano (~100-1100 years old), which in turn had higher concentrations than ancient guano (~30,000 years old).

The dominant insectivorous bat species between the caves and bat houses in this study are different, and therefore could have significantly different mercury concentrations in their guano. The dominant bat species roosting in Florida and Georgia caves are the maternity/wintering colonies of the Southeastern myotis (*Myotis* *austroriparius* – MYAU) (Gore and Hovis, 1998), with lesser contributions from Tri-colored bats (*Perimyotis subflavus* – PESU – formerly known as eastern pipistrelle, *pipistrellus subflavus*). The endangered Gray bat (*Myotis grisescens* – MYGR) was formerly abundant in some caves in the Florida, but the Florida population has decreased in the last few decades and the species may no longer be present in the state (Gore *et al*., 2012). The caves in Georgia have MYAU and PESU as the dominant species (pers. comm. K. Morris, Georgia Department of Natural Resources, April 24, 2013). Thus, MYAU is assumed the dominant species contributing to the guano piles, which forage near water (Barbour and Davis 1969) and consume Coleptera and Lepidoptera arthropods and culicidae (Zinn 1977). PESU also forage near water (Fujita and Kunz, 1984; Whitaker and Hamilton, 1998) and consume insects in the orders of Trichoptera, Homoptera, Coleoptera, Hymenoptera and Lepidoptera (Sherman 1939; Ross 1961; Whitaker 1972; Carter et al., 1999). The dominant species roosting in bat houses in Florida is the Brazilian free-tailed bat (*Tadarida braziliensis* – TABR), with MYAU present to a lesser degree. In the southeastern United States, the TABR diet includes insects in the order of Coleoptera, Diptera, Lepidoptera and Hymenoptera (Sherman 1939).

The guano in this study was assumed modern (<100 years old, as in the Hagan 2014 study), since caves in this region are prone to flooding and none of the guano produced cores over 11 inches (279.4 millimeters), indicating a lack of long-term accumulation of guano piles. The effect of flooding on guano piles in regards to mercury mobility is also unknown, as is bioturbation from fauna. Stratigraphically dated guano piles also provide inconsistent results (Zukal *et al*., 2015). The guano piles from one of the bat houses were also known to be disturbed, as the bat house guano piles at University of Florida, Gainesville are collected in 55 gallon drums and given away in 5 gallon buckets to gardeners several times per week (Kenneth Glover, per comm, 4/26/2013). Therefore, the guano cores in this study were not dated and analyzed for temporal concentrations of Hg.

Detailed data on the bat population in the caves or bat houses was not collected during the study and is not available to the authors. The goal of the study was not to be a comprehensive study on mercury in bat guano correlated with bat species, sex, or age, but to make a preliminary analysis of mercury concentrations in bat guano from Florida and Georgia. This preliminary analysis hypothesizes that mercury concentrations in guano from the Florida and Georgia bat populations will be similar to modern/fresh guano and greater than historic and ancient concentrations in the Hagan (2014) study at Mammoth Cave National Park. The main bat species utilizing caves and bat houses is different, so these two environments were used for a general species comparison, hypothesizing that guano representing different predominant species of bats might have significantly different bioaccumulation of mercury, as seen in the guano.

This data is valuable for monitoring potential mercury contamination in bat populations and also cave ecosystems. Many species of cave fauna ingest bat guano, and therefore mercury may bioaccumulate in the cave food web.

MATERIALS AND METHODS

The guano from insectivorous bats in this study was collected from ten caves and two bat houses in Florida and caves in southwestern Georgia (Fig. 1). The total number of guano samples collected from caves was 95, and 17 for bat houses. All samples were collected between January 12, 2013 and February 13, 2014. Sample locations were approximated on cave survey maps. Collecting an equal number of samples among the caves was a theoretical part of the study design before sampling started. However, due to the heterogeneous nature of the quantity and depth of guano available in the caves sampled, the number of samples were different. Calculating the amount of guano present in the caves would have been difficult, as the breadth and depth of guano was different for every cave and would have required extensive time and damage to the cave environments. Bats were also present in several of the caves during guano collection, and the authors wanted to keep disturbance to the bats minimal. Core sampling was chosen randomly based on the depth of the guano piles, and only a few guano piles in the caves or bat houses were deep enough to use the corer. Core samples from caves and bat houses were collected with a Russian sampler to avoid compaction of guano (Maher, 2006, Johnston *et al*., 2010). Cores were divided into 1 inch (25.4 mm) subsamples starting from the top of the core. Guano samples from cave surfaces were collected with plastic spoons and put into clear, reclosable plastic bags, with a new spoon and bag for each sample.

