The importance of long-term capture-mark-recapture archives for wildlife monitoring and research: Two examples from bat populations

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.
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Abstract

Long-term capture-mark-recapture (CMR) projects provide useful data to study and monitor wildlife. Specifically, CMR data can help identify how an animal interacts with its environment and how these interactions change throughout its life. In this thesis, I use data and sample archives from temperate hibernating bats captured and tagged as part of a long-term project in Newfoundland and Labrador, Canada. In Chapter 2, I use fur collected from adult female *Myotis lucifugus* captured multiple times from 2012-2017 to investigate age dependent changes in the concentration of the toxic compound, methylmercury (MeHg). Results suggest total mercury concentrations, which are highly correlated with MeHg, decrease with age in the fur of adult female bats. This pattern indicates that adult female bats can eliminate enough MeHg from their tissues to have steady or decreasing concentrations in their fur. In Chapter 3, I use forearm measurements taken by multiple observers from captured and tagged *M. lucifugus* and *Myotis septentrionalis* to quantify the measurement error and observer bias associated with this morphometric variable. Results suggest measurement error can add enough variation to mask relationships between forearm length and related variables. Further, observer bias can cause type I errors when comparing populations with small differences in forearm length that were measured by different observers. These two studies exemplify the use of long-term CMR projects as an invaluable tool to assess research techniques, study wildlife biology, and monitor ecological changes.

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List of Abbreviations

1. AIC_c – Akaike's Information Criterion corrected for small sample size
2. CMR – Capture-mark-recapture
3. CRM- Certified reference material
4. dw – Dry weight
5. EPA – Environmental Protection Agency
6. FAL – Forearm length
7. ME – Measurement error
8. MeHg – Methylmercury
9. NL – Newfoundland and Labrador
10. OB – Observer bias
11. PIT – Passive integrated transponder
12. SNP – Salmonier Nature Park
13. THg – Total mercury
14. WNS – White-nose syndrome

List of Symbols

1. w_i – Akaike weight
2. Δi – Difference between the top-ranked model and the i^{th} model
3. $\sum w_i$ – Cumulative Akaike weight
4. k – Number of parameters
5. %ME – Percent measurement error
6. s^2_{wi} – Within individual variance
7. s^2_a – Among individual variance
8. MS_{wi} – Mean squared deviation within individuals
9. MS_a – Mean squared deviation among individuals
10. m – Mean number of repeated measurements per individual

Chapter 1
Introduction to long-term capture-mark-recapture studies in bats

Long-term capture-mark-recapture (CMR) studies offer valuable data which can be used to answer a wide range of biological questions and make predictions that are difficult or impossible to test with short-term studies of unmarked animals (Lettink & Armstrong, 2003). These CMR methods allow for repeated measurements and/or sampling of known individuals over time. There are a variety of methods for marking individuals, all of which allow for the unique identification of individuals upon recapture (Bendik, Morrison, Gluesenkamp, Sanders, & O'Donnell, 2013; Ellison, 2008; Gibbons & Andrews, 2004). These methods are especially useful as a tool for species that are difficult to study through visual observation techniques. Specifically, CMR methods can provide insight into inter-individual variation of ecological traits, such as dietary preferences (Berl, Flaherty, Danielson, Kellner, & Swihart, 2017), social behaviours (Ripperger et al., 2019), and responses to natural (Noonan et al., 2014) or experimental (Lloyd, Moehrenschlager, Smith, & Bender, 2013) changes in the environment. Since any of these variables may be dependent on life history traits such as age (Burgett et al., 2018) and reproductive history (Culina, Linton, Pradel, Bouwhuis, & Macdonald, 2019), capturing and sampling an individual at multiple times is crucial to understanding how an animal's interactions with the biotic and abiotic environment change throughout its life.

In addition to investigating inter- and intra-individual variability in ecological traits, CMR methods can be used to estimate population-level parameters including abundance, density, and recruitment, which are essential to understanding population viability (Pollock

& Alipizar-Jara, 2005). Through long-term CMR studies, population parameters can be tracked over time and used to inform policy and conservation decisions by predicting the future viability of a population (Lettink & Armstrong, 2003). Further, monitoring population parameters can assess the effectiveness of conservation and restoration efforts designed to improve population viability (Stem, Margoluis, Salafsky, & Brown, 2005).

