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Mercury Exposure, Fuel Stores and Torpor Use in Silver Haired Bats

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Abstract

Methylmercury (MeHg) is an environmental contaminant with a high biomagnification potential and potent neurotoxic effects. Insectivorous bats may be exposed to MeHg through their diet. I measured total mercury (THg, combination of inorganic and methylmercury) in fur samples of migratory silver-haired bats (*Lasionycteris noctivagans*), and used existing data collected with quantitative magnetic resonance analysis and temperature sensitive radio tags, from the same bats, to test the effects of mercury exposure on body composition and torpor use. THg levels varied 20-fold, with heavier and fatter bats having higher levels, suggesting that individuals that routinely eat more accumulate more THg. THg was not related to torpor use at 25 and 17 °C, however, at 10 °C, bats with higher THg levels used more torpor. This was characterized by fewer but longer torpor bouts. This study provides insight into the tolerance of MeHg in bats, and potential adverse effects that it may elicit.

Keywords

Chiroptera, *Lasionycteris noctivagans*, silver-haired bat, bat, torpor, fuel stores, body composition, mercury, methylmercury, neurotoxin

Summary for Lay Audience

Heavy metals have many potent toxic effects on organisms that encounter them. One such metal is methylmercury (MeHg), which primarily affects the brain and nervous system when it is ingested. Bats that feed on insects may be readily exposed to MeHg through their diet. This is because many insects that bats feed on begin their lives near aquatic environments, which is the main habitat of mercury methylating microorganisms. The tolerance levels and changes that bats exhibit when exposed to MeHg are not well understood, and thus, this study aimed to determine the effect of MeHg on body composition and torpor in silver-haired bats, a migratory bat species in North America. Torpor is a state of reduced metabolic rate that leads to a drop in body temperature. Bats heavily rely on torpor to save energy as they have high metabolic rates. Torpor is especially important during migration, as this is an energy intensive process. I received fur samples, as well as data on body mass, body composition, and torpor use in silver-haired bats from Jonasson (2017) and Baloun (2019), that were collected during previous studies at Long Point, ON, Canada. By testing these fur samples for mercury content, I was able to construct a variety of models that investigated the relationships between the fur mercury content and the body mass, body composition, and torpor use in these bats. From this, I concluded that bats that were heavier and fatter had higher total mercury concentrations than bats that were not as well nourished. This may suggest that bats that consistently eat more accumulate more mercury. In Jonasson's and Baloun's studies, torpor use was measured at multiple temperatures, and I determined that at warmer temperatures, the mercury content of the bat did not significantly affect its torpor usage. However, at colder temperatures, bats with higher mercury concentrations engaged in

more torpor, where they used fewer but longer torpor bouts. This study provides insight into a bat's tolerance of heavy metals and potential adverse effects that this may elicit.

Co-Authorship Statement

A version of this thesis will be submitted to the Canadian Journal of Zoology—with Dr. Christopher G. Guglielmo, Brian Branfireun, Kristin Jonasson and Dylan Baloun as co-authors. Both Dr. Guglielmo and Dr. Branfireun were directly involved in experimental design, provided equipment, assisted in data interpretation, and provided crucial editorial feedback. Kristin Jonasson and Dylan Baloun provided the data sets and fur samples as well as invaluable insights into the collection process.

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List Of Abbreviations

A_{Torpor}	area of torpor
DMA	milestone direct mercury analyzer
DOM	dissolved organic matter
dw	dry weight
EnTot	total energy
fw	fresh weight
GEM	atmospheric gaseous elemental mercury
Hg^0	elemental mercury
MeHg	methylmercury
POA	preoptic area
QMR	quantitative magnetic resonance
THg	total mercury
ww	wet weight

Chapter 1: Introduction

Chiroptera is one of the most diverse mammalian orders, with over 1400 recognized species of bats that are distributed across six continents (all excluding Antarctica) in a variety of habitats (Altringham 2011). With this wide range of habitats comes an equally broad range of adaptations and life history strategies. One pressure that many bat species are confronted with, when residing in more temperate or sub-arctic habitats, is how to survive harsh winters with low temperatures and low food (e.g. flying insect) availability. The most ubiquitous energetic strategy employed by temperate bats is hibernation (Beer and Richards 1956), but certain species migrate to lower latitudes to escape the demanding weather conditions (Fleming 2019).

Although many bats may make migratory movements to winter hibernacula, such as caves (Furey and Racey 2016), there are three "tree-roosting" bat species common in Canada that are widely acknowledged to make seasonal, long-distance migrations of up to several thousand kilometers (Fleming 2019). The hoary bat (*Lasiurus cinereus*) is the largest species in size and weight and ranges from 20 – 35 g (Koehler and Barclay 2000). The other two species, the eastern red bat (*Lasiurus borealis*) and the silver haired bat (*Lasionycteris noctivagans*) both weigh between 6 – 15 g (O'Keefe et al. 2009; McGuire and Guglielmo 2010).

In Ontario, Canada, the fall migration of silver haired bats appears to have two distinct pulses, one in late August and another in mid-September (McGuire et al. 2012). The greater temperature variation in the spring, when compared to the fall, is reflected in the

spring migration timing, which also tends to have a greater variation than fall migration timing (Jonasson and Guglielmo 2016). Generally, spring migration is centered around the end of April, with females tending to migrate before males (Jonasson and Guglielmo 2016). During these migrations, bats will usually stay at a stopover site for only one day where they may opportunistically forage in the evening before departing (McGuire et al. 2012).

The geographic range of silver-haired bats encompasses most of North America; they are found from the east to west coasts between 65 °N and 20 °N latitudes in Alaska and Mexico, respectively (Kunz 1982; Kays and Wilson 2009). Solitary bats most commonly reside in boreal or coniferous and deciduous forests; with a strong preference for areas near bodies of fresh water, as their preferred prey inhabit these areas (Kunz 1982). The diet of the silver haired bat consists mainly of soft-bodied insects with a bias for Lepidoptera and Diptera, but they will opportunistically forage on other insects such as Homoptera, Hemiptera, Hymenoptera, Coleoptera and Neuroptera (Reimer et al. 2010).

Torpor is an active process defined as a profound reduction of metabolic rate and, therefore, body temperature (McGuire et al. 2014). It is integral to many bat species, as they use it throughout the year on a daily or seasonal basis to reduce their energy requirements, as they have extremely high metabolic rates (Fenton and Rydell 2023). Since bats are strictly nocturnal, migratory bat species use torpor extensively during daytime rest periods when they cannot forage or travel to save critical energy for nocturnal flight (Baloun and Guglielmo 2019). During the remainder of the year, torpor

can also be used during the day when bats are inactive, to compensate for the inability to forage. Depending on the ambient temperature, migratory bats at stopover can save 8-91% energy through the use of torpor (McGuire et al. 2014). This would correlate to an extra 20-90 km of possible flight per night, which is 36% of the average distance traveled in a given night (McGuire et al. 2014).

During migration, bats may travel large distances to get to their southern non-breeding grounds, which increases their chance of encountering areas that have been subject to anthropogenic degradation or pollution such as methylmercury (MeHg) (Chételat et al. 2018). Bats migrating through the eastern part of North America are at a particularly high risk of encountering MeHg, due to the natural characteristics of Hg sediment distribution and atmospheric deposition patterns (Rasmussen et al. 1998; Durnford et al. 2010). When Hg is emitted into the atmosphere, it can be carried long distances by prevailing winds. Certain areas have higher rates of deposition, which can occur through either precipitation, or dry processes (Durnford et al. 2010). Other factors such as high lake and soil acidity or poorly buffered soils can also increase the MeHg exposure in areas (Nasr and Arp 2017).

1.1 Mercury

Mercury is a heavy metal and a pollutant, that can be found as inorganic mercury salt, organic mercury compounds and metallic mercury (Langford and Ferner 1999). All three forms have a variety of toxicological effects on organisms, but organic mercury

compounds, and particularly MeHg, is of special concern, as it readily accumulates and has a high trophic biomagnification potential (Chen et al. 2005; Lavoie et al. 2013; Rice et al. 2014). This stems from the fact that MeHg, unlike the other forms, has a strong affinity for thiol groups, is somewhat lipophilic, and readily binds to proteins, leading to a high biological persistence and adsorption in organisms (Jeong et al. 2024). Consequently, predators at higher trophic levels in a food web experience a greater potential for MeHg exposure (Jeong et al. 2024).

Mercury, in all its forms, naturally occurs in the environment at low levels, however anthropogenic emissions have far exceeded these levels. Atmospheric Hg concentrations have increased by 300–500% over the past century (Gworek et al. 2020). The main anthropogenic sources of mercury come from activities such as fossil fuel combustion, mining (both industrial and artisanal), dumping, garbage incineration, run-off from industrialized areas, and even some rice-based agricultural endeavors located near abandoned mines (Qiu et al. 2008; Yates et al. 2014). Elevated mercury levels do not only occur near these point sources, however, as mercury can travel far distances in the atmosphere and be deposited thousands of kilometers away from its origin (Driscoll et al. 2013). Once mercury has been deposited into a particular environment, it can remain there for a long time (Driscoll et al. 2013). This means that MeHg can be found in high concentrations in any environments due to the deposition of atmospheric gaseous elemental mercury (GEM) and subsequent methylation (Iverfeldt 1991)

The methylation of inorganic mercury in soils, lake sediments, and submerged plant matter occurs through sulphate- or iron-reducing bacteria, as well as methanogenic microorganisms (Bigham et al. 2017). This process is especially favored in anaerobic wetlands, but it can happen in a wide variety of environments. Certain environmental features can facilitate the methylation of Hg, increasing its bioavailability. One example of such features is high water temperatures, which can stimulate the release of contaminants from the sediment into the water column and increase the metabolic rate and respiratory volume of the methylating bacteria (Jeong et al. 2024). Another factor that can facilitate methylation is the presence of dissolved organic matter (DOM). DOM affects the bioavailability of Hg, as it plays an important role in the photodegradation of MeHg (Qian et al. 2014).

Consequently, high levels of MeHg in riparian environments lead to aquatic emergent insects accumulating high amounts of MeHg (Jensen et al. 2007). Since these insects are a food source for many species, they become a key route for MeHg to enter food webs (Riva-Murray et al. 2020). Bats may consume aquatic emergent insects either directly, or indirectly by consuming the predators of these insects, such as spiders or dragonflies. The high metabolic rates of bats, when coupled with their long lifespan, suggest that they are at an increased risk of accumulating MeHg. Thus, they are also at an increased risk of experiencing adverse effects caused by mercury poisoning, such as, but not limited to, oxidative stress, growth suppression, reproductive disruption, neurotoxicity, genotoxicity, changes in the gut microbiota and metabolites (Hoffman et al. 2002).

1.2 Fur as a Route of Mercury Depuration

Fur and hair growth play a vital role in depurating MeHg in mammals (Budtz-Jørgensen et al. 2004; Brookens et al. 2007; Cardona-Marek et al. 2009). Fur is inert once formed, and thus reflects the accumulated body burden at the time that the hair was grown (Chételat et al. 2018). This has led to the analysis of fur samples being a commonly used, non-invasive method of measuring the MeHg exposure of a given individual (Hickey et al. 2001). When analyzing fur samples for MeHg, total mercury (THg) is often used as a proxy. These two measurement forms are not significantly different to each other in bat fur, as the average fur THg is comprised of about 86 % MeHg (range 71–95 %) (Yates et al. 2014). The benefits to using THg as opposed to MeHg are lower costs, time, and work involved.

Fur and blood mercury (both THg and MeHg) levels are closely correlated, ($r = 0.870$, $p<0.001$) with the concentrations in the bat fur averaging to be 260 times greater than in the blood (Wada et al. 2010). However, there are some fluctuations in the blood concentration throughout the year, due to the migratory movements of silver haired bats which leads to differential exposure. The reason for the large concentration increase in the fur, is that most bats, including silver haired bats, molt once a year. The molt happens only in the late summer, leading to the fur concentration being an accumulation of the body burden (Fraser et al. 2013). Blood concentrations can also represent the body's burden over an extended period in time as MeHg has a long half-life in biological tissues. There has been a wide range of biological half-life values recorded for MeHg (30 to 120

days), as it varies by species and tissue type. The range spans from around 30 to 120 days (Rand and Caito 2019).