Guano samples from Florida caves were from Big Mouth Cave (7/13/2013, bat pellets collected throughout cave and compiled into 1 sample); Cottondale Cave (10-30-2013, 5 surface samples taken throughout the cave and a core that was 6 in (152.4 mm) deep and subsampled at 1 in (25.4 mm) intervals; Florida Caverns Old Indian Cave (2/13/2014, two 8 in (203.2 millimeters) cores subsampled at 1 in (25.4 mm) intervals in the Rotunda Room, 5 surface samples taken throughout cave); Jerome’s Bat Cave (10/30/2013, 12 surface samples taken throughout cave); Judges’s Cave (10/30/2013, one 11 in (279.4 mm) core subsampled at 1 in (25.4 mm) intervals, and one 8 (203.2 mm) core at 1 in (25.4 mm intervals), Newberry Bat Cave (9/15/2013, 1 sample of pellets), Snead’s, also known as Pope’s Bat Cave (10/30/2013, 6 surface samples), and Thornton’s Cave, also known as Sumter Bat Cave (7/13/2017, 1 sample at one of the entrances). Caves from Georgia were Climax Cave (2/28/2013, 3 surface samples from the Barrel Room; 7/6/2013, 10 in (254 mm) core subsampled at 1 in (25.4 mm) intervals from Barrel room, 4 surface samples, 2 composites of 2 separate 11 in (279.4 mm) cores, two separate samples that were the bottom inches of two separate 11 in (279.4 mm) cores; and Waterfall Cave (8/17/2013, 2 surface samples).

Guano samples were taken from two bat houses in central Florida. Two cores were taken at the University of Florida bat house on 9/13/2013. One core had 5 intervals of 1 in (25.4 mm) each. The other core had 7 intervals, with the first six intervals at 1 in (25.4 mm) and the 7th interval comprising the last 1.5 inches of the core. The guano piles at the Lower Suwanee National Wildlife Reserve were not deep enough to use the corer, so samples were taken as compilations within different locations under the bat house. Depths were measured in inches with a ruler from the top of the pile to the concrete bottom. A 4 in (101.6 mm) sample was compiled from the middle under the bat house, a 3 in (76.2 mm) sample was compiled from the back left corner, a top inch (25.4 mm) was taken from the back middle, and the bottom inch (25.4 mm) was taken from the back middle.

All samples were stored in a freezer until freeze dried. All samples were freeze dried to constant weight, and analyzed for Total Mercury (THg) by thermal decomposition, gold amalgamation and atomic absorption spectroscopy (EPA method 7473) using a Milestone DMA80 mercury analyzer. This method provided detection limits in the sub-parts per billion range. The QA/QC included blanks, replicates and matrix spikes. All duplicates had <10 percent difference and were averaged. The DMA80 was calibrated with NIST-traceable standards, and the calibration was verified using standards purchased from NIST and the National Research Council of Canada.

RESULTS

For analysis and graphics, we used R (R Core Team, 2016), an open-source statistical computing package. Since some samples were taken from cores and others from the surface, we first compared the variability of each set of core samples against surface samples or samples from another core collected in the same cave. We used the Fligner-Killeen test of homogeneity of variances (Fligner and Killeen, 1976) to determine that the variances among the types of samples (core or surface) taken in a single cave or bat house were not significantly different. In addition, we checked samples from the same core for systematic autocorrelation. Given the lack of significant differences in variability or systematic autocorrelation, the following analysis does not distinguish between core samples and surface samples taken from the same cave.

Figure 2 shows boxplots of the mercury concentrations separately for samples taken in caves and in bat houses, along with the respective sample sizes for each group. Because more than five times as many samples were collected in caves as in bat houses, a traditional confidence interval for the overall mercury concentration across locations would be dominated by the samples from the caves. Instead, we investigate differences among the individual caves and the bat houses before proposing an estimation method.