No matter the objective, a long-term study's design should be optimally suited to the questions being asked, such that rigorous statistical tests can effectively test predictions and elicit robust conclusions. Data collected from long-term CMR studies are often flexible and can be used to address questions outside the original purposes of the study, adding to the value of such datasets (Lindenmayer et al., 2012). Since little extra effort is required, and a small amount, if any, stress is added to the captured animal, collecting minimally-invasive samples and data to be archived is a relatively cost-effective and ethically sound practice that can optimize the efficacy of a long-term study. The flexibility of the data means many researchers collect measurements and samples which do not have immediate value to the current objective, with the aim that the archives can be used in future research and monitoring. Even if the study design does not allow for robust conclusions to be made, the low cost enables low-risk exploratory studies which may open new lines of inquiry to be pursued with more rigorous study designs (Whitlock, 2011).

Bats (Chiroptera) are an order of flying mammals of which CMR methods have greatly increased our understanding (Ellison, 2008). The small size, cryptic nature, and nocturnal activity of bats makes CMR methods a useful tool to study bat ecology. Studies on bats have used CMR methods since bat research was in its infancy, with the first published

banding of a bat occurring in 1916 (Allen, 1921). Since then, CMR methods have played a crucial role in uncovering important aspects of bat biology including longevity (Florko, Bohn, Kalcounis-Rueppell, & Brigham, 2017), migration (Simal et al., 2015), and life history traits (Jan et al., 2019). Moving forward, CMR methods will continue to play a critical role in bat research with advancements in technologies, such as passive integrated transponder (PIT) tags, GPS tags (Castle, Weller, Cryan, Hein, & Schirmacher, 2015), and proximity sensors (Ripperger et al., 2019), allowing for the remote 'recapture' of bats, eliminating the need to handle individuals after marking them. Although these remote 'recapture' methods are vital for questions regarding sociality and movement, physical recaptures are still necessary to collect morphometric information and to collect physical samples (e.g., wing tissue, fecal matter, fur) that can be used for questions requiring genetic or chemical information. Since these morphometric measurements and physical samples are not known to impact the animal's survival (Pollock et al., 2016), they are usually collected whenever possible. Long-term CMR studies can use these data and samples to investigate inter- and intra-individual variation in parameters such as dietary preference, exposure to toxic compounds, and body condition.

One of the useful aspects of CMR studies of bats is their ability to estimate and document age of live animals, since molecular age estimation techniques for bats are not fully developed (Wilkinson et al., 2020). As bats do not show obvious morphological signs of aging once they mature, performing age-based analyses on live bats is difficult (Wilkinson & Brunet-Rossinni, 2009). A juvenile bat can be differentiated from an adult based on the degree of ossification of the metacarpal-phalanges joint which become indistinguishable

from an adult in autumn (Kunz & Anthony, 1982). Thus, individual bats must be captured as a juvenile to be assigned an 'exact' age, whereas individuals first captured as an adult are assigned an 'at least' age.

In this study, I use archived fur samples and morphometric data from captured and tagged bats as part of an ongoing long-term CMR project in Newfoundland and Labrador (NL), Canada. This long-term project was initiated to monitor the temperate hibernating bat species *Myotis lucifugus* (little brown myotis), to detect its population trends, and to research basic biological aspects of the species. Although Chapter 2 and 3 of this thesis fall under the general objective of this long-term project, the specific goals of these chapters were determined after data collection. Thus, these chapters provide examples of the versatility of long-term CMR studies and the important contributions they make towards understanding how wildlife are affected by environmental change.

I used fur samples taken from adult female *M. lucifugus* who were captured multiple times throughout the study to quantify age related patterns of methylmercury (MeHg) accumulation in the tissues of bats (Chapter 2). Alterations to earth's natural biogeochemical cycles have increased the availability of harmful compounds, such as MeHg, to humans and wildlife (Pirrone et al., 2010). Adult bats in the NL study population have been previously shown to have concentrations of MeHg in their tissues higher than the threshold where toxic effects occur (10 μg/g; Nam et al., 2012) with a large amount of variation among individuals in the population (Little, Burgess, Broders, & Campbell, 2015a). Very little is known about the traits which cause this variation in MeHg concentrations among bats (Chételat et al., 2018). Since MeHg bioaccumulates (Wolfe, Schwarzbach, & Sulaiman, 1998), and bats are

long-lived (Wilkinson & South, 2002), age may be an important factor causing this variation. The investigation of age dependent mercury concentrations presented in Chapter 2 can be used to assess the risk mercury poses to bats as they age.

