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

Amy E. Edwards(1), Jenise L. Swall(2), Charles H. Jagoe(3), and Jeffery Gore(4)

(1)Hanover County Government, 9015 Pole Green Park Lane, Mechanicsville, VA 23116

(2)Department of Statistical Sciences and Operations Research, Virginia Commonwealth University, 1015 Floyd Avenue, Richmond, VA 23284

(3)School of the Environment, Florida A&M University, 1515 S. Martin Luther King Jr. Blvd., Tallahassee, FL 32307

(4)Florida Fish and Wildlife Conservation Commission, 3911 Highway 2321, Panama City, FL 32409

Corresponding author: Amy Edwards, [aeedwards@hanovercounty.gov](mailto:aeedwards@hanovercounty.gov)

Running head: Hg in bat guano

**Abstract** Increasing concentrations of mercury in the environment from anthropogenic sources is a health threat for both humans and wildlife, and insectivorous bats are particularly vulnerable to this heavy metal via trophic transfer. A portion of the mercury bats ingest is excreted in their guano, which serves as a record of mercury contamination. Increased mercury levels are of particular concern in Florida, a state with abundant waterbodies and high levels of mercury from local and global sources via atmospheric deposition. We measured total Mercury (THg) concentrations in bat guano from caves and bat houses in two cave regions in Florida and one in nearby Georgia to compare THg concentrations between cave regions and between caves and bathouses, which have different dominant species. Results show significant differences in concentrations between all three regions, but results were inconclusive for differences between concentrations of THg in bat guano between caves and bat houses. The mean concentration for all samples from all locations was estimated at 0.5410 +/- 0.0461 ppm. This has implications for both the health of bats and fragile cave ecosystems, as guano is an important food source for other cave species and can lead to increased Hg levels throughout the cave ecosystem.

**Keywords** Mercury, bat guano, bioaccumulation

**Introduction**

Increasing concentrations of mercury (Hg) 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 bio accumulates in the aquatic and terrestrial food webs (Selin 2009). Methylmercury can then enter terrestrial food webs through consumption of fish or aquatic invertebrates. For example, Brasso and Cristol (2008) reported very high Hg concentrations in tree swallows (*Tachycienta bicolor*) which fed on insects that emerged from a Hg-polluted river in Virginia.

Due to several factors, insectivorous bats are particularly susceptible to Hg bio accumulation via trophic transfer through the food chain (Iskali and Zhang 2015). Bats take up Hg when feeding on large quantities of insects (e.g. mosquitoes) that accumulate Hg during their aquatic larval stages in Hg-contaminated waterbodies, as well as when feeding on terrestrial insects that bio accumulate Hg (e.g. spiders) (Brack and Whitaker 2001; Cristol et al. 2008). Bats have unquestionably been affected by this heavy metal, since several studies have found the presence of Hg 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). Heavy metal contamination has been linked to bat population decreases (Mickleburgh et al. 2002), and studies have shown sub lethal biological effects of impaired reproduction, chronic health issues and death in bats exposed to high contaminant loads of heavy metals (Clark and Shore 2001; Hickey et al. 2001).

One region in the United States where insectivorous bats are exposed to high levels of Hg in the environment is the southeastern U.S. coastal plain. This region includes the states of Florida and Georgia. In particular, Florida receives high levels of mercury from local and global sources via atmospheric deposition (Prestbo and Gay 2009). The Florida Atmospheric Mercury Study (FAMS) of air monitoring stations during 1994-1995 found that Florida had a yearly average of 900 pounds of mercury coming from rainfall (Stephenson 1997). The rainfall pollutes surface waters throughout the state and contributes to the trophic transfer of Hg from insects to bats. Once the bats ingest Hg, some of the metal is excreted in their feces (called guano), which primarily consists of bat hair, insect remains and bat mucus (Maher 2006). Over time, guano deposits in sheltered environments, such as caves, may accumulate vertically to sizeable depths and offer a chronostratigraphic record in a range of hundreds to thousands of years (Mizutani et al. 1992; Maher 2006). Florida and Georgia have several cave regions which provide habitat for several insectivorous bat species, and guano is found in many of these caves.

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 Hg 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 the Florida panhandle, 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 (Hg 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 Hg than historical guano (~100-1100 years old), which in turn had higher concentrations than ancient guano (~30,000 years old).