Figure 3 shows the concentrations from caves, separated by location and excluding caves with fewer than 3 samples. Although ten caves were surveyed, four of them had too few observations to ascertain the mean or variability of mercury concentrations in that location. (Only one sample was taken from Big Mouth Cave and Newberry Bat Cave, and only two samples were available from Thornton’s Cave and Waterfall Cave). Given the differences among the boxplots in Figure 3, it appears that the means and variances of concentrations likely differs between at least some of the caves. Figure 4, which shows boxplots for the samples from the two bat houses, also shows evidence of a difference in mean and potentially variance.

Given the likelihood of heterogeneous variance, the traditional analysis of variance (ANOVA) approach is excluded. Instead, we use generalized least squares (e.g. Sen and Srivastava, 1990, Chp. 6) to fit a model which allows each individual cave and bat house to have its own means and variance. After fitting this model, we combine the estimates (and the accompanying variability) to estimate the overall mean concentration of mercury at all sites. We then use linear contrasts between the fitted means to estimate the difference in the mean concentration in bat houses vs. caves.

We first consider comparisons between caves. With Tukey’s method of adjusting for multiple comparisons, we conclude that there are significant differences in mean mercury concentrations between Climax Cave and Florida Caverns Old Indian Cave (p < 0.001) and between Climax Cave and Judge’s Cave (p< 0.001). The mean mercury concentration in Florida Caverns Old Indian Cave is estimated to be 0.20 +/- 0.09 ppm higher than that in Climax Cave, while the mean in Judge’s Cave is estimated to be 0.21 +/- 0.11 ppm higher. Climax Cave is the largest cave in the southeastern United States coastal plain, and the largest one sampled in this study. This cave also had the largest observable number of bats and amount of guano in this study period, which could affect the differences in mercury concentrations between this cave and the other caves.

The mean concentrations in the two bat houses are also significantly different (p < 0.0001), with the mean at the bat house at Lower Suwanee National Wildlife Reserve (NWR) estimated as 0.37 +/- 0.17 ppm higher than that at the bat house at the University of Florida at Gainesville. Given the smaller sample sizes for the bat houses, the validity of the normal distribution assumed in the model may reasonably be questioned. However, the Wilcoxon-Mann-Whitney non-parametric test procedure, which does not depend on the normality assumption, provides additional evidence that the concentrations are significantly different in the two bat houses (p < 0.001). We note that this aspect of the analysis could be improved by increasing the number of samples from bat houses. This could include increasing the sample size from each bat house, particularly as we had only 5 samples from the Lower Suwanee NWR bat house, or increasing the number of bat houses from which samples were obtained. The Lower Suwanee bat house is a much smaller bat house than the bat house at University of Florida at Gainesville, which has the largest occupied bat house in the world and two structures for bat houses instead of the one structure at Lower Suwanee.

Lastly, we used linear contrasts to estimate the difference in mean concentrations between caves and bat houses. The estimated means from the six caves with sufficient numbers of observations were weighted equally to obtain an estimate for the mean mercury concentration in caves. The estimates from the two available bat houses were also weighted equally in determining the estimated concentration for bat houses. Using the estimates and their associated variability from our generalized least squares model, we estimated the difference between caves and bat houses as 0.04 +/ 0.07, which is not significant (p = 0.29). To obtain an estimate of the overall mean concentration, we assigned equal weight to the estimated mean for caves and the mean obtained for bat houses. This overall mean concentration was estimated to 0.53 +/- 0.04 ppm.

DISCUSSION

Since the main bat species utilizing caves and bat houses is different, we hypothesized that guano representing different predominant species of bats might have significantly different bioaccumulation of mercury, as seen in the guano. Indeed, the concentrations from bat houses (dominant species TABR) were estimated to average 0.1104 ppm lower than the concentration in the caves (dominant species MYAU). This slight difference could be due to the different diets in the two predominant species, which would impact trophic transfer of mercury and subsequent bioaccumulation in the bats. The other insects making up the dominant diet in TABR could possibly be less susceptible to mercury uptake, or mercury hotspots could exist in Florida and Georgia to make some insects, and therefore guano, have more mercury.

