Mercury concentrations in bat guano from caves and bat houses in Florida and Georgia

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ABSTRACT

Several pollutants are bioaccumulating in insectivorous bats, including the heavy metal mercury. This has resulted in an increased presence of mercury in bat waste (guano), which is thereby potentially increasing mercury in guanitic food webs such as those in cave ecosystems. The objectives of this study were to analyze mercury concentrations in guano collected from caves and bat houses in Florida and Georgia to compare concentrations between caves and also a different bat habitat (bat houses) with a predominantly different species of bat than the caves. Data were highly variable and analyzed statistically in R using Fligner-Killeen test of homogeneity of variances, generalized least squares, Tukey’s method of adjusting for multiple comparisons, Wilcoxon-Mann-Whitney non-parametric test procedure, and linear contrasts. Accounting for differences among all sampling locations, the mean concentration of mercury was estimated at 0.53 +/- 0.04 ppm. There were no significant differences between the average concentrations of mercury in bat guano from caves and bat houses, 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. Results indicate that mercury bioaccumulation in bats is causing similar levels of mercury concentrations in bat guano in both caves and bat houses in Florida and Georgia. But variability exists between all locations, which means other variables (e.g. sex and age of bats, geographic hot spots for mercury exposure) also affect mercury concentrations in guano.

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

INTRODUCTION

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 bioaccumulation via trophic transfer (Iskali and Zhang, 2015; Syaripuddin et al., 2014). They 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; Becker et al., 2018). Increasing concentrations of mercury in the environment have unquestionably affected bats, 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; Yates et al., 2008; Wada et al., 2010; Yates et al., 2012; Syaripuddin et al., 2014). 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). Bats with white-nose syndrome have been found with elevated levels of contaminants and mercury exposure that potentially predisposes bats to this disease (Kannan et al., 2010).

Part of the mercury load in bats is excreted in their fecal matter, called guano, which primarily consists of bat hair, insect remains and bat mucus (Maher, 2006). Guano can therefore be examined for for indications of the presence of mercury in a habitat or ecosystem that contain bats, such as a cave or bat house. Several studies have already shown the presence of mercury in bat guano from caves. 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. A recent study by Hagan (2014) analyzed three age-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 concentration of mercury in bat guano has implications for both the health of bats and cave ecosystems. The presence of mercury in guano allows potential for mercury to bioaccumulate in guanitic 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).

Since mercury in guano can be used as an indicator of potential mercury pollution in guanitic food webs, this study had two objectives. The first objective was to analyze mercury concentrations in guano collected from caves in Florida and Georgia to compare concentrations between locations. Given that the caves play host to the same predominating species of bat, any significant differences in mean mercury concentrations among locations is a possible indicator of differing pollutant levels in the surrounding environment. Guano was also collected below bat houses, which are inhabited by bats of a different species. The second objective was to compare guano from these two habits with different predominating specieswith the hypothesis that different species will have different diets and possibly different levels of bioaccumulation of mercury, as indicated by their guano.

Data resulting from this study is valuable for monitoring potential mercury contamination in both bats and cave and karst ecosystems in Florida and Georgia.

MATERIALS AND METHODS

The guano from insectivorous bats in this study was collected from eight caves and two bat houses in Florida and two caves in southwestern Georgia (Figure 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 from each cave were not the same. Bats were also present in several of the caves during guano collection, and the authors wanted to keep disturbance to the bats minimal.

Both core and surface samples were collected for the study. Statistical analysis revealed no significance between core layer concentrations of mercury. Core samples were collected with a Russian sampler to avoid compaction of guano (Maher, 2006, Johnston et al., 2010). 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. 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). 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).

Guano samples were taken below 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. 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).

All samples were stored in a freezer until they 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 (Global headquarters in Sorisole, Italy). This analyzer has a detection limit as low as 0.001 nanograms of mercury and as high as 300 ppm (mg/kg). 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. The open-source statistical computing package R (R Core Team, 2016) was used to make graphics and conduct statistical analyses.