For this study, we analyzed total mercury concentrations (THg) in bat guano from caves and bat houses in Florida and Georgia. We hypothesized that concentrations between regions would not be significantly different, since the same dominant bat species are using these caves with similar diets. Since the dominant species of bats differs between caves and bat houses, we hypothesized that concentrations would be significantly different between these locations. This data is valuable for monitoring potential mercury contamination in economically significant bat populations (Kasso and Balakrishnan 2013) and fragile cave ecosystems.

**Materials and Methods**

The guano from insectivorous bats in this study came from caves and bat houses in Florida and caves in southwestern Georgia (Fig. 1). The guano collected was assumed modern/fresh since caves in this region are prone to flooding, which likely transports guano after deposition and prevents long-term accumulation of guano piles. The effect of flooding on guano piles in regards to mercury mobility is also unknown, and stratigraphically dated guano piles provide inconsistent results (Zukal et al. 2015). Therefore, the guano cores in this study were subsampled to find spatial, but not temporal correlations of Hg with depth.

The main bat species which use caves in this region are the Southeastern myotis (*Myotis austroriparius -* MYAU), tri-colored bat (*Perimyotis subflavus* - PESU) and Gray bat (*Myotis grisescens* - MYGR). The main bat species which use bat houses are the Brazilian free-tailed bat (*Tadarida braziliensis* – TABR), with some MYAU.

To protect the bats and caves, numbers from the state cave surveys are used instead of cave names for sampling locations. Regions 1 and 3 are in Florida. Region 1 includes the caves Florida Cave Survey (FCS 2016) 229, 338, 440, 535, 537, 555, 556, 557, 565, 872 and 925. The dominant bat species roosting in Florida caves is MYAU (Gore and Hovis 1998). The endangered MYGR was formerly abundant in some caves in region 1, 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). Region 3 includes the caves FCS 3, 84, 188, 213, 265, 1373, 1390, and 1630. Region 2 includes the caves from the Georgia Speleological Survey (GSS 2016) GGR 56, GSS 36, GSS 250, and two caves without survey numbers, with MYAU and PESU as the dominant species identified during bat surveys (pers. comm. K. Morris, Georgia Department of Something, Month day, 2013).

Two bat houses, one at University of Florida at Gainesville and one at the Lower Suwanee National Wildlife Reserve (both Region 3) were sampled. TABR is the predominant species at these bat houses, with MYAU also present. Both bat houses are in Region 3 (Fig. 1).

Core guano samples in 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 subsamples starting from the top of the core. Sediment and guano from cave surfaces were collected with non-metal utensils in ~5 g amounts and put into plastic bags. All samples were stored in a freezer until freeze dried. All samples were collected between January 12, 2013 and February 13, 2014.

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 was rapid and inexpensive, and provided detection limits in the sub-parts per billion range. 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**

All measured mercury concentrations in units of parts per million (ppm) are depicted in box plots in Figure 2. The leftmost (gray) boxplot shows all measurements (without respect to region), with the minimum observation at 0.0628 and the maximum at 2.1750. The mean concentration is estimated at 0.5410 +/- 0.0461 ppm. This boxplot shows three outlying points, which consist of measurements from two caves in region 1 (FCS 565 and FCS 556) and one cave in region 3 (FCS 188). These outliers may come from sampling bias from mercury “hotspots” in the guano piles. If they were removed from the analysis, the estimate of the mean concentration would be lowered to 0.5123 +/- 0.0316 ppm.

The three white boxplots in Figure 2 show mercury concentrations grouped by region. Of the three regions, region 2 typically has lower concentrations, with less variability than those in regions 1 and 3.

The samples in regions 1 and 2 came exclusively from caves, in which the typical species present are predominantly of species MYAU, PESU, and/or MYGR. Region 3 differs in that it includes guano samples from caves, bat houses, and an interstate underpass. While the caves in region 3 are populated by MYAU bats, similar to the caves in the other regions, the bat houses and the interstate overpass are predominantly populated by the TABR and/or MFT species. For comparison with the study from Mammoth Cave (Hagan 2014), the estimated mean mercury concentration for caves in all three regions is 0.5611 +/- 0.0511 ppm. Outliers again have a notable impact, as their exclusion would result in a reduced estimate of 0.5279 +/- 0.0330 ppm.