This study also hypothesized that mercury concentrations in guano from the Florida and Georgia bat populations would be similar to modern/fresh guano and greater than historic and ancient concentrations in the Hagan (2014) study at Mammoth Cave National Park. The estimated mean concentration of all bat guano in all three regions (caves and bat houses) is 0.5306 +/- 0.0403 ppm. In comparison with the Mammoth Cave study, the guano from Florida and Georgia is within the mean range of the modern/fresh guano (0.7 +/- 0.2 ppm) from Kentucky. Kentucky is in the southeastern U.S. near Florida and Georgia, and is likely experiencing the same phenomenon of increasing deposition and trophic transfer of mercury in the environment. Historical (~100-1000 years old) and ancient (~30,000 years old) guano from the Hagan (2014) study had lower than modern/fresh values of 0.20 +/- 0.05 ppm and 0.01 +/-0.0 ppm, respectively. The higher average concentrations in both the Kentucky and Florida bat guano indicates an increase in mercury bioaccumulation in bats in more recent history, although a different composition of bat species populations at Mammoth Cave could impact the comparison.

Syaripuddin *et al*. 2014 found levels of mercury in insectivorous bat fur above and below the 10 mg/kg threshold in which detrimental effects occur in bats. The relationship between mercury levels in bat fur and bat guano is unknown, but guano in this study didn’t reach levels that high. The highest concentration was 1.6793 ppm from Jerome’s Cave, in the Florida panhandle.

Weaknesses of the study include the low sample size and the differences in the age of the guano among locations, possibly skewing the results. The two bat houses in this study are known to give away guano on a routine basis to gardeners, thereby removing the older guano. Although guano in cave environments may be disturbed by flooding, cavers, and cave scientists, caves are a more protected environment from the elements that bat houses, so the guano from caves in this study are likely older and less disturbed than the guano under the bat houses. There are also no established guidelines for guano collection.

The concentration of mercury in bat guano has implications for both the health of bats and cave ecosystems. Bats with white-nose syndrome have been found with elevated levels of contaminants and mercury exposure could potentially predispose bats to this disease (Kannan *et al*., 2010). The presence of mercury in guano affects not only the health of bats, but contaminated guano may allow mercury to bioaccumulate in cave ecosystems and food webs for trogloxenes, troglophiles and troglobites. Coprophagy of guano has been observed in cave-adapted salamanders (Fenolio *et al*., 2006), dermestid cave beetles (Mizutani *et al*., 1992), and even meat ants who enter caves to collect and transport guano back outside to their mounds (Moulds, 2006). Macroinvertebrate communities in caves have been found to increase after fresh guano is deposited (Poulson and Lavoie, 2000), and the nutrient quality of guano has been found to influence biodiversity of macroinvertebrates in caves (Iskali and Zhang, 2015).

Future studies should evaluate methylmercury concentrations in both fresh guano and hair from bats roosting above the guano to correlate concentrations between the bats and the bat waste. It would also be beneficial to know if the bacteria that convert inorganic forms of mercury to methylmercury existed in caves, as the methylmercury is the bioavailable form.

LITERATURE CITED

ANDERSON, M. J., and C. J. F. ter Braak. 2003. Permutation tests for multi-factorial

analysis of variance. Journal of Statistical Computation and Simulation, 73: 85-113.

BARBOUR, R. W., and W. H. DAVIS. 1969. Bats of America. Lexington, KY: University

Press of Kentucky. 286 p.

BOGDANOWICZ, W., M. B. FENTON, and K. DALESZCZYK. 1999. The relationships

between echolocation calls, morphology and diet in insectivorous bats. Journal of

Zoology, 247: 381-393.

BRACK, V., and J. O. WHITAKER. 2001. Foods of the northern myotis, *Myotis*

*septentrionalis*, from Missouri and Indiana, with notes on foraging. Acta Chiropterologica, 3(2): 203-210.

CARTER, T. C., M. A. MENZEL, R. R. CHAPMAN, and K. V. MILLER. 1999. Summer

foraging and roosting behavior of an eastern pipistrelle, *Pipistrellus subflavus*. Bat Research News, 40: 5-6.