To date, the highest recorded THg concentration measured in bat fur is 274 mg/kg fw (fresh weight; weight of a sample in fresh condition. This is done immediately after the sample has been collected as biological samples may lose weight over time due to evaporation or other releases of water. However, one should note that water is not a large constituent of fur) (Nam et al. 2012) and the highest recorded blood concentration is 707.64 mg/kg ww (wet weight; total weight of a plant, animal, or other material containing the chemical of interest that has not been dried to remove water) (Yates et al. 2014). These were both collected near point sources of Hg emissions in the northeastern United States. It is important to note that these samples were not taken from the same bat, and the studies that collected the samples did not collect both fur and blood mercury. This is the reason that the highest fur and blood levels do not correlate with the average 260 fold increase.

Silver haired bats have two distinct types of fur: overhairs, which are straighter, coarser, and measure about 10 mm in length, and underhairs, which are 5-8 mm long (Nason 1948). In river otters (*Lontra canadensis*) it has been shown that overhairs tend to have smaller THg variances when compared to the underhair, whose variance was at least 10 times greater than the overcoat in individuals (Eccles et al. 2019). This means that the offloading of THg into the undercoat is more variable, leading to the mean topcoat Hg

concentrations being a more reliable indicator of the body burden. All the samples used for my study contain both under- and overhairs. Each sample was taken from the same location on the bat's upper back, which minimizes the possible variation of THg depuration to different body parts.

1.3 The Toxicology of Mercury

The main route for MeHg uptake in wildlife is through diet (Wiener 2013). Over 90% of ingested MeHg is absorbed into the body in the gastrointestinal tract and once this has occurred, it can stay in the body for a long period of time (Clarkson 2002). As stated, the half-life of MeHg in biological tissue can be quite high (Rand and Caito 2019), which can lead to a buildup of the toxin, as the ingestion rate can easily exceed the depuration rate. MeHg can penetrate many regions of the body as it can cross cell membranes and other barriers (Li et al. 2021). Methylmercury also has a high affinity for the anionic form of thiol, which is found in many biomolecules such as proteins (receptors, transporters, enzymes, structural proteins), lipids (membrane constituents and intracellular messengers), and nucleic acids (DNA and RNA) (Farina and Aschner 2017; Li et al. 2021). The interaction contributes to the occurrence of oxidative stress and impaired function of the molecules (Farina and Aschner 2017). One of the main body systems impaired by MeHg is the central nervous system. As the nervous system's tissues have a high lipid content, it readily allows for an accumulation of MeHg to occur, which also has the consequence of the toxin easily crossing the blood-brain barrier (Raposo et al. 2020).

The blood-brain barrier is comprised of specialized endothelial cells that form a continuous layer with tight junctions (Aschner and Aschner, 1990). Here, the neighboring cells fuse, which inhibits most substances from crossing in between cells. This also nullifies the oncotic and osmotic forces which usually allow for the exchange of substances between blood and tissues. However, some substances are able to cross the barrier via highly specific carrier-mediated transport systems or facilitated diffusion. Many factors determine whether a substance can utilize these, including their lipid solubility, and as previously mentioned, MeHg expresses this characteristic.

Once in the brain, MeHg can accumulate in multiple regions of the cerebrum including the hippocampus (Raposo et al. 2020). The hippocampus is an important site for the generation and integration of new neurons, as the neurogenic niches, where the formation happens, are especially active here (Raposo et al. 2020). The cellular mechanisms in these neurogenic niches are negatively impaired and/or altered by MeHg, leading to poor neurogenesis and ultimately leading to a wide range of cognitive deficits and abnormalities (Raposo et al. 2020).

The hippocampus is also associated with regulating memory, behaviour, and cognitive functions such as spatial cognition (Sherry et al. 1992). Studies have found negative correlations between MeHg levels and learning, special memory, and even magnetic orientation (Falluel-Morel et al. 2007; Farina et al. 2011; Landler et al. 2017; Swaddle et al. 2017). Bats orient through an internal magnetic compass (McGuire et al. 2012). This

suggests that MeHg could cause a disruption between the sensory inputs of the magnetic signals, the visual surroundings, and integrating both, in order to successfully navigate during their migration. This could lead to changes in activity patterns such as a different onset of migration, or migration speed. These changes in activity patterns could potentially have negative repercussions such as not reaching the migratory destinations at the optimal time, or at all. The use of torpor may also be altered to account for the shifts in migration timing. Both of these effects could lead to changes in body composition due to an energy mismatch caused by stress, shifted torpor regime or flight patterns.

Another abnormality brought about by MeHg contamination that has been documented in many species is an increase in restlessness and hyperactivity (Maximino et al. 2011; Boucher et al. 2012; Ma 2018; Seewagen et al. 2019). If bats exhibit restlessness and hyperactivity when exposed to MeHg, their ability to enter and/or stay in torpor may be reduced. A single arousal event lasting an hour can use as much energy as hibernating for two months (Fenton and Rydell 2023). This impaired torpor ability could lead to bats catabolizing their fat stores at an increased rate, which could result in not having enough energy to migrate as well as risking starvation.

Another important area of the brain that could be negatively affected in migrating bats is the anterior hypothalamus, and more specifically the preoptic area (POA). The POA is the key integratory site for thermoregulation in the brain (Tan and Knight 2018). The hypothalamus is also associated with other functions such as, but not limited to, sleep,

circadian rhythm, and behavior. If MeHg were to alter mechanisms in this area of the brain, torpor use could be affected from a thermoregulatory aspect. This could possibly happen in two ways, either the ability to cool the body off below the torpor threshold could be impeded or the torpor threshold may be shifted.

The use of torpor in mammals is at least partially under voluntary control and is affected by extrinsic factors such as ambient temperature, food availability or pregnancy (Dietz and Kalko 2006). This would classify it as a thermoregulatory behavior, which is flexible and goal-oriented (Tan and Knight 2018). When bats want to become torpid, they often seek out cool microclimates to have an ideal environment for entering and remaining in torpor. This may be another facet that could be altered with elevated MeHg levels, as the bat's decision making may be impacted in the hypothalamus.

The risk of starvation becomes compounded by the fact that many studies show that MeHg can detrimentally impair motor coordination and balance (Bellum et al. 2007; Fujimura et al. 2012). This could potentially affect a bat's ability to forage, as silver-haired bats are aerial insectivores, so the act of catching prey requires precise movements and quick reaction time. To add to this, they also rely heavily on echolocation when hunting. Echolocation is processed and integrated in the midbrain superior colliculus (Kothari et al. 2018) and as the brain is a main organ affected by MeHg, it would stand to reason that echolocation may also be adversely affected. Furthermore, studies in birds have also shown that MeHg contamination impairs overall foraging behavior, leading to

poor nutrition and body condition, which then could have carry-over effects such as subsequent migration performance and fitness (Kobiela et al. 2015). All these factors could create a positive feedback loop of decreasing nutrition and fat stores, as bats with higher MeHg loads may already have less available energy due to the reduction of torpor use.

Based on previous studies conducted on various mammals and bird species, 3–5 μg/g dw (dry weight; weight after all the water has been removed) of MeHg in brain and 10 μg/g THg in fur has been suggested as a threshold for when non-lethal neurochemical effects arise in bats (Dietz et al. 2013; Yates et al. 2014; Little et al. 2015). Yet in the literature, there have been multiple documentations of bat fur THg levels that surpass this threshold by a significant amount, with the neurochemical effects of these levels being a lot less pronounced, if at all present, than when compared to other similar sized organisms (Wada et al. 2010; Nam et al. 2012; Chételat et al. 2018). This, and the fact that brain MeHg concentrations of bats correspond to about 10% of the fur MeHg concentration, has led to the suggestion of raising the fur MeHg threshold in bats to 30-50 μg/g dw (Chételat et al. 2018). The observation that bats seem to be able to tolerate higher levels of MeHg than many other species, has also led to the question of whether realistic environmental exposure levels (especially in areas where higher bioaccumulation occurs) can be high enough to induce the aforementioned non-lethal toxicological effects.

A potential pathway that may aid in maintaining normal neural function during high MeHg exposure was proposed by Nam et al (2012). When comparing enzyme activities and receptor densities of contaminated vs. reference bats, the numbers were similar, yet the correlations regarding MeHg exposure were opposing. The inflection point was set at around 1–5 mg/kg dw of THg in the brain, depending on the neurochemical. This led to the postulation that Hg-induced neurochemical changes in chronically exposed bats are different than those seen in reference bats. Chronically exposed bats may either phenotypically adjust to these high MeHg levels, or the long-term exposure may exhaust the brain's ability to counteract the toxin. One significant change observed was in cholinergic function. Chronically exposed bats exhibited reduced acetyl cholinesterase activity, which is an enzyme involved in terminating nerve impulses at cholinergic synapses by breaking down acetylcholine (Sarangle et al. 2023). This may be a strategy of maintaining neurotransmitter homeostasis, analogous to how various medications operate, that improve cognitive function in Alzheimer's and dementia patients (Ellison 2002).

1.4 Thesis Objectives

The objective of my study was to use fur THg levels in conjunction with previously collected body temperature and body composition data to quantify the effects of THg on torpor use and fuel stores. I hypothesized that THg will negatively impact torpor use and body condition. I predicted that bats will become more restless and hyperactive with increasing THg exposure, impeding their ability to adequately use torpor. This would then have negative downstream effects on their fuel stores as their metabolic rate would not be dampened.

For my analysis I used fur samples and data collected by Dr. Kristin Jonasson and Dylan Baloun during their graduate studies in 2012-2014 and 2016, respectively, at Long Point, Ontario, a stopover for migratory bats (Jonasson 2017; Baloun and Guglielmo 2019). Baloun's samples were taken while he was investigating the use of torpor during stopover, and the correlated energy expenditure in conjunction with ambient roosting temperature. Through the use of temperature sensitive radiotelemetry and quantitative magnetic resonance analysis (QMR) of body composition analysis, he measured torpor characteristics and energy expenditure of bats roosting at different temperatures. He concluded that a bat's energy use was independent of the roosting temperature, and that there was a causal relationship between body mass and energy use for spring females and fall male juveniles. He also discovered that bats with higher body fat used less torpor during spring migration, but in the fall, this only held true for warm roosting temperatures. By incorporating the measured THg concentrations into Baloun's models, I tested if the variation in torpor use in captive bats could be explained, at least in part, by THg.

Jonasson studied differences between the sexes of silver-haired bats during migration by examining spring migration phenology, stopover behaviour, seasonal differences in daytime torpor use, and spring and autumn long-distance migratory movements (Jonasson 2017). She also used QMR technology to determine the body composition of individual bats and incorporated this into her analysis. The results showed that bats sometimes conduct multi-day stopovers where they actively refuel, and that depending on the sex and season, the use of daytime torpor use may vary to accommodate different

energy needs of the bat. Unlike Baloun, these bats were not held captive for the torpor analysis, rather they were released and monitored in the wild. Due to the fact that certain facets of Jonasson data set had very small sample sizes, I was limited in the analysis. There were not enough migratory flight tracks to do a robust analysis on the migration routes and stopover behavior. I was able to look at the torpor usage in these wild bats, just not to the extent of what I was able to do with Baloun's data.

Chapter 2: Methods and Materials

Collection of fur samples, torpor and body composition data were done by Jonasson and Baloun in 2012-2014 and 2016 respectively, in conjunction with other research projects (Jonasson 2017; Baloun and Guglielmo 2019). I received 168 fur samples and corresponding torpor and body composition data from Baloun and 59 fur samples and corresponding torpor and body composition data from Jonasson, for a total of 227 fur samples. Of these, there were 100 samples from males and 127 from females, which were from 191 adults and 36 juveniles. In the data set, 119 bats were caught in the spring and 108 were caught in the fall. Juveniles were only present in the fall, as bats are born in the summer and by springtime, they have already matured to the point that they are indistinguishable from older adults. The majority of bats were caught in 2016, totaling 168, while 3, 23 and 33 were caught in 2012, 2013 and 2014, respectively.

I conducted the mercury analysis in conjunction with the Biotron staff at the University of Western Ontario.

