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Distribution of mercury in archived fur from little brown bats across Atlantic Canada

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ABSTRACT

Total mercury (Hg) concentrations were measured in archived fur from adult female little brown bats sampled at maternity roosts across Atlantic Canada. Mercury concentrations varied significantly among regions and roosts. Bats from Nova Scotia and Newfoundland had the highest median Hg concentrations (9.67 μg/g and 9.51 μg/g) among regions, and individuals from Kejimkujik National Park had the highest Hg (median: 28.38 μg/g) among roosts. Over one third of individuals sampled had fur Hg concentrations exceeding thresholds associated with neurochemical responses. Within-roost examinations of stable carbon and nitrogen isotopes in fur showed inconsistent associations with Hg concentrations. Therefore, the hypothesis that within-roost variation in Hg is driven by variation in diet is not supported by this data, and it is recommended that key prey items be included in future mercury bioaccumulation studies for bats. The elevated mercury fur concentrations for bats from southern Nova Scotia remains an anomaly of concern even when placed in the larger context of Atlantic Canada.

1. Introduction

Mercury (Hg) is a pervasive environmental contaminant, with a long atmospheric residence time and subsequent capacity to travel long distances (Driscoll et al., 2013; Schroeder and Munthe, 1998). Consequently, elevated concentrations of Hg have been documented in various terrestrial and aquatic species associated with freshwater ecosystems in northeastern USA and southeastern Canada far from point sources of Hg (e.g., Burgess et al., 2005; Drysdale et al., 2005; Edmonds et al., 2010; Spencer et al., 2011; Yates et al., 2013). Five biological Hg hotspots have been identified in these regions as a result (Evers et al., 2007), and toxicological responses such as decreases in the reproductive success of common loons have been noted in these areas (Burgess and Meyer, 2008). The primary exposure route for Hg in wildlife is via diet (Harris and Bodaly, 1998). When methylated, methylmercury (MeHg) has the capacity to biomagnify within terrestrial and aquatic ecosystems, placing top-level predators at elevated risk of Hg exposure.

Examination of Hg in North American little brown bat (*Myotis lucifugus*) populations in northeastern USA, Ontario, and Nova Scotia (Hickey et al., 2001; Little et al., 2015; Yates et al., 2013) reveal that individuals roosting near contaminated sites and biological Hg hotspots also have elevated Hg concentrations (Little et al., 2015; Yates et al., 2013). Although laboratory-established lowest observable adverse effect level guidelines do not currently exist for bats, Nam et al. (2012) noted Hg-associated biphasic responses in neurochemical biomarkers in little brown bats, corresponding to an inflection point between 10 and 40 μg/g (fresh weight) Hg in fur. Above this threshold, Nam et al. (2012) noted Hg-associated increases in monoamine oxidase (MAO) activity, and an absence of acetylcholinesterase (ChE) activity. The potential adverse physiological responses to elevated Hg concentrations in bats is of particular concern given that populations throughout eastern North America are already in dramatic decline due to white-nose syndrome (WNS) (Frick et al., 2010). Currently, the northwards spread of the WNS fungus has strongly impacted bat populations throughout the Maritime provinces (Nova Scotia, New Brunswick and Prince Edward Island) with some colonies being eradicated. However, WNS has not yet affected populations in Newfoundland and Labrador. In light of these marked population

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declines and the ethics of sampling affected populations, archived fur samples provide an important opportunity to assess Hg trends in little brown bats across Atlantic Canada.

A high proportion of the diets of little brown bats across Canada include emergent aquatic insects, especially Ephemeroptera, Trichoptera, and Chronomidae, in addition to terrestrial adult insects such as Lepeidoptera and Diptera (Clare et al., 2011, 2013; Belwood and Fenton, 1976). Emergent adult invertebrates can contain elevated concentrations of Hg accumulated during their larval and nymph stages within freshwater ecosystems (Kraus et al., 2014). In our case, due to different research goals at the time of sampling, we do not have data for prey items for all roosts. In lieu of missing prey information for the little brown bat roosts in this study, we examined stable carbon and nitrogen isotope values in the same fur samples. Previously published mammalian studies have successfully used the variability and range of $\delta^{15}N$ and $\delta^{13}C$ values for single species (without any prey data) to interpret niche width and infer possible feeding patterns (e.g. Segers and Broders, 2015; Crawford et al., 2008). In addition, to place in context the stable isotope data, we reviewed literature for typical ranges of $\delta^{13}C$ and $\delta^{15}N$ values of potential key prey items from North American temperate forested ecosystems (in Supplementary data). Because $\delta^{13}C$ undergoes little trophic fractionation, comparisons of $\delta^{13}C$ in bat fur can help to characterize the variety of primary production sources. By contrast, $\delta^{15}N$ exhibits step-wise enrichments (2–4‰) with trophic level, providing a means for establishing consumer positions within food webs (Peterson and Fry, 1987). Many studies have demonstrated that $\delta^{13}C$ and $\delta^{15}N$ data can be used to assess potential sources and transfer routes of contaminants such as Hg to wildlife (Jardine et al., 2006). Although $\delta^{13}C$ and $\delta^{15}N$ values in the tissues of two Atlantic Canada bat species, including little brown bats, have been related to their dietary niches (Broders et al., 2014), they have not yet been used to interpret dietary-related variations in Hg concentrations in bats.