CLARK, D. R., Jr, A. S. WENNER, and J. F. MOORE. 1986. Metal residues in bat colonies,

Jackson County, Florida, 1981-1983. Florida Field Naturalist, 14: 38-45.

CLARK, D. R., J. R., and R. F. SHORE. 2001. Chiroptera. Pp. 159-214, *in* Ecotoxicology in

wild mammals (R. F. SHORE and B. A. RATTNER, eds). John Wiley & Sons, New York.

CUCULIC, V., N. CUKROV, Ž KWOKAL, and M. MLAKAR. 2011. Distribution of trace

metals in anchialine caves of Adriatic Sea, Croatia. Estuarine, Coastal and Shelf.

Science, 95(1): 253-263.

FENOLIO, D. B., G. O. GRAENIN, B. A. COLLIER, and J. F. STOUT. 2006. Coprophagy in a

cave-adapted salamander; the importance of bat guano examined through nutritional and

stable isotope analyses. Proceedings of the Royal Society B, 273: 439-443.

FLIGNER, M. A, and T. J. KILLEEN. 1976. Distribution-free two-sample tests for scale. Journal of the American Statistical Association, 71: 210-213.

FUJITA, M. S., and T. H. Kunz. 1984. *Pipistrellus subflavus*. Mammalian Species, 228: 1-6.

FUKUI, D., M. MURAKAMI, S. NAKANO, AND T. AOI. 2006. Effect of emergent aquatic

insects on bat foraging in a riparian forest. Journal of Animal Ecology, 75: 1252-1258.

GAMES, P. A., J. F. HOWELL. 1976. Pairwise Multiple Comparison Procedures with

Unequal N's and/or Variances: A Monte Carlo Study. Journal of Educational

Statistics, 1: 113-125.

GORE, J. A., L. LAZURE, and M. E. LUDLOW. 2012. Decline in the winter population of

gray bats (*Myotis grisescens*) in Florida. Southeastern Naturalist, 11: 89-98.

GORE, J. A., and J. A. HOVIS. 1998. Status and conservation of southeastern myotis maternity

colonies in Florida caves. Florida Scientist, 61: 160-169.

HAGAN, S. 2014. Mercury bioaccumulation in bat populations in Mammoth Cave National

Park: Modern, Historical, and Ancient Samples. Thesis. Western Kentucky University.

Kentucky.

HICKEY, M. B. C., M. B. FENTON, K. C. MACDONALD, and C. SOULLIERE. 2001. Trace

elements in the fur of bats (Chiroptera: Vespertilionidae) from Ontario and Quebec,

Canada. Bulletin of Environmental Contamination and Toxicology, 66: 699-706.

ISKALI, G., and Y. ZHANG. 2015. Guano subsidy and the invertebrate community in Bracken

Cave: The World’s largest colony of bats. Journal of Cave and Karst Studies, 77: 28-36.

JOHNSTON, V. E., F. MCDERMOTT, AND TAMAS. 2010. A radiocarbon dated bat guano

deposit from N.W. Romania: Implications for the timing of the Little Ice Age and

Medieval Climate Anomaly. Palaeogeography, Palaeoclimatology, Palaeoecology, 291:

217- 227.

KANNAN, K., S. H. YUN, R. J. RUDD, and M. BEHR. 2010. High concentrations of

persistent organic pollutants including PCBs, DDT, PBDEs and PFOs in little brown bats with white-nose syndrome in New York, USA. Chemosphere, 80: 613-618.

MAHER, L. J., JR. 2006. Environmental information from guano palynology of insectivorous

bats of the central part of the United States of America. Palaeogeography, Palaeoclimatology, Palaeoecology, 237: 19-31.

MICKLEBURGH, S. P., A. M. HUTSON, AND P. A. RACEY. 2002. A review of the global

conservation status of bats. Oryx, 36: 18-34.

MIURA, T., T. KOYAMA, and I. NAKAMURA. 1978. Mercury content in museum and recent

specimens of chiroptera in Japan. Bulletin of Environmental Contamination and Toxicology, 20: 696-701.

MIZUTANI, H., D. A. MCFARLANE, AND Y. KABAYA. 1992. Nitrogen and carbon isotope

study of bat guano core from Eagle Creek Cave, Arizona, U.S.A. Mass Spectroscopy,

40: 57-65.