2.1 Quantitative Magnetic Resonance Analysis

Both Baloun and Jonasson used quantitative magnetic resonance analysis (QMR), to quantify dry fat mass, wet lean mass, and total body water (free water plus water bound in lean tissues) (Jonasson 2017; Baloun and Guglielmo 2019). This is done by placing the bat in a small holding tube, and subsequently placing that tube into a magnetic resonance scanning machine for 1-3 min. This strategy of determining body composition is noninvasive, as the animal does not need to be anesthetized or restrained, and the technique has been validated in bats (Taicher et al. 2003; McGuire and Guglielmo 2010). The different tissues are detected using the principle that all atomic nuclei possess a degree of "spin", which is dependent on the number of protons (Grover et al. 2015). This spin induces a signature magnetic field, also known as the relaxation rate, which the machine correlates to different tissue types (McGuire and Guglielmo 2010). Energy expenditure can be calculated from the loss of fat mass (39.6 kJ/g) and wet lean mass (5.3 kJ/g) over a period of activity, such as daytime roosting.

2.2 Experimental Torpor Measurements

Baloun used body temperature to measure torpor use (Baloun and Guglielmo 2019). Skin temperature is considered an accurate and minimally invasive alternative way of measuring core body temperature (McGuire et al. 2014). To measure the skin temperature and analyze torpor use throughout the trials, each bat was outfitted with temperature sensitive radio-transmitters (0.3g; ±0.1 °C; BD-2XT, Holohil Systems Ltd., Carp, ON, CAN), which were temporarily attached to a clipped area on the back of the bat using a non-toxic latex glue (Ostobond; Ostomy Quebec). This process was implemented at three

different temperature treatments, 25, 17, and 10 °C, which were chosen to reflect the naturally occurring temperature range of the spring and fall migration periods. For each trial and temperature treatment, the bats that were caught on that day were first measured for fat and lean mass by QMR and outfitted with the temperature sensitive radio-transmitter. Then they were individually placed into cloth bags, which were hung in a temperature-controlled cabinet (±0.2 °C; model PTC-1 with PELT-5 temperature controller; Sable Systems, Las Vegas, NV, USA) for approximately 12 hours. The treatment temperature was measured every 30 seconds during each trial using a Temperature/RH smart sensor cable attachment on a HOBO Micro Station (±0.01 °C; S-THB-M002, H21-002, Onset Computer Corporation, Bourne, MA, USA). Each trial started at 07:00 and ended at 19:00 to mimic the natural daytime stopover time. At the end of each trial, the fat and lean mass of each bat was remeasured and the radio tag removed. After sunset, the bats were released at the capture site.

Jonasson used similar methods in her study, the main difference being that she did not place the bats in a cabinet (Jonasson 2017). Instead, she released them prior to dawn and by using the radio transmitter attached to the bats, handheld antennas (3- element, Yagi) and receivers (SRX 600, Lotek Wireless Inc, Newmarket, Ontario, Canada), she was able to trace them to their daytime roost. Once located, she monitored the body temperature and the ambient temperature of the roost using a temperature data-logger (HOBO Pendant, Onset, Cape Cod, Massachusetts).

2.3 Fur Samples

In both Baloun and Jonasson's studies, they trimmed the fur dorsally from the center of the back of bat to attach a radio tag (Jonasson 2017; Baloun and Guglielmo 2019). Then they collected the fur samples and archived the fur for later analysis.

Jonasson's fur samples (N= 59) were previously analyzed for THg content at the Biotron at Western University (Faculty of Science, Biotron Research Center, London, ON, Canada). I measured the THg of the Baloun's archived fur samples (N= 168) using the same facility and equipment. This was done using a Milestone Direct Mercury Analyzer (DMA) 80 employing US EPA method 7473 (United States Environmental Protection Agency 2007). The equipment's method detection limit was 0.24 ng. The precision of measurement from each replicate analyses was documented to be greater than 80%. A sample with known concentration and a blank were run at least once every 10 samples to assess analysis accuracy.

Prior to analysis, the fur samples were wrapped in aluminum foil to secure them. The aluminum foil was tested in the DMA 80 for Hg and no THg was detected. To measure the Hg content in the sample, it was first heated to 650 °C. The combusted gaseous sample was then swept through a platinum catalyst along with a carrier gas (room air), which converted all the Hg into elemental mercury (Hg^0). This gas mixture was then run through a gold coated quartz amalgamator, which binds with the gaseous Hg to form an amalgam. Once the rest of the sample were cleared from the system, the amalgamator is heated. This breaks the bond between the Hg^0 and the gold and the Hg^0 is moved into the

flow cell where atomic absorption spectrophotometry occurs to determine the amount of Hg^0.

2.4 Statistical Analysis

I constructed a variety of statistical analyses in R (version 4.4.2, R Core Development Team, 2022) to investigate whether there were relationships between the mercury load of silver-haired bats and various parameters associated with body composition and torpor. I combined the data from Jonasson and Baloun for the body composition models. In the torpor models, I kept the data sets separate as they were not compatible. The parameters that I used from Baloun's study were fur THg content, body composition (overall mass, lean wet mass, and dry fat mass), sex, age, season of capture (spring or fall), forearm length (proxy of body size), treatment temperature, A_{torpor} (defined as the total area in between the torpor threshold line and the body temperature curve of the individual bat on a graph depicting the body temperature plotted against time; Figure 1), EnTotal (total energy use defined as fat energy + lean energy in kJ), maximum torpor depth (the difference between lowest skin temperature and the torpor threshold), number of torpor bouts (number of times the skin temperature dipped below the torpor threshold and back up again), and the mean torpor bout duration (average length of a single torpor bout). From Jonasson studies I used data on fur THg content, body composition (overall mass, lean wet mass, and dry fat mass), sex, forearm length, mean ambient temperature, torpor depth (mean difference between skin temperature and ambient temperature), torpor duration (total time spent in torpor) and torpor percent (percentage of time spent in torpor out of total monitored time).

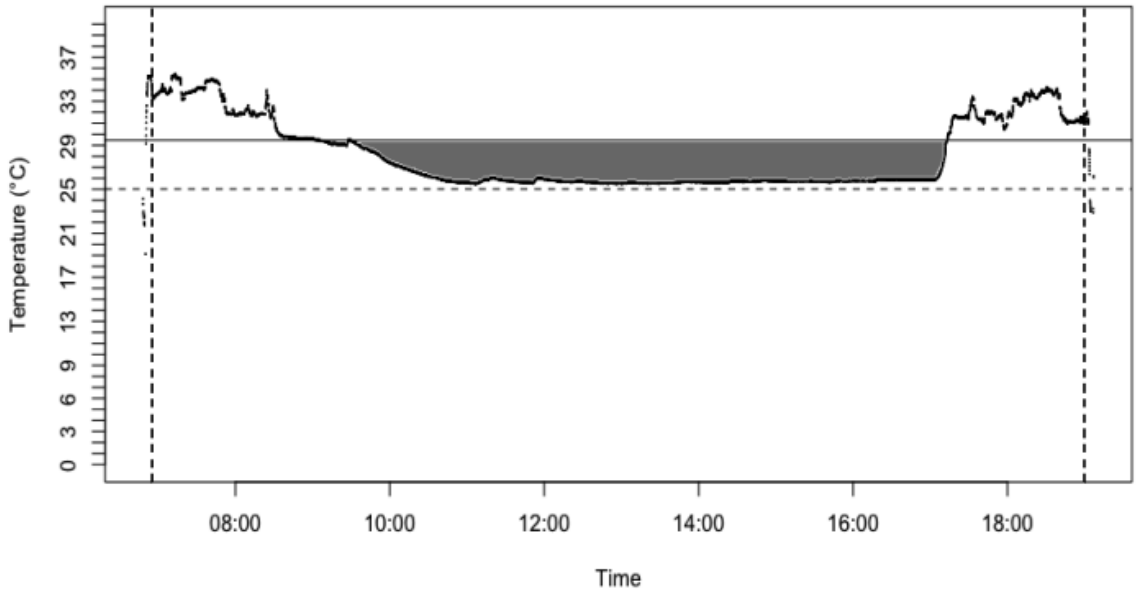


Figure 1: Example quantification of torpor use (A_{Torpor}) from skin temperature (°C; thick black points) of a silver-haired bat during a daytime stopover roosting at 25 °C (horizontal dashed line. The torpor threshold (horizontal solid black line) is calculated based on the lower limit of a 99% confidence interval around the mean of the euthermic skin temperature. A_{Torpor} is represented by the dark grey shaded area under the torpor threshold and above the skin temperature trace) (Baloun and Guglielmo 2019).

All statistical analyses were run in R (version 4. 2. 2, R Core Development Team, 2022). Shapiro-Wilk normality tests (shapiro.test function: R) and histograms (ggplot function: R) were used to explore the distribution of the data. Variables that were not normally distributed were transformed, in order to satisfy the assumption of normality that is necessary for computing linear models. The EnTotal, and A_{Torpor} were both square root transformed, and THg concentration, mass and fat were transformed using the log10 function. I used linear models (analysis of variance: ANOVA) to analyze the interplay between the variables. The correlations that I tested for the body composition section

were the effects of season, sex, age, total body mass, and forearm length on fur THg concentration; and sex, season, age, fur THg and forearm length on both fat and lean mass. For torpor use, mass, temperature treatment, sex, season and fur THg were tested for relationships with A_{Torpor}, as well as EnTotal in captive bats. This data set was then split up by temperature treatment to create new linear models (ANOVA) using the same predictor variables and interactions from the original linear model. In free ranging bats, fur THg, mean ambient temperature and mass were tested against torpor depth, -duration, -percent and minimum skin temperature. By using the drop1 function, backwards-step-elimination was employed to remove all non-significant terms from the various models (α=0.05) until only significant terms remained. All the results were reported using Type III sum of squares to allow for a randomized order of terms in each linear model. The function qqplot in R was used to assess the normality of residuals from each linear model. Student's t-tests (t.test function: R) were also used to determine if there were significant differences between the means of two separate groups within the data set.

Chapter 3: Results

The THg fur concentration for the whole data set (N= 227) ranged from 1.11 mg/kg to 22.27 mg/kg with an overall mean of 4.53 mg/kg, meaning that the data was highly skewed (Figure 2). Females (N= 127) ranged from 1.33 mg/kg to 22.27 mg/kg with a mean of 5.01 mg/kg and males (N=100) ranged from 1.11 mg/kg to 11.12 mg/kg with a mean of 3.93 mg/kg. This indicated that females tended to have a higher THg concentration than males with the means of the two sexes being significantly different ($t=2.61$, $df=198.04$, $p=0.01$). Juvenile bat's (N= 36) fur concentrations ranged from 1.50 mg/kg to 4.45 mg/kg with a mean of 2.90 mg/kg whereas adults (N=191) ranged from 1.11 mg/kg to 22.27 mg/kg with a mean of 4.84 mg/kg. The adults had significantly higher THg mean ($t=6.64$, $df=218.85$, $p<0.001$). Spring bats (N=119) ranged from 1.19 mg/kg to 16.57 mg/kg with a mean of 5.22 mg/kg and fall bats (N= 108) ranged from 1.11 mg/kg to 22.27 mg/kg with a mean of 3.78 mg/kg, where the difference in means was significant as well ($t=3.32$, $df=222.55$, $p=0.001$). An overview of the data is shown in Table 1. Year of capture did not play a significant role in THg concentration (-0.18 ± 0.20, $t=-0.91$, $F_{1,225}=0.83$, $p=0.36$); thus, it was not included in the table or any of the models.

Table 1: Body mass, dry fat mass, wet lean mass, fur THg concentration and forearm length (mean and standard deviation) of silver haired bats caught at Long Point, Ontario in spring and fall of 2012-2014 and 2016.