RESULTS

A total of ten caves were surveyed during the data collection phase of this project. However, in four of these caves, we were able to collect fewer than three samples. This is too little information to reliably ascertain the mean of mercury concentrations in these four caves, so we excluded these caves from our comparison of mean mercury concentrations at the various locations. The left panel of Figure 2 shows the concentrations from the remaining six caves, along with the number of observations available for each.

The Fligner-Killeen test of homogeneity of variances (Fligner and Killeen, 1976) indicated that variances in mercury concentrations among the six caves cannot be assumed to be equal. Therefore, we cannot compare the mean concentrations among the caves using the traditional one-way analysis of variance (ANOVA) approach, since ANOVA assumes homogeneity of variances. Instead, we use generalized least squares (e.g. Sen and Srivastava, 1990, Chp. 6) to estimate a mean concentration for each cave, while also accommodating the differing variability among the caves. Since we are hunting for significant differences between any pair(s) of caves among the six with sufficient observations, we use Tukey’s method of adjusting for multiple comparisons. We used the “emmeans” package in R (Lenth, 2018) to facilitate the comparison of all pairs of caves.

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.

A similar concern is illustrated in Figure 3, which depicts the observations taken from bat houses. The variances of the observations taken from the two groups cannot be assumed to be equal, and the small number of samples taken from the Lower Suwanee NWR Bat House. Given the different species predominating in caves and bat houses, as well as the differences in habitat, differences between these observations and those from the cave-dwelling bats

Figure 4, which shows boxplots for the samples from the two bat houses, also shows evidence of a difference in mean and potentially variance.

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.

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 versus 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.

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 can be 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 would be improved by increasing the number of samples from bat houses. This would 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

In the first hypothesis, we predicted that since similar dominant species roost in caves, than concentrations of mercury among caves would be similar. The data rejected this hypothesis, as there was a significant difference of mean concentrations between caves. This indicates that mercury bioaccumulation has geographic hot spots of mercury pollution or affect these bat populations differently due to factors more involved than simply species, such as sex and age of bats and colony size. For example, Climax Cave is the largest cave in the southeastern United States coastal plain, and the largest one sampled in this study. This cave had the largest observable number of bats and amount of guano in this study period, which would potentially affect the differences in mercury concentrations between this and other caves.

The second hypothesis was also rejected, as the mercury concentrations in guano were not significantly different between caves and bat houses. This would indicate that mercury pollution is bioaccumulating in both predominant species (MYAU for caves, TABR for bat houses) to a similar degree.

It is interesting to note that the results of the estimated mean concentration of all bat guano in this study area of Florida and Georgia was 0.53 +/- 0.04 ppm, within the mean range of the modern/fresh guano (0.7 +/- 0.2 ppm) from the Hagan 2014 study in Kentucky. The guano collected for this study was assumed modern (<100 years old, as in the Hagan, 2014 study), since none of the guano produced cores over 11 inches (279.4 millimeters), indicating a lack of long-term accumulation of guano piles. The guano piles from the bat houses were also known to be disturbed. The guano at the University of Florida, Gainesville bat houses 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).

We note that analysis would be improved by increasing the number of samples from bat houses. This would 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.

Other 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 are disturbed by cavers and cave scientists, caves are a more protected environment from the elements than 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 effect of flooding on guano piles in regards to mercury mobility is also unknown, as is bioturbation from fauna.

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. A more detailed study should correlate these concentrations with bat species, sex, and age. 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. A study of mercury mobility in guano would also be interesting, as our study found a lack of significant difference in variances among the core and surface samples, as well samples from the same core.

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STATEMENT OF RESPONSIBILITY: Participation and contribution of each author: Amy Edwards designed research study and completed all field work and laboratory analysis of mercury, Jenise Swall ran statistical analyses, and Charles Jagoe provided Direct Mercury Analyzer 80 laboratory support.

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FIGURE LEGENDS

Figure 1: Location Map

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

Figure 3: Boxplots of mercury concentrations by cave; excludes caves with fewer than 3 observations

Figure 4: Boxplots of mercury concentrations by bat house