Our goal was to investigate the potential and feasibility of using archived fur samples and field measurements for spatial analyses of Hg concentrations in female little brown bats in maternity roosts across Atlantic Canada. New data on Hg and stable isotopes bats Prince Edward Island, Newfoundland and Labrador and stable isotope values for Nova Scotia bats were combined with previously published Hg results for little brown bats in Nova Scotia (Little et al., 2015). As archived fur samples are available for many researchers globally, we also assessed whether stable isotope data from fur samples would provide any useful insights into Hg trends for a species with a diverse dietary range, even without prey data.

2. Methods

2.1. Field collection

For this project, we accessed an archive of fur samples maintained by HGB, who led sampling trips across Atlantic Canada prior to WNS-related declines. Since samples were collected for different research goals, to enable more robust statistical comparisons, we deliberately restricted our samples to adult female bats of reproductive age from maternity roosts. We chose this restriction for several important reasons: (a) Hg concentrations in females of most mammalian species are of increased concern because maternal Hg burden can be transferred to vulnerable fetuses (Dietz et al., 2013); (b) pregnant and lactating female bats in maternity roosts likely do not travel as far as other bats not taking care of young (Encarnação, 2012); (b) *Myosis* spp. frequently return to the same maternity roost location each year, e.g., exhibit strong philopatry (Moussy et al., 2012).

Bats were captured at roost sites located in buildings before populations showed significant declines due to WNS. Capture and handling procedures were conducted under sampling permits from provincial wildlife authorities, and in accordance with guidelines approved by the Saint Mary's University Animal Care Committee. Captures were conducted at dusk, using mist-nets (Avinet Inc, Dryden, New York, USA) and harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia), or directly by hand. Fur was sampled from the mid-dorsal area using stainless steel scissors that were disinfected between individuals with a sequential wash of diluted bleach, water, and ethanol as per genetic research protocols. While not typical of conventional trace-metal analyses (e.g. acid washing, ultra-pure water), this protocol does prevent cross contamination transfer of oils and proteins between samples, and which would also prevent transfer of significant Hg associated with proteins and biological materials. Following collection, samples were stored in sterile polypropylene microcentrifuge tubes and kept frozen at −20 °C until analyses. Each bat was assessed for sex, forearm length, general age (Kunz and Anthony, 1982), and reproductive status (pregnant, lactating, or not pregnant) prior to release, as described by Little et al. (2015).

We selected 25 maternity roosts throughout four regions in Atlantic Canada: Nova Scotia (NS), Prince Edward Island (PEI), Newfoundland (NL) and Labrador (LAB). We did not include New Brunswick (NB) because there were not sufficient samples in the archive. Since research suggests Hg concentrations in bat fur can vary with sex and age (adult vs. juvenile) (Yates et al., 2013), fur samples were selected from adult female little brown bats to minimize within-set variability. Samples were also limited to the same evening of sampling for each site whenever possible. We attempted to have 15 fur samples from each roost site, although due to inherent limitations, we could only get 5 to 9 fur samples from 4 roosts (Table 1).

Given associations between Hg concentrations in insectivorous songbirds and body weight (Rimmer et al., 2005), we assessed whether among-roost variations in Hg concentrations were related to individual body size. We could not use total weight for bat size ranges because often it was not possible to ascertain the pregnancy status of adult females which would influence total mass of the bat (Little et al., 2015; Supplementary information). So instead, we used forearm length (as opposed to mass) was used as a proxy for size. We hypothesized that larger bats would have higher Hg burdens due to larger bats' assumed ability to eat larger prey and more food (e.g., accumulate more Hg). Samples were selected from across the entire breadth of forearm lengths.

2.2. Hg analyses

Total Hg (THg) concentrations in archived fur were measured using a Direct Mercury Analyzer (Milestone Tricell DMA 80.3, Milestone, Inc., Shelton, CT), in accordance with the United States Environmental Protection Agency (USEPA) method 7473 (USEPA, 2007). Static in samples was eliminated using an antistatic balance beam, antistatic gun, and connected antistatic mat. External certified reference material NIST 2976 (mussel tissue), National Institute of Standards and Technology (mean percent recovery 110.3 ± 3.1% SE (n = 15)), was included in each run to assess analytical precision and accuracy. Between-run variance was further monitored using 4 replicates of 6 fur samples (mean relative percent difference, RPD; 7.0 ± 3.8% SD, n = 24). Intra-run variability was assessed using a series of blanks, and 14 fur samples were run in triplicate (mean RPD, 4.6 ± 3.6% SD). Mercury concentrations in fur are measured in μg/g, dry weight (dw).