Sex	**Season**	**Age**	**N**	**Mass (g)**	**Fat mass (g)**	**Lean mass (g)**	**THg (mg/kg)**	**Forearm (mm)**
Female	Spring	Adult	83	11.69±1.25	1.54±0.68	8.75±0.69	5.56±3.89	41.65±1.03
Female	Fall	Adult	28	12.14±1.78	2.08±1.24	8.89±0.77	5.07±4.92	41.10±0.91
Female	Fall	Juvenile	16	11.35±0.64	1.39±0.22	8.83±0.69	2.87±0.86	41.49±0.82
Males	Spring	Adult	36	10.10±0.94	0.88±0.31	8.01±0.79	4.79±2.44	41.10±1.45
Males	Fall	Adult	44	11.32±1.38	1.92±1.17	8.33±0.55	3.68±1.99	41.76±1.06
Male	Fall	Juvenile	20	10.24±0.80	1.18±0.26	8.01±0.54	2.93±0.81	40.93±1.17

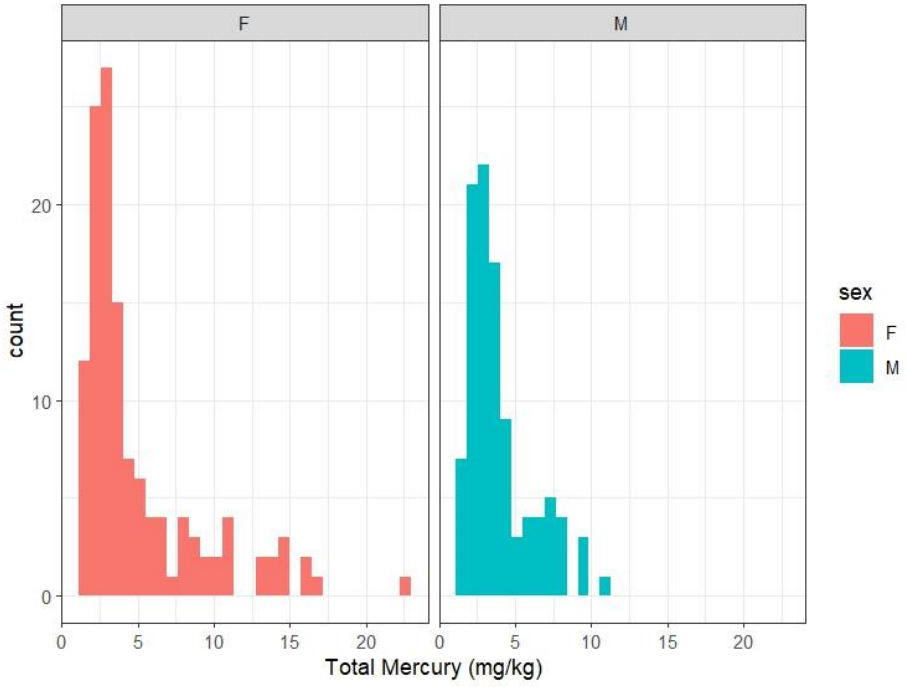


Figure 2: Fur THg concentration (mg/kg) in silver haired bats by sex; overall range of 1.11 mg/kg - 22.27 mg/kg with a mean of 4.53 mg/kg; female range of 1.33 mg/kg – 22.27 mg/kg with a mean of 5.01 mg/kg; male range of 1.11 mg/kg – 11.12 mg/kg with a mean of 3.93 mg/kg

3.1 Variation in Fur Total Mercury

To begin, I analyzed only bats caught in the fall, as adults and juveniles were both present here. This allowed me to determine if age class was a significant factor in predicting mercury exposure. The results showed that there was no age class effect in the fall when controlling for body size and mass. The model indicated that forearm length, which is a measure of body size, and mass were significant in predicting THg exposure (log(mass)= 2.28 ± 0.38, t=6.00, p<0.001; forearm= -0.06 ± 0.02, t=-2.95, p=0.004; $F_{2,104}$=18.45, p<0.001). Thus, when accounting for body size, as mass increased, so did the THg concentration. Since age class was not a significant factor, I was able to proceed in combining the age classes into one data set, as long as body size and mass was accounted for.

In the full model, season, mass and forearm length significantly predicted the fur THg concentration (season (spring)= 0.14 ± 0.03, t=4.41, p<0.001; log(mass)= 1.60 ± 0.31, t=5.14, p<0.001; forearm= -0.05 ± 0.01, t=-3.27, p=0.001; $F_{3, 219}$= 14.63, p<0.001; Figure 3). No interaction terms were significant. Thus, the mercury load of the fur was affected by both season and mass, with spring bats having higher THg concentrations and when controlling for body size, heavier bats had higher THg concentrations.

Originally, I had planned to analyze the data with THg being the independent variable. This would have been more in line with my predictions, as I had thought that THg would

be a driving factor for lower body mass. However, when I started to explore the data, it became quite clear that the opposite pattern was being expressed. Bats with higher THg concentrations had higher body mass. Thus, I changed my approach to having THg acting as the dependent variable. In light of the data, this scenario would be the more plausible one, as the likely explanation for this is that the heavier bats are accumulation more THg by eating higher quantities of food.

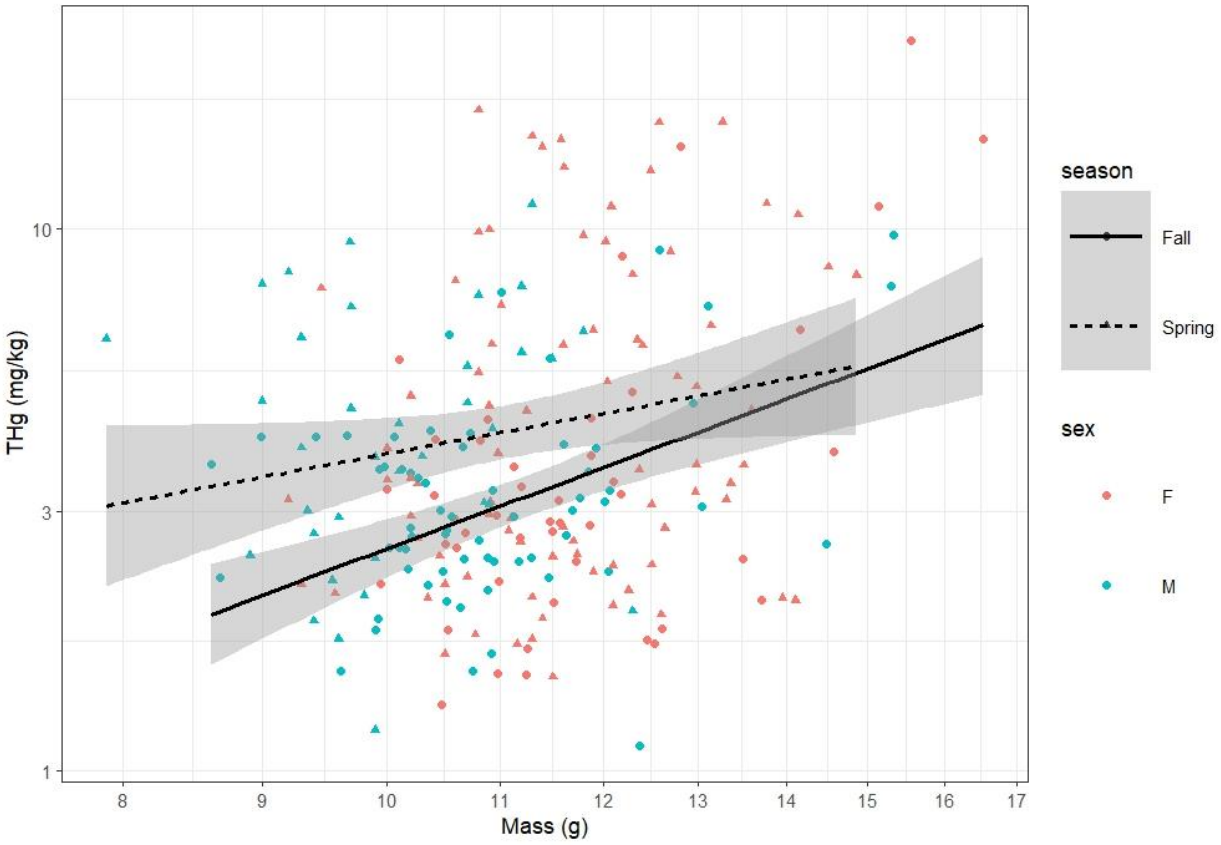


Figure 3: Total mercury concentration (mg/kg) in fur as a function of mass (g) in silver haired bats. When accounting for body size, THg concentration increased with increasing mass and this varied by season (season (spring)= 0.14 ± 0.03, t=4.41, p<0.001;

log(mass)= 1.60 ± 0.31, t=5.14, p<0.001; forearm= -0.05 ± 0.01, t=-3.27, p=0.001; $F_{3, 219}$= 14.63, p<0.001). The figure plots the raw data points and best fit lines without accounting for a small effect of forearm length.

3.2 Body Composition

Overall, females had greater body mass and greater lean mass than males (mass: t=2.61, df=198.04, p=0.01; lean mass: t=6.84, df=208.35, p<0.001). Adults also had greater body mass and fat mass than juveniles (mass: t=3.44, df=74.08, p=0.001; fat: t=3.85, df=195.19, p<0.001). Seasonality played a role in body composition with fall adult bats having greater fat mass than in the spring (t=4.19, df=101.01, p<0.001). To determine how THg was related to specific facets of body composition and to get a deeper understanding of the mechanisms at play, I created two body composition models. The first focused on dry fat mass and the other focused on wet lean mass.

3.2.1 Fat Mass

THg content was significantly affected by the amount of dry fat mass, season, forearm length, as well as the interactions season*fat ($F_{4, 204}$=10.42, p<0.001). The p value of fat mass and season*fat were less than 0.001 and 0.01 respectively (log(fat)=0.48 ± 0.11, t=4.35; season(spring)*log(fat)=-0.37 ± 0.15, t=-2.49). This shows that when controlling for body size, there is a positive correlation between the THg concentration in the fur and

the fat mass of a bat, with the characteristics of the effect varying by season (forearm=-0.03 ± 0.01, t=-2.03, p=0.04; Figure 4). In spring, bats had lower overall fat mass and the THg levels did not increase as rapidly with increasing fat content as they did in fall bats (season=0.23 ± 0.04, t=5.94, p<0.001). This model also showed that larger bats had lower THg levels by percentage (-0.03 ± 0.01, t=-2.03, p= 0.04).

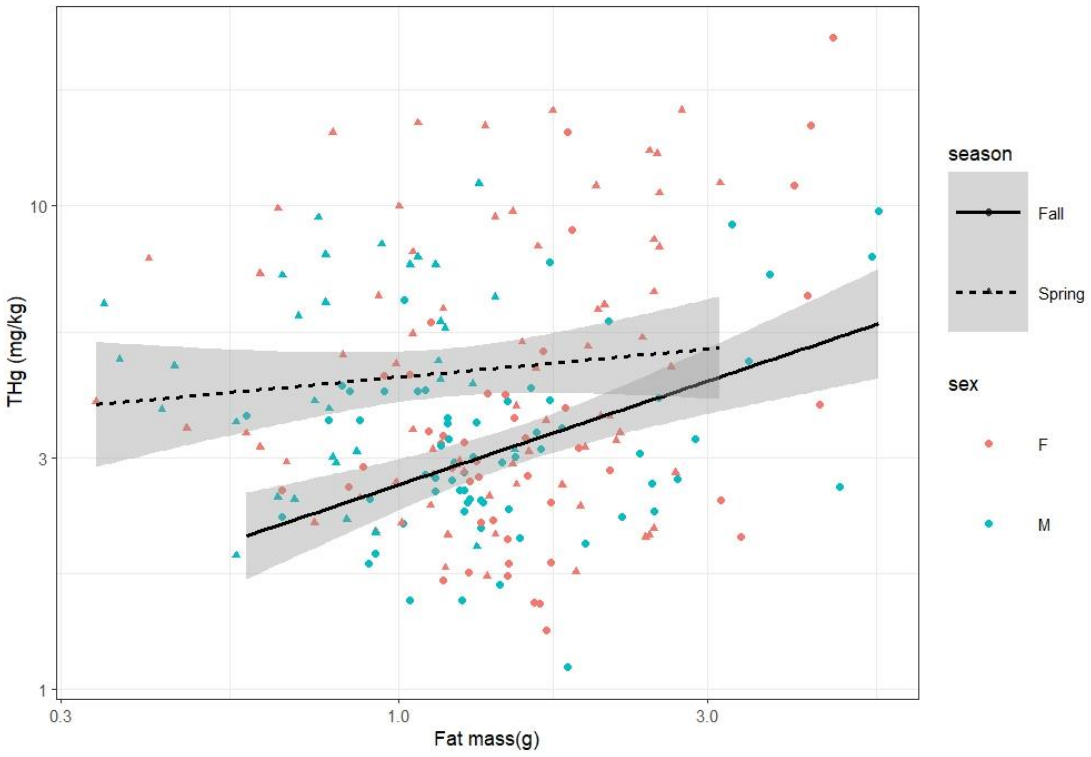


Figure 4: Fur THg concentration as a function of dry fat mass in silver haired bats. When controlling for body size, THg concentrations increased with increasing fat mass, and the trend differed by season. There was also a fat by season interaction (forearm=-0.03 ± 0.01, t=-2.03, p=0.04; log(fat)=0.48 ± 0.11, t=4.35, p<0.001; season(spring)*log(fat)=-0.37 ± 0.15, t=-2.49, p<0.01; season=0.23 ± 0.04, t=5.94, p<0.001; $F_{4, 204}$=10.42, p<0.001). The figure plots the raw data points and best fit lines without accounting for a small effect forearm length.