MOULDS, T. 2006. The first Australian record of subterranean guano-collecting ants.

Helictite, 39: 3-4.

O’SHEA, T. J., A. L. EVERETTE, and L. E. ELLISON. 2001. Cyclodiene Insecticide, DDE,

DDT, Arsenic, and mercury contamination of Big Brown Bats (*Eptesicus fuscus*)

foraging at a Colorado Superfund site. Archives of Environmental Contamination and Toxicology, 40: 112-120.

PETIT, M. G. 1975. A Late Holocene chronology of atmospheric mercury. Environmental

Research, 13: 94-101.

PETIT, M. G., and J. S. ALTENBACH. 1973. A chronological record of environmental

chemicals from analysis of stratified vertebrate excretion deposited in a sheltered

environment. Environmental Research, 6: 339-343.

POULSON, T. L., and K. H. LAVOIE. 2000. The trophic basis of subsurface ecosystems. Pp.

231-249, *in* Ecosystems of the world (H. WILKENS, D. C. CULVER, and W.

F.HUMPHREYS, eds.). Volume 30: subterranean ecosystems. Elsevier, Amsterdam.

POWEL, G. V. N. 1983. Industrial effluents as a source of mercury contamination in terrestrial

riparian vertebrates. Environmental Pollution, 5: 51-57.

R CORE TEAM. 2016. R: A language and environment for statistical computing. R

Foundation for Statistical Computing, Vienna, Austria.

URL <https://www.R-project.org/>.

ROSS, A. 1961. Notes on food habits of bats. Journal of Mammalogy, 42: 66-71.

SEN. A. and M. SRIVASTAVA. 1990. Regression Analysis: Theory, Methods, and Applications. Springer-Verlag, New York.

SELIN, N. E. 2009. Global Biogeochemical Cycling of Mercury: A Review. Annual Review of

Environment and Resources, 34: 43-63.

SHERMAN, H. B. 1939. Notes on the food of some Florida bats. Journal of Mammalogy, 20:

103-104.

SYARIPUDDIN, K., A. KUMAR, K. SING, M. A. HALIM, M. NURSYEREEN, AND J.

WILSON. 2014. Mercury accumulation in bats near hydroelectric reservoirs in Peninsular Malaysia. Ecotoxicology, 23: 1164-1171.

WADA, H., D. E. YATES, D. C. EVERS, R. J. TAYLOR, and W. A. HOPKINS. 2010. Tissue

mercury concentrations and adrenocortical responses of female big brown bats (*Eptesicus*

*fuscus*) near a contaminated river. Ecotoxicology, 19: 1277–1284.

WELCH, B. L. 1951. On the comparison of several mean values: an alternative approach.

Biometrika, 38: 330-336.

WHITAKER, J. O., Jr. 1972. Food habits of bats from Indiana. Canadian Journal of Zoology,

50: 877-883.

WHITAKER, J. O., Jr., and W. J. Hamilton, Jr. 1998. Mammals of the Eastern United States.

Ithica, NY: Cornell University Press. 583 p.

YATES, D., M. MOORE, T. KUNZ, and D. C. EVERS. 2008. Pilot assessment of

methylmercury availability to bats on the South River, Virginia. Report BRI 2008.

BioDiversity Research Institute, Gorham.

YATES, D., S. ANGELO, T. DIVOLL, AND D. C. EVERS. 2012. Assessment of mercury

exposure to bats at Onondaga Lake, New York. Report BRI 2010-11. BioDiversity Research Institute, Gorham.

ZINN, T. L. 1977. Community ecology of Florida bats with emphasis on *Myotis austroriparius*.

Gainesville, FL: University of Florida, 88. M. S. Thesis.

ZUKAL, J., J. PIKULA, and H. BANDGUCHOVA. 2015. Bats as bioindicators of heavy metal

pollution: history and prospect. Mammalian Biology, 80: 220-227.

Figure 1: Location Map

Figure 2: Boxplots of mercury concentrations, caves versus bat house

Figure 3: Boxplots of mercury concentrations by cave

Figure 4: Boxplots of mercury concentrations by bat house