**Comparisons of mean mercury concentrations between regions 1, 2 and 3 from caves**

Given the geographical differences among the regions, the data were examined for evidence of differences in mean mercury concentrations among the caves in regions 1, 2, and 3. Since no bat houses are present in regions 1 and 2, we limited this comparison to cave-based measurements.

The presence of the outlying observations and the different variability among the regions means that the traditional one-way analysis of variance analysis is likely not appropriate here. Instead, we utilize an exact permutation test, which is a non-parametric statistical testing procedure. In addition, the structure of the data is nested; for example, measurements within each cave may be correlated, and are nested within cave and then within region. To accommodate potential correlational structure, we utilize an approach which considers all possible permutations of caves among regions, rather than individual measurements among regions (Anderson and ter Braak 2003). The resulting p-value is 0.0037, indicating a significant difference in mean mercury concentration in caves among regions 1-3. If our interests are limited to the surface measurements, we can conduct the same analysis, simply excluding measurements which came taken from the lower levels of guano core samples. In this case, the resulting p-value is 0.0173, in agreement with a finding of significant differences in the mean cave concentrations.

**Comparisons of mean mercury concentrations between caves and bat houses**

In region 3, three of the eight locations from which samples were drawn were manmade structures. These include 2 bat houses (at the University of Florida at Gainesville and Suwannee National Wildlife Refuge) and one interstate overpass (I-75 in Florida). The populations of bats inhabiting these areas are of different species than are found in the caves; they are predominantly of species TABR and MFT. For the purposes of this analysis, we group these structures together and, for simplification, refer to all three locations as “bat houses”. Figure 3 shows that in region 3, the mercury concentrations taken from the bat houses were generally somewhat lower than those taken from caves.

Another permutation test was used to investigate the question of whether the mercury concentrations differ between the bat house and cave-based populations in region 3. This test is considering all possible permutations of the 8 locations to the status of “cave” or “bat house”, comparing our actual results to those obtained with the various permutations. With no bat houses located outside region 3, we can only consider concentrations from the eight locations sampled in region 3. Even though we have 22 measurements in total, the permutation test accommodates the fact that the individual samples are nested within the sampling location (whether cave or bat house). This restriction limits the power of our testing procedure. The p-value for this test is 0.25, indicating that, based on this study, there is not enough evidence to indicate a significant difference in mean mercury concentration between bat house and cave populations in region 3.

**Discussion**

The estimated mean concentration of all bat guano in all three regions (caves and bat houses) is 0.5410 +/- 0.0461 ppm. In comparison with the Mammoth Cave study (Hagan 2014)l the guano from Florida and Georgia is within to the mean range of the modern/fresh guano (0.7 +/- 0.2 ppm). This indicates that the modern/fresh guano from Florida and Georgia have similar Hg concentrations to the modern/fresh guano in Kentucky. Kentucky is geographically not far from Florida and Georgia, and is likely experiencing the same phenomenon of increasing deposition and trophic transfer of Hg in the environment.

The mean concentration of mercury was significantly different for guano in all three cave regions. This could represent a mercury deposition “hotspot” in one region over the others. Greater atmospheric deposition of mercury in one region would increase bioaccumulation of mercury in the bat’s food supply, which could lead to greater bioaccumulation of mercury in the bats. Even among the measurements used in this study, we saw some outlying measurements from FCS 565, FCS 556, and FCS 188, with concentrations above 1.2 ppm. This may indicate that there are localized extreme hotspots within the regions we studied. While these measurements are difficult to obtain, investigation of this possibility would likely require more measurements in these localities.

The concentration of mercury in bat guano has implications for both the health of bats and fragile cave ecosystems. Bats with white-nose syndrome have been found with elevated levels of contaminants and could potentially predispose bats to this disease (Kannan et al. 2010). The presence of Hg in guano affects not only the health of bats, but contaminated guano may allow Hg to bio accumulate 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 could look at the methylmercury (MeHg) concentrations in both fresh guano and the hair from the 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 converts inorganic forms of mercury to methylmercury (MeHg) existed in caves, as the MeHg is the bioavailable form.

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Figure 1: Location Map

Figure 2: Measured mercury concentrations in ppm from all locations (left, gray box) and grouped by region (white boxes). Number of measurements shown in gray.

Figure 3: Mercury concentrations in ppm in samples taken from region 3, grouped by location type.