3.2.2 Lean Mass

THg content of the fur was significantly affected by lean mass, season and forearm length ($F_{3, 205}$=9.84, p<0.001), with the p value of lean mass being 0.003 (0.07 ± 0.02, t=2.96). Thus, the concentration of THg in the fur and the lean mass of a bat were positively correlated, although not as strongly as fat mass (Figure 5). Other information that was concluded from the lean mass model was that just like the fat mass model, larger bats had lower THg levels (forearm=-0.04 ± 0.02, t=-2.35, p=0.02) and bats in the spring had higher THg levels (season(spring)=0.15 ± 0.03, t=4.56, p<0.001).

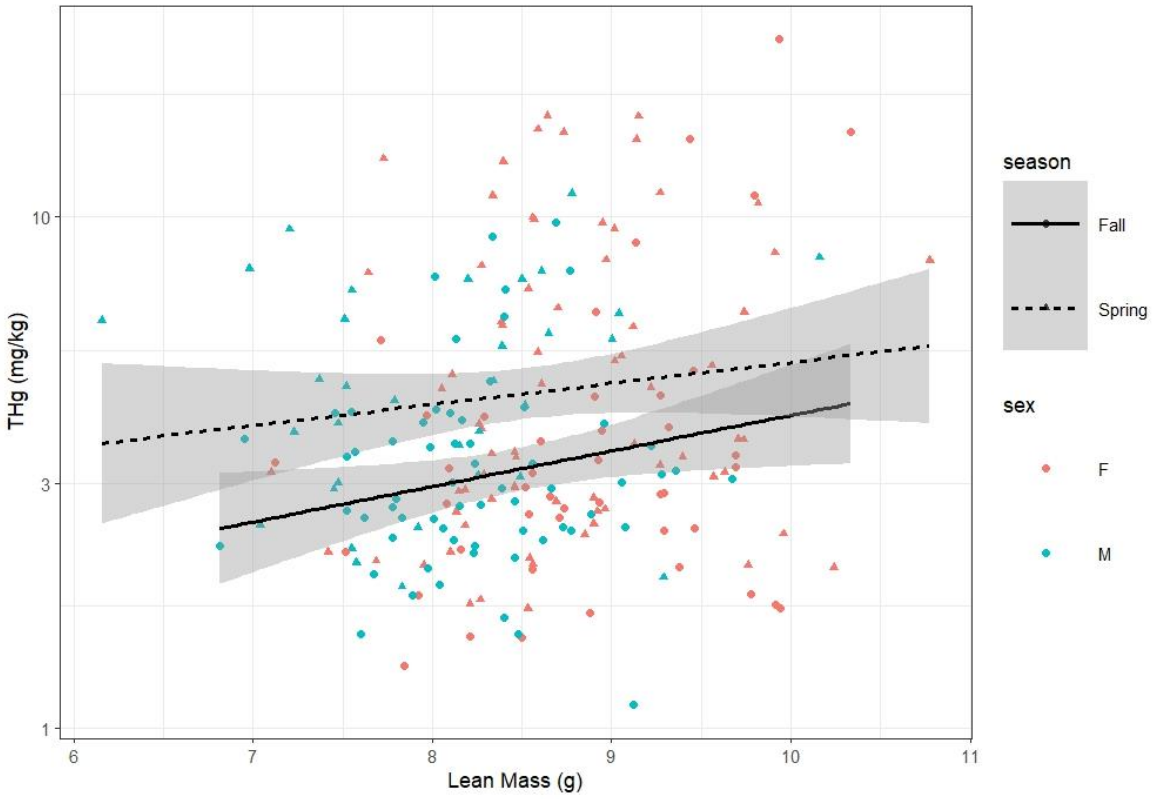


Figure 5: Fur THg as a function of lean wet mass in silver haired bats. When controlling for body size, THg concentrations increased with increasing lean mass, which differed by season (lean=0.07 ± 0.02, t=2.96, p=0.003; forearm=-0.04 ± 0.02, t=-2.35, p=0.02; season(spring)=0.15 ± 0.03, t=4.56, p<0.001; $F_{3, 205}$=9.84, p<0.001). The figure plots the raw data points and best fit lines without accounting for a small effect forearm length.

3.3 Torpor Use in Captive Bats

To analyze the Baloun's torpor data, I recreated the models that he used for his analysis of the effect of mass, season, temperature, age class and sex on torpor use and energy expenditure and I added THg as an independent variable to see if it would provide any further explanatory information. Following Baloun's analysis method, I started with a global model that included the A_{Torpor} as the dependent variable and the mass, treatment, sex, season, THg and all two-way interactions as the independent variables. The results were identical to Baloun's, with mass, treatment, season, and mass*season proving to be significant (Baloun and Guglielmo 2019). Continuing with Baloun's method, the data was split into spring and fall bats due to the mass*season interaction. In the fall, the results were also identical to Baloun's, with mass and treatment being significant. However, in the spring, the interaction treatment*THg was significant (-38.52 ± 18.04, t=-2.14, p=0.039) along with the variables mass and treatment ($F_{6,38}$=108.2, p<0.001). This led me to investigate the temperature treatments individually. In doing so, I recombined the seasons because keeping them separate would have led to very small sample sizes.

The temperature treatments that Baloun had subjected the bats to were 10, 17 and 25 °C. At the warm and medium temperature treatments, there were no relationships between fur THg concentration and the A_{torpor}. However, at the 10 °C temperature treatment, there

was a significant positive relationship between A_{torpor} and fur THg concentration, when controlling for mass (log(THg)=28.81 ± 11.83, t=2.44, p=0.020; $F_{2, 37}$= 8.56, p<0.001; Figure 6). As the THg concentration in the fur increased, so did the A_{torpor}.

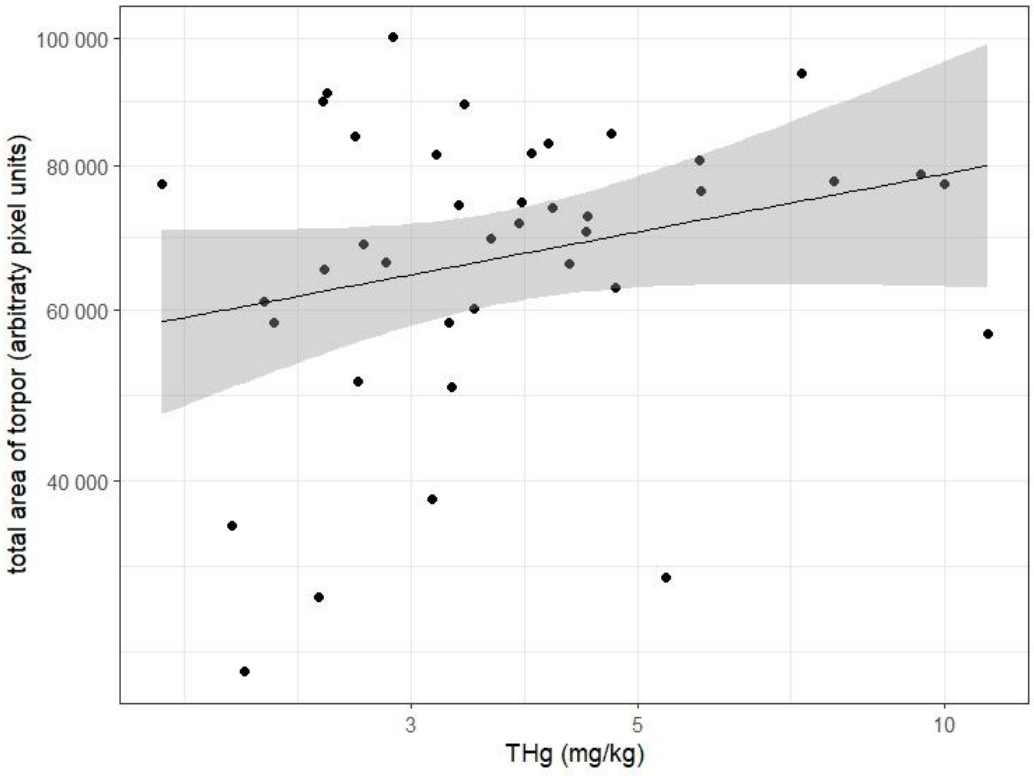


Figure 6: A_{torpor} as a function of fur THg content in silver-haired bats at 10 °C. When controlling for mass, the use of torpor increased with increasing THg concentration (log(THg)=28.81 ± 11.83, t=2.44, p=0.020; log(mass)=-184.44 ± 50.12, t=-3.68, p=0.001; $F_{2, 37}$=8.56, p<0.001). The figure plots the raw data points and best fit line without accounting for the effect of mass.

Another torpor metric that Baloun investigated was total energy used over a 12 h experiment calculated from losses of fat and lean mass measured by QMR (EnTotal). He analyzed this in the same way as A_{torpor}, where first a global model was created, followed by an analysis of the individual seasons. When adding THg into these models, the results were again identical to Baloun's, and THg was not significant. Mirroring the A_{torpor} analysis, the temperature treatments were subsequently divided. Cold and warm treatments showed no relationship between EnTotal and fur THg concentration, although the 10 °C treatment again showed a negative relationship with energy use ($p=0.066$). In the 17 °C treatment, THg concentration and the interaction season*THg expressed a significant relationship with EnTotal, along with the metrics sex, season, mass*sex and mass*season ($\log(THg)=-0.68 \pm 0.26$, $t=-2.62$, $p=0.013$; $\log(THg)*season(spring)=1.13 \pm 0.37$, $t=3.06$, $p=0.004$; $F_{7, 33}=9.36$, $p<0.001$; Figure 7). The general trend was that as THg concentration increased, the total energy use decreased. However, this pattern differed by season where fall bats held true to the overall trend, whereas bats in the spring increased their energy expenditure with increasing THg levels.

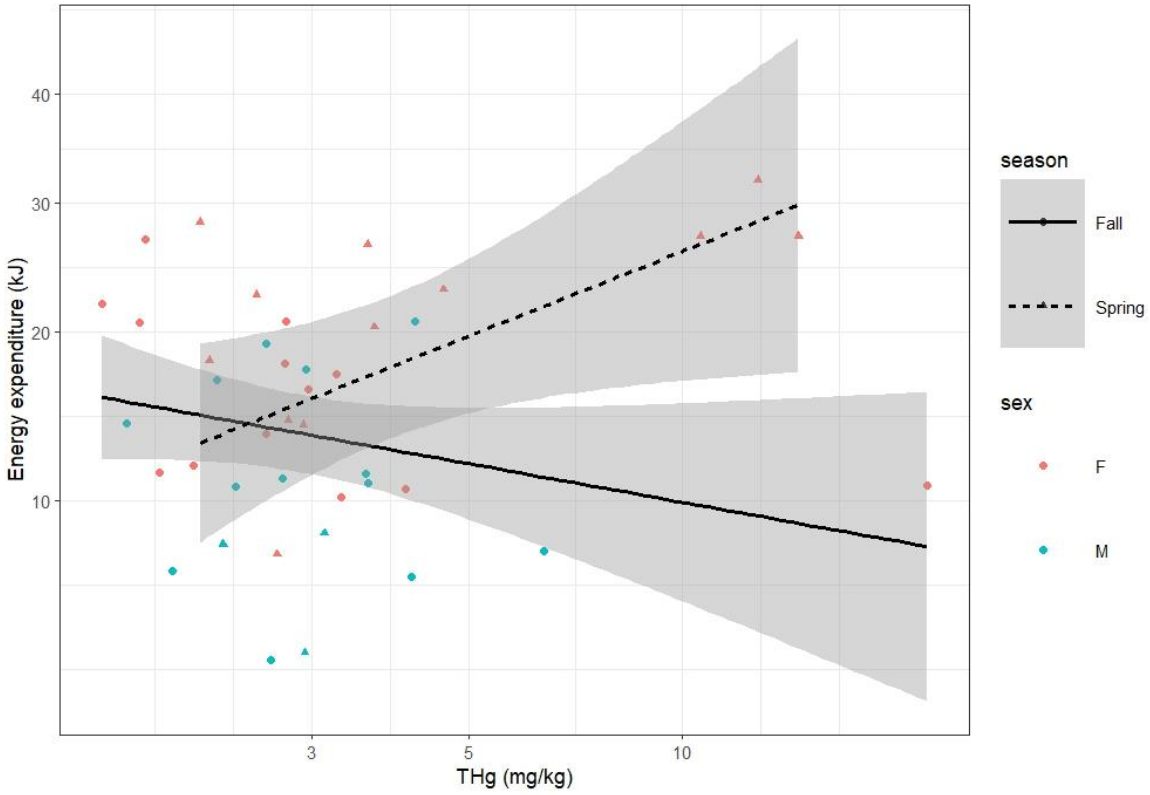


Figure 7: Total energy expenditure as a function of THg concentration in silver-haired bats at 17 °C. Overall, energy expenditure decreased with increasing THg concentration, although this differed by season, where spring bats actually increased their expenditure (log(THg)=-0.68 ± 0.26, t=-2.62, p=0.013; log(THg)*season(spring)=1.13 ± 0.37, t=3.06, p=0.004; $F_{7, 33}$=9.36, p<0.001).

Following these results, I investigated how metrics that measure specific facets of torpor behavior differed among the temperature treatments. The metrics that I included in the analysis were the number of torpor bouts (measured as the number of times body temperature went below the torpor threshold and then back up to it), the maximum depth of torpor (measured as the maximum difference between skin temperature and the torpor

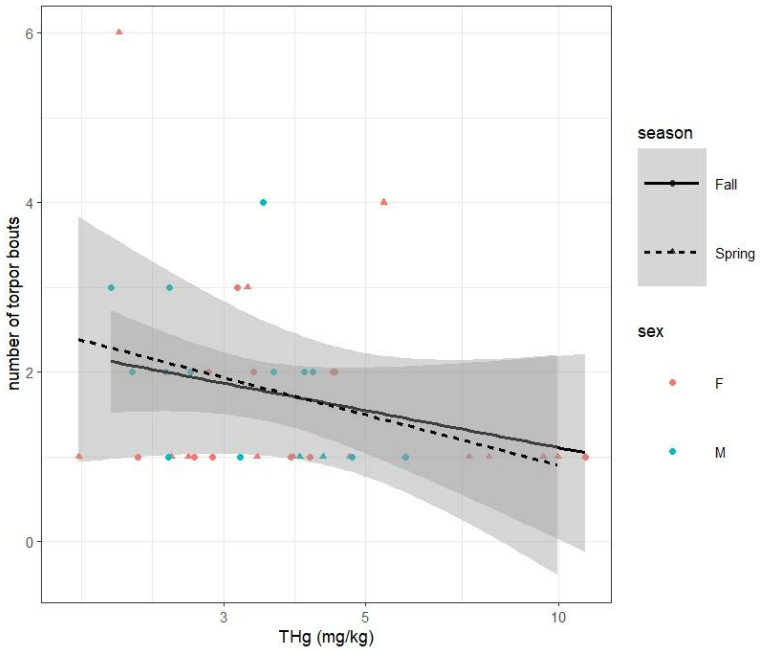
threshold) and the mean torpor bout duration (measured as the mean number of minutes that a single bout of torpor took).

When considering the number of torpor bouts, THg was not significant at the 25 °C temperature treatment, however, it was significant at the 17 and 10 temperature treatments. In the 10 °C treatment, if all other values were held constant, bats with higher THg values entered more torpor bouts meaning that they aroused more frequently than bats with lower THg exposure (log(THg)=12.06 ± 5.26, t=2.45, p=0.020). However, due to the significant interaction terms, the reality of the pattern is not straight forward. The effect of THg on the number of torpor bouts varied with mass, in that as mass increased, the THg exposure had less of an effect on the number of torpor bouts. This led to an overall negative trend in numbers of torpor bouts where the number of torpor bouts decreased as THg concentration increased (log(mass)*log(THg)=-5.64 ± 2.20, t=-2.57, p=0.015, Figure 8a). The other variables that significantly affected the number of torpor bouts were log(mass), sex, season, log(mass)*sex and log(mass)*season ($F_{7, 33}$=7.05, p<0.001).

In the 17 °C temperature treatment, log(THg) and log(mass)*THg were also significant, but curiously the relationship was the opposite to the cold treatment, as bats with higher THg values had fewer torpor bouts and thus aroused less frequently (log(THg)=-18.36 ± 6.93, t=-2.65, p=0.012), but as mass increased, the THg concentration had a greater effect on the number of torpor bouts the individual bat took. Thus, the overall trend was a positive relationship with the number of torpor bouts increasing as THg increased

(log(mass)*log(THg)=7.27 ± 2.75, t=2.748, p=0.012; $F_{3,33}$=8.23, p<0.001, Figure 8b). However, when inspecting the graph of the relationship, most of the data points seem to be adhereing to a negative slope except for the three data points in the top right corner. These may be spurious results, and if this were the case, then the bats held at 17 °C would follow the same trend as the bats at 10 °C.

A



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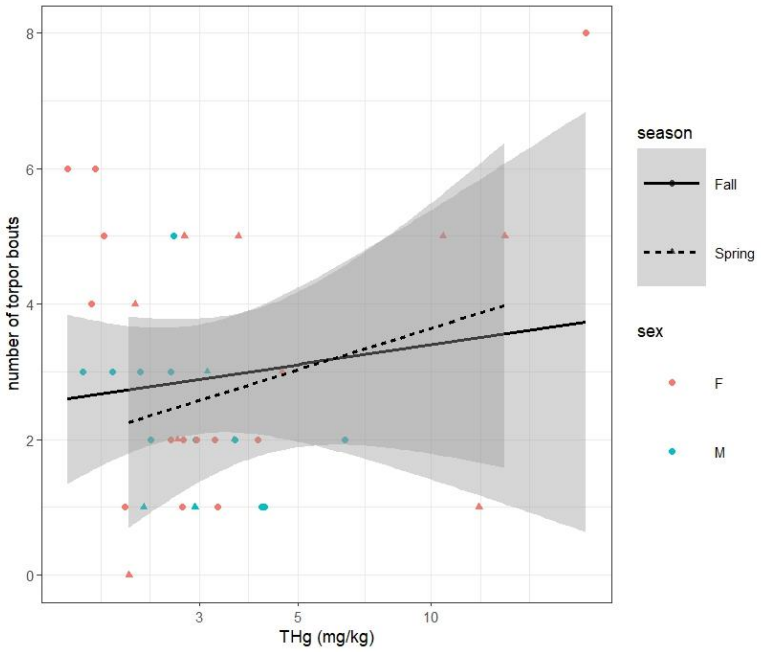
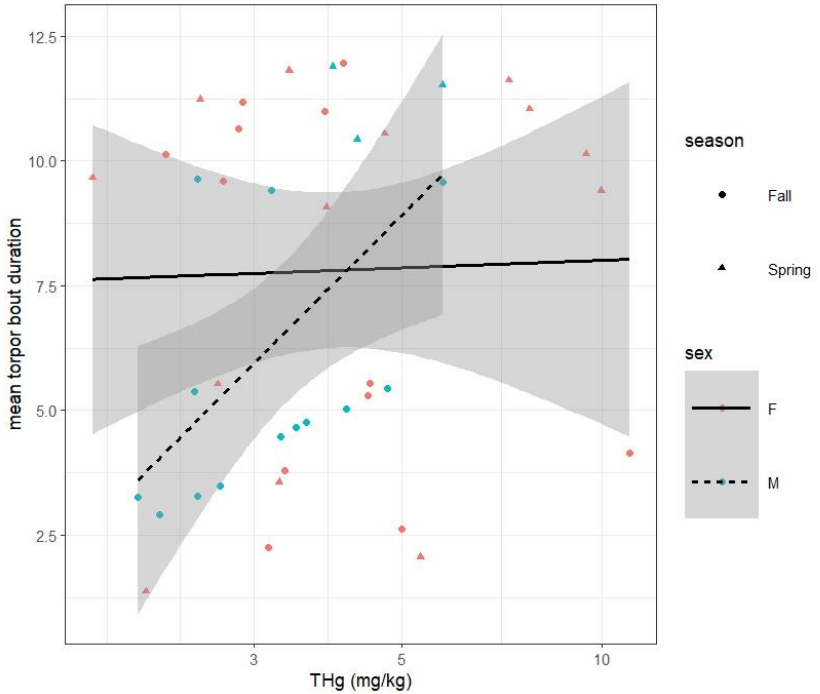


Figure 8a: Number of torpor bouts as a function of THg concentration in silver haired bats at 10 °C. When controlling for all other variables, the number of torpor bouts increased with increasing THg levels (log(THg)=12.06 ± 5.26, t=2.45, p=0.020). However, due to the significant interaction with mass, the overall trend was negative (log(mass)*log(THg)= -5.64 ± 2.20, t=-2.57, p=0.015; $F_{7, 33}$=7.05, p<0.001). Figure 8b: Number of torpor bouts as a function of THg concentration in silver haired bats at 17 °C. When controlling for all other variables, the number of torpor bouts decreased with increasing THg levels (log(THg)=-18.36 ± 6.93, t=-2.65, p=0.012). However, due to the significant interaction with mass, the overall trend was positive (log(mass)*log(THg)=7.27 ± 2.75, t=2.748, p=0.012; $F_{3,33}$=8.23, p<0.001).

A



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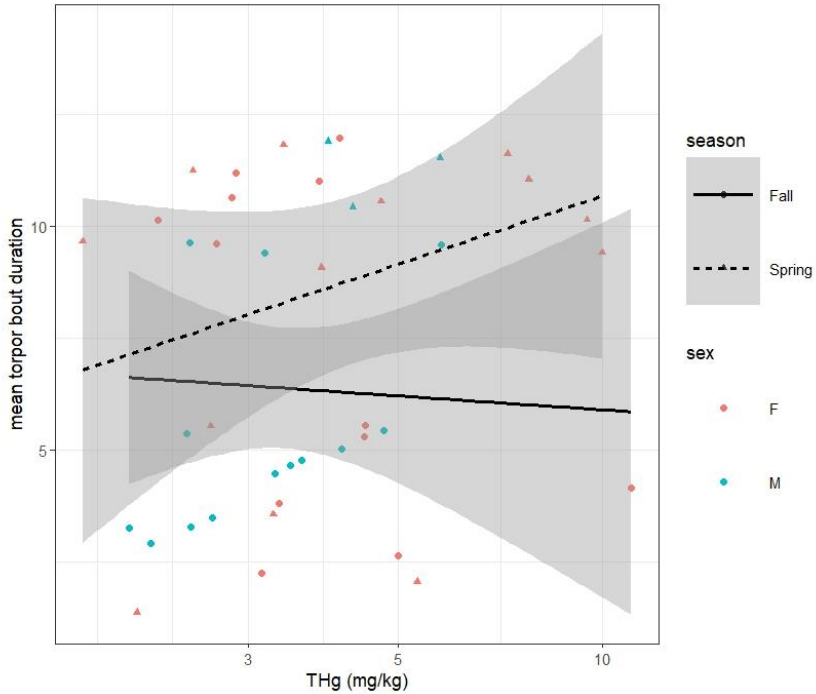

Figure 9a: Mean torpor bout duration as a function of THg concentration in silver haired bats split by sex. Males increased their torpor bout duration at a higher rate than females (log(THg)*sex(M)=8.09 ± 2.73, t=2.96, p=0.006).

Figure 9b: Mean torpor bout duration as a function of THg concentration in silver haired bats split by season. Spring bats increased their torpor bout duration, whereas fall bats exhibited a slight decrease (log(THg)*season(spring)=5.45 ± 2.59, t=2.10, p=0.043; $F_{7, 33}$=5.52, p<0.001).

The mean torpor bout duration was only affected by THg at the 10 °C temperature treatment. On its own, THg was not significant, however, the interactions THg*sex and THg*season were highly significant, with males expressing a positive relationship between THg and the mean bout duration whereas females showed a weaker relationship (log(THg)*sex(M)=8.09 ± 2.73, t=2.96, p=0.006, Figure 9a). In the spring, the data revealed that there is a strong positive relationship between the THg concentration and the mean bout duration, as opposed to the fall where there was a slight negative relationship (log(THg)*season(spring)=5.45 ± 2.59, t=2.10, p=0.043; $F_{7, 33}$=5.52, p<0.001; Figure 9b).

The last parameter I investigated was the maximum depth of torpor. Again, only the 10 °C treatment showed any indication of an effect from the THg concentrations. (log(max.depth torpor) ~ season, $F_{1,35}$=4.50, p=0.04) However, there was an issue with the residual plot, indicating that the data did not fit the model very well for the lower negative quantiles. These outliers were 4 female bats who only used extremely light torpor. If the skewed residual plot were to be ignored, then bats with higher THg burdens would have lighter depths of torpor. In an attempt to fit the data to the model, I reran the model without the four outliers. This produced a normal distribution but had the consequence of the THg not being significant anymore. This suggests that these four observations were driving a spurious pattern. As these points were real observed individuals and biologically relevant, I am hesitant to remove them from the model. Thus, I believe this aspect of torpor needs further investigation to make a robust conclusion.

3.4 Torpor use by Bats in the Field

Jonasson's data set was much smaller than Baloun's, especially in regard to the torpor traces. In total, she sampled 59 bats, but only 19 of those were tested for torpor use. Thus, I was limited in the analysis I was able to do. This led me to create very simplistic statistical models, without any transformations or two-way interactions and only as few variables as possible. The independent variables that were included were the THg concentration, mean ambient temperature, and the mass of the bat. The dependent variables I tested were torpor depth, torpor duration, torpor percent, and minimum skin temperature.

Of all tested independent variables, none exhibited a significant relationship with THg. For torpor depth or duration, none of the tested independent variables were significant and these models did not fit the data well (depth: $F_{1,17}=1.64$, $p=0.218$; duration: $F_{1,17}=1.89$, $p=0.187$). The percentage of torpor that bats used during the monitored time only showed a significant relationship with the mean ambient temperature, with lower temperatures leading to a greater torpor use ($F_{1,17}=12.11$, $p=0.003$). The last metric I analyzed was minimum skin temperature, which mirrored the torpor percentage, where only the mean ambient temperature was significant. The relationship between the two variables was that higher temperatures led to higher minimum skin temperatures ($F_{1,17}=18.04$, $p<0.001$).

Chapter 4: Discussion

This study is the first to test how body condition and the use of torpor in bats relate to variation in THg exposure. With respect to body condition, I found that the body burden of THg increased with increasing mass when controlling for body size, regardless of age or sex, with THg loads being higher in the spring than in the fall. The body burden of THg also increased with increasing fat mass and lean mass, with the correlation between fat mass and THg concentration varying by season.

In bats taken into captivity for one day and exposed to three experimental temperatures (25, 17, and 10 °C), torpor use was almost exclusively affected by THg at the coldest temperatures, where bats with higher THg concentrations used more torpor. Interestingly, energy use was only affected at the 17 °C temperature treatment and not the cold treatment, where bats with higher THg concentrations used significantly less energy. This pattern was driven by fall bats, as spring bats revealed a slight opposite trend. When all other metrics are held constant, bats with higher THg loads aroused from torpor more frequently, but due to a positive significant interaction between mass and THg, the overall relationship was that bats with higher THg loads took fewer torpor bouts. Similarly, at 10 °C temperature treatment, THg on its own led to shorter mean torpor bout duration which differed by sex and season, but when factoring in the mass interaction, the average torpor bout was longer for higher THg bats. Bats that were held at 17 °C expressed the exact opposite relationship between the THg concentration and the number of torpor bouts for both torpor on its own, and the torpor mass interaction. In wild bats,

torpor use was independent of THg concentration, but the sample size was very small, thus limiting the conclusions.

4.1 Body Condition and Composition

The body mass of an individual bat was a strong predictor of THg body burden, when controlling for body size. The direction of the relationship was contrary to my prediction, as I expected that due to the negative toxicological effects of MeHg, that bats in poorer body condition would show higher THg concentrations. The resulting positive relationship can be explained by the possibility that heavier bats are those that consistently ingest more prey items, increasing their exposure potential to THg. However, this increase in dietary exposure may have not been enough to elicit a large significant effect in the bats. Chételat et al (2018) theorized that bats may have a higher-than-average tolerance for THg than other mammals, as they found that some bats in in high mercury areas of Canada, can have levels of up to 54.23 mg/kg of fur THg with a mean 10.61 ± 13.38. The THg levels found in my samples were a lot lower than this, suggesting that I was not able to capture the full spectrum of effects elicited on bats.

The amount of THg measured in the fur samples differed by season, in that spring bats had higher THg concentrations than fall. A possible explanation might involve the molting cycle of the bat. Since fur is one of the main routes of depurating THg, bats will unload a large amount of the THg during the molt, which happens in the summer. This

would lead to the fur's chemical composition being representative of these conditions, as once the fur is formed, it is inert (Fraser et al. 2013). Thus, the THg concentration does not change from the time it is grown to the time that it is measured (Chételat et al. 2018). However, all fur bearers will still lose and regrow some amount of fur throughout the year, and this could provide an additional opportunity for depurating THg. If the dietary intake of THg is different over the winter than during the summer, this may lead to differential data between the seasons.

Another explanation for the seasonal difference might be that in the spring, females eat more due to pregnancy, resulting in an increase in their exposure potential. Pregnant females will also decrease their torpor use to facilitate the development of the fetus. The lack of torpor drives up their energy use even further. In the fall, females will then have recently given birth and lactated, which are other routes of eliminating THg. This increase in exposure due to pregnancy could be supported by my results, as I found that females had higher THg concentrations than males. Although the females in my study were palpated to check for pregnancy and none were detected (Jonasson 2017; Baloun and Guglielmo 2019), both Baloun and Jonasson warned that because it was so early in the season, it would be likely to not detect the fetus. If the fetuses where undetected, my results would be congruent with those from Yates et al (2014). They found that on average, females accumulate two times the amount of THg than males. It has yet to be proven what the driving process for this sex discrepancy is, but as stated, the increase in food intake and decrease in torpor usage, to compensate for gestation and lactation are predicted to be the main factors, along with females being larger in size. Although sex

was not a significant predictor in determining THg burden in my model, it is reasonable to conclude that females are driving this pattern, as they tended to have higher masses and THg concentrations.

Fur THg concentration was strongly related to fat mass, a good measure of the excess energy stores of the bat. Fatter bats had higher THg concentrations, which is congruent with my model of body mass. Seasonality also played a role in the relationship between THg and fat mass. Overall, in the spring, bats had lower fat mass and the THg concentration did not increase as much with increasing fat mass as it did in the fall. This may be explained by females increasing their intake for pregnancy in the spring. However, this increase in energy does not get turned into fat, instead it is used by the growing fetus, but the females will still experience the increased THg body burden. In the fall, all the energy that females consume will be used by her own body, thus the THg concentration of a bat will scale more closely with fatness.

When investigating how fur THg related to wet lean mass, it followed the same general pattern as fat mass where, when controlling for body size, bats with more lean mass had higher THg concentrations. The explanation for this pattern could be the same as for the fat mass model. Bats that consume more food and maintain greater tissue masses are at a greater risk of being exposed to THg, but with the increased caloric intake, they are able to build and maintain more muscle and organ mass. There was no seasonal effect on THg for lean mass.

Fat mass had a stronger correlation to THg concentration than lean mass, even with the higher number of significant variables and two-way interactions in the fat model. This was to be expected as fat mass tends to be a better predictor of caloric intake than lean mass (Weise et al. 2014). As stated previously, one can reasonably conclude that bats with more fat mass have been eating more, and consequently have a higher total intake of THg, contributing to a higher bioaccumulation.

Another factor that may play a role in the stronger correlation of THg to fat mass would be the age of the bats. My models showed that bats that exhibited higher fat mass were significantly older. By this I mean they were not in the juvenile age class, as determining the age of a bats in a noninvasive manner beyond the two categories of juvenile and adult is often impossible. This is why Baloun and Jonasson were only able to make the distinction between these two age classes. That being said, this argument still holds weight as adults are more successful at foraging due to having greater experience. Adults are also not growing in size anymore, so they appear to be able to maintain and add mass to a greater extent than juveniles. To add to this, they have also had a longer time period to accumulate Hg.

The fat load during both the spring and fall migration was correlated with the THg in fur, which should have been mostly grown during the previous summer with some potential replacement over time. The majority of fur growth would be either a few months or almost a year prior to the sampling, suggesting that certain bats are maintaining a higher

mass and body fat levels than others, and consequently bioaccumulating more THg. This leads to the question of whether these are high quality individuals, that are better at maintaining a good body condition, leading to more Hg, or if they are highly stressed individuals that maintain higher fat, to buffer against food uncertainty?

There have yet to be any published studies of a bat's propensity to employ risk sensitive foraging in controlled laboratory environments. There are field studies that touch on this topic, but results vary by species and location (Korine et al. 2023). In many organisms, a chronic stress response can lead to an increase in energetic demands, which could elicit downstream effects such as a lower body mass (Rauw 2012). Body mass has also often been used as a non-invasive consistent biomarker of chronic stress (Dickens and Romero 2013). This would support the hypothesis that these individuals are of high quality and are not encountering prolonged stressors.

A potential stressor that bats may face is food insecurity. This is closely tied to the type of environment that the bat inhabits (Geggie and Fenton 1985). There are several published studies that have recorded that in urban environments, bats spent less time foraging, leading to the conclusion that there could be a greater abundance of food (Jung and Threlfall 2018). Contrasting these findings, other studies have found bats spend more time foraging in urban areas (Geggie and Fenton 1985). The differences in these findings may be in part attributed to the variation of resource abundance, accessibility, and patchiness of different urban areas. The presence of parks or streetlights may provide dense and predictable resource sites, which "may buffer bats against energetic stress,

enabling them to cope with consistent high exposure to anthropogenic stressors" (Korine et al. 2023). This would support the alternative explanation that the heavier bats are experiencing greater stressors. This would align with the fact that the silver-haired bat is a species that does not tolerate urban environments as well as other species of bats (Coleman and Barclay 2012). The type of environment (rural vs. urban) that the bat inhabited, may also help explain the difference in THg concentrations, as areas with high anthropogenic disturbance also have the potential to have high levels of THg (Zhang et al. 2012).

4.2 Torpor in Captive Bats

The use of torpor in silver haired bats was not affected by THg body burden when all three temperature treatments and both seasons were pooled into a global model. When this model was split up by season, there was a significant interaction between temperature treatment and THg concentration in the spring. Subsequently, the temperature treatments were analyzed separately to get a better understanding of the interplay between torpor use, THg burden and roosting temperature. Overall, bats rooting at the coldest temperature, 10 °C, appeared to be more affected by THg, whereas at 17 °C only the number of torpor bouts were affected and at 25 °C there was no significant effect of THg detected.

In the global model, the two higher temperature treatments masked the effect of the coldest temperature treatment. A contributing factor to why the effect of THg was not significant at these higher temperatures is that bats do not rely as heavily on torpor to depress their metabolic rate as temperatures increase (McGuire et al. 2014; Baloun and Guglielmo 2019). With a reduced use of torpor comes a lower statistical power. Torpor is used to a greater extent at colder temperatures (McGuire et al. 2014) and thus also has a greater potential to show deviations from the norm. The reason why bats use torpor less frequently at higher temperatures is that bats do not need to expend as much energy thermoregulating, as their body temperature is closer to the ambient temperature (McGuire et al. 2014). Since they are expending less energy, they are not as inclined to save extra energy through the use of torpor.

At the coldest roosting temperature, the integrated measure of total torpor use (A_{torpor}) was predicted by the THg concentration and body mass. Bats with higher THg concentrations used significantly more torpor than bats with lower THg concentrations and as previously stated in the body composition models, heavier bats had significantly higher THg content in their fur. Baloun's study (Baloun and Guglielmo 2019) and my body composition model showed that heavier bats used less torpor when controlling for THg. Thus, this discrepancy leads me to predict that THg is a contributing factor in how torpor is used, in that an increased ingestion of THg would cause a bat to use more torpor. Therefore, I would expect a heavy bat with low THg fur levels to use less torpor than a heavy bat with high THg levels.

A compelling reason for this pattern could be that the neurotoxic effects of THg are prohibiting the proper function of the anterior hypothalamus' POA (Tan and Knight 2018). As previously stated, the POA is a key integratory site for thermoregulation and sleep in the brain. Since torpor is characterized by a depression of body temperature, it is regulated by the POA.

Just like A_{torpor}, the total energy each bat used was not influenced by THg in the global model, nor was it significant when broken up by season. When each treatment was analyzed independently the 17 °C temperature treatment was the only one where THg has a significant effect. However, it is noteworthy to mention that at 10 °C, THg was very close to being significant. At 10 °C there was a strong trend for bats with higher THg concentrations to use less energy. Considering that in the cold treatment analysis of A_{torpor}, bats with higher THg used more torpor, it would be a logical conclusion that they would therefore use less energy.

At 17 °C, bats with higher THg concentrations used significantly less energy. This may be because in the A_{torpor} analysis, these bats displayed a trend to use more torpor (but it was just not enough to be significant). This would lead to a lower energy expenditure and since A_{torpor} was at the cusp of being significant, it was enough to make EnTot be significant. The interaction between THg and season was also significant, where bats with higher THg used less energy in the fall but more energy in the spring. The best explanation for this result is pregnancy. Female bats seemed to be driving this pattern and

pregnancy is an energy intensive life phase that happens in the spring. This is supported by Baloun (2019), who also determined that females have an increased energy expenditure in the spring (McGuire et al. 2014).

At 10 °C, the number of torpor bouts a bat took was significantly affected by their THg concentration. When controlling for every other variable, bats with higher THg concentrations took more torpor bouts than bats with lower THg concentrations. However, due to the interaction between mass and THg the overall pattern was reversed. Here, as the mass of the bat increased, the effect of THg on the number of torpor bouts decreased. This led to the overall trend being bats with higher THg levels making fewer torpor bouts and thus staying in torpor longer. The interaction could be hinting at the fact that increased fat mass (which is closely tied to increased THg contamination) may be providing a buffer against the toxic effects of THg, as bats in better condition may be able to counteract the toxin more effectively. This is assumes that all bats were reentering torpor after the similar amount of arousal time. If the arousal time was vastly different between bats with high and low THg levels, the pattern may be complicated to interpret. For example, if bats with higher THg concentrations arose less frequently but remained awake for an extended period in time they could be using less torpor than a low THg bat that arises frequently but returns to a torpid state immediately. However, the global model would support the situation where the time spent out of torpor was lower for high THg bats, thus, I will assume that their arousal periods are either equal in length or shorter.

The overall pattern of torpor use was quite clear. Bats that took the most torpor bouts were leavier ones in the low THg category. This is because bats with lower THg concentrations were able to follow their natural trend, where heavier bats use less torpor than lighter bats (Baloun and Guglielmo 2019). The heavier bats in the high THg concentration category used fewer bouts and thus used more torpor, aligning themselves with my previous results that indicate that THg increases torpor use. The issue this analysis presents is in the interpretation and mechanisms behind what is causing the pattern. Thus, this should be further investigated experimentally in future research.

The situation becomes more convoluted when including the number of torpor bouts in the 17 °C temperature treatment. Here, the pattern expressed was the exact opposite of the 10 °C temperature treatment, as high THg bats took fewer torpor bouts when all other variables were held constant. However, when factoring in the mass by THg interaction, the overall trend was that higher THg individuals took more torpor bouts and thus aroused more frequently. One explanation for this is that at warmer temperatures, bats generally use less torpor, leading to a more limited data set. The variation in the number of torpor bouts and THg levels was also a lot higher at 17 °C. Another factor that may be playing a role in the opposing results is that at 17 °C, it may not be “cold enough” for the bats to experience adverse effects of THg. Cold temperatures provide a degree of stress for bats, and there may be a threshold temperature between 10 and 17 °C, at which they succumb to the cold challenge and their body no longer prioritizes the THg contamination.

When roosting at 10 °C, the effect that the THg body burden of an individual bat had on the mean torpor bout duration differed significantly between the sexes and seasons. On its own, the THg concentration was not significant, but when including the interactions, the overall trend was that the mean bout duration increased with increasing THg. This would mean that they are engaging in more torpor.

In both sexes there was a positive relationship between the THg load and mean bout duration, however slopes of the trend lines differed. In males, the slope was very steep, whereas females only exhibited a very small difference in mean bout duration as THg concentration increased. Overall, there was not a significant difference between the averages of the two sexes mean bout duration. A plausible reason for males having a greater variation in torpor bout duration is that they may be more sensitive to changes in THg concentration. Females tend to consume more food due to pregnancy, and all the bodily changes that come along with it, and they tend to have a larger body size, meaning that they have a greater chance to be exposed to higher concentrations of THg. This could lead to building up a tolerance over time (Nam et al. 2012).

In the spring, bats exhibited a positive relationship between the THg concentration and mean bout duration as opposed to the fall, where there is a slightly negative trend. This could in part be due to the fact that the temperature is generally colder and more variable in the spring than in the fall. This may lead bats to use torpor for longer periods of time as insurance for the colder periods. Insects are generally less abundant in the spring as well,

limiting the caloric intake of the bats. This would further drive the bats to use more torpor to avoid an energy deficit. Another factor that may be playing a role in this pattern is that bats molt in the summer, providing them with an opportunity to unload a large amount of THg from their body. The THg would still be present in their fur, but they may have less THg in their body, which could shift the use of torpor.

Since the last metric, depth of torpor, did not fit the model well, I am hesitant to make any concrete conclusions. However, I suspect that the four bats that were driving the pattern may have been pregnant. This would explain the lack of torpor use. They would then be eliminated from the model as they do not fit into the testing pool. Then the THg concentration would not have an effect on the depth of torpor. Nevertheless, I believe this topic warrants further investigation of whether there is a real effect from the THg concentration at play.

In revisiting the topic of urban vs. rural environmental impacts on bats, the roost types that the individual bats occupy in their respective home range could play a role in the patterns this study unveiled. Roost type can have a significant effect on the energy expenditure of a bat, where individuals that occupy more buffered roosts, have a lower heat tolerance as opposed to ones in more exposed roosts that experience larger fluctuations in temperature (Czenze et al. 2022). Urban environments can experience a “urban heat island effect”, which is the occurrence of cities exhibiting higher temperatures than outlying areas (Shochat et al. 2006). As silver haired bats are tree

roosting bats, they would be directly affected by this difference in air temperature. Due to the lack of trees in urban settings, bats may also be forced to roost in other structures such as bat houses or things like garden umbrellas or attics. These may provide better temperature buffering than their naturally preferred roosts. If this were true, then silver-haired bats residing in urban areas may have a higher use of torpor than rural bats, when both are exposed to a rural setting (which is where this study took place), as these urban bats are not as tolerant to the colder temperatures. Thus, to save the extra energy that would be used to thermoregulate, they may become torpid instead.

4.3 Torpor use of Bats in the Field

No significant effect of THg contamination was found when the use of torpor in silver haired bats was measured in a field setting. The limited sample size may have played a large role in these results, which would warrant further investigation with a larger sample size. The fact that not even the mean ambient temperature influenced torpor depth or duration also supports the suspicion that the sample size may have not been sufficient, as it is well established that ambient temperature generally plays an integral role in torpor usage (Audet and Fenton 1988; McGuire et al. 2014; Fjelldal et al. 2021). However, in the bats from Baloun's experiment (2019), there was only a consistent significant effect of THg at 10 °C. In Jonasson's data set (2017), none of the mean ambient temperatures were that low. The temperatures in her study ranged from 12 - 21.5 °C with a mean of 16.5 °C. This begs the question of at what temperature THg effects become apparent. This probably varies from individual to individual, but if the average threshold were 10

°C, then the changes in torpor use would not have had the opportunity to show up in Jonasson's data. Even if the temperature threshold was encapsulated in Jonasson's data, the other bats who roosted at higher temperatures could have the potential of masking the effect as there were more of them.

Regardless of whether THg affected the use of torpor in the wild, it is quite plausible that bats behave differently in captivity than in the wild. Being exposed to novel environments, such as the torpor chambers used by Baloun, could pose a stressful situation. Stress is known to activate the autonomic nervous system, which leads to an increase in body temperature (Dallmann et al. 2006). To enter torpor, bats must significantly lower their body temperature, leading to the notion that stress may inhibit the use of torpor.

On the other hand, in the wild, tree roosting bats may be exposed to greater fluctuations in environmental conditions compared to a captive setting. In the wild, there may be large gusts of wind, loud noises, and shifting exposure to sunlight, that could also elicit a stress response and hinder torpor use. At this point it is difficult to say which stress response would outweigh the other, or if there is even a significant difference. To my knowledge, this situation has not been studied yet. Furthermore, it is not feasible to directly compare Baloun's and Jonasson's torpor data due to the large differences in the methods of their studies. Nonetheless, I would predict that bats in captive studies would use torpor to a lesser extent than bats in the wild.

Overall, if torpor were not affected by THg in the wild, the most logical reasoning behind this would be that the increase in stress from recent handling and novel environments has negative downstream effects. Studies have shown that one form of stress may decrease the body's ability to handle another stressor (Sokolova 2013). This additional stress could then lead to a decreased tolerance of THg stress.

Chapter 5: Conclusion

Rather than conducting experimental dosing experiments, my study used the natural variation in Hg exposure to test the hypothesis if mercury contamination would have negative effects on silver haired bat's body composition and torpor use. Some bats had high fur THg (> 20 mg/kg), and I found 20-fold variation among individuals which suggest that bats from different summer breeding areas experience large differences in MeHg exposure. Given all the extraneous sources of potential noise in the analysis, it is surprising that I was able to detect strong and significant relationships among fur THg, body condition, and torpor use. I determined that larger and heavier bats with more fat and lean mass had higher THg concentrations in their fur, and that age class did not play a role in the body burden when controlling for weight and body size. Season affected THg levels in fur, with bats sampled in the spring having higher concentrations. Furthermore, in bats experimentally tested for a full day at three roosting temperatures, torpor use was consistently affected by THg at the coldest roosting temperatures. Body composition played a large role in this relationship, where overall there was a trend for greater torpor use in the heavier and higher THg bats. In free-ranging bats in the field there was no detectable effect of THg on torpor, however, the sample size was very small, limiting the possible analysis.

This study has furthered the field of ecotoxicology as well as our understanding of the way Chiroptera are affected by MeHg contamination. It also has brought to light numerous different directions that future bat research could go in order to aid in

conserving bat populations, and to further our understanding of their threats. In the future, this study should be repeated but as a controlled dosing experiment in a laboratory to eliminate confounding variables. Although I found minimal effects of THg at higher temperatures, a more controlled and focused study may reveal certain details at these temperatures that this study was not able to due to a more in-depth analysis. Another topic that future studies should examine is the relationship between THg body burden and its effect on migration. Torpor is a large part of migration, and this would be a logical next step in determining the impact of THg on bats. Lastly, THg tolerance in terms of neurotoxic effects should be assessed for a wider variety of species in order to determine if certain species are at higher risk than others. This should be coupled with developing potential mitigation and detoxification techniques, which would allow for more focused conservation measures to be implemented in key habitats.

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