I use forearm length measurements from captured and tagged *M. lucifugus* and *Myotis septentrionalis* (northern myotis) to quantitatively characterise the measurement error and observer bias associated with these forearm measurements (Chapter 3). Rapid changes to ecosystems are expected to affect wildlife health and morphology (Acevedo-Whitehouse & Duffus, 2009; Birnie-Gauvin, Peiman, Raubenheimer, & Cooke, 2017; Tomassini, Colangelo, Agnelli, Jones, & Russo, 2014) and having robust morphometrics is necessary to monitor these changes. The analysis of measurement error and observer bias presented in Chapter 3 can help researchers determine if observed differences in morphology, within or among populations, are large enough to make valid inferences. Chapter 4 is a summary of the results and conclusions of the manuscript chapters.

Collectively, this thesis exemplifies the importance of long-term CMR studies. As humans continue to alter the landscape, atmosphere, soil, and water chemistry, ecological systems are undergoing rapid change. The establishment and continuation of long-term CMR studies is crucial for monitoring the effects these changes are having on the environment and wildlife. Established CMR studies can help gauge the effectiveness of policy changes and conservation efforts, ultimately providing baselines and important biological data to scientists and decision makers (Lindenmayer et al., 2012).

Chapter 2
Declining mercury concentrations with age in the fur of an insectivorous terrestrial mammal (*Myotis lucifugus*)

2.1 Overview

Methylmercury (MeHg) is a naturally occurring, toxic form of mercury that can bioaccumulate in organisms and is abundant in some aquatic environments. These characteristics of MeHg put high trophic level predators who derive energy from aquatic environments at the greatest risk of experiencing toxic effects. Due to the potential for bioaccumulation of MeHg over an individual's life, the risk of MeHg toxicity may increase as terrestrial mammals age. Total mercury (THg) concentrations were measured from the fur of adult female *Myotis lucifugus* collected between 2012-2017 in Salmonier Nature Park, Newfoundland and Labrador. Using linear mixed-effects models, the effect of age, year, and day of capture on THg concentrations was determined. I expected that, due to MeHg's ability to bioaccumulate, THg concentrations would increase with age, and that due to annual summer moulting, individuals captured earlier in the season would have lower THg concentrations than individuals captured later in the season. Models were compared using AIC_c, and multimodel inference was used to compute estimates for the parameters found in the top models. Contrary to expectations, THg concentrations decreased with age and were not affected by day of capture. Among individuals, there was a negative relationship between the initial THg concentration of an individual and the rate of change in THg concentrations with age. Using a trend analysis, I found no evidence of population level changes in fur THg concentrations over the study period. Together, the results indicate that adult female bats can

eliminate enough MeHg from their tissues to have decreasing THg concentrations in their fur. The results from this study have implications regarding the effects of MeHg at different stages in the life of terrestrial female mammals, with young adults potentially at the greatest risk of experiencing toxic effects from high MeHg concentrations resulting in reduced reproductive output.

2.2 Introduction

Mercury (Hg) is a naturally occurring heavy metal which is toxic to organisms at high concentrations (Scheuhammer, Meyer, Sandheinrich, & Murray, 2007). Anthropogenic activity has altered the Hg cycle through processes such as fossil fuel combustion and precious metal extraction (Pirrone et al., 2010), which release inorganic Hg from the lithosphere, increasing the concentration of Hg in the atmosphere, soils, and water (Pirrone et al., 2010). Once in the atmosphere, Hg may be transported globally and deposited in remote locations (Selin & Jacob, 2008). Deposited inorganic Hg can be methylated via sulfate- or iron-reducing bacteria to methylmercury (MeHg), its bioavailable form (Paranjape & Hall, 2017; Robinson & Tuovinen, 1984). Methylation occurs primarily in aquatic environments, and rate of net methylation is determined by interacting factors including water pH and the concentration of dissolved organic carbon (Zhu, Zhang, & Žagar, 2018). People and wildlife living in regions with high rates of Hg deposition and environmental conditions that promote high net methylation rates are at risk of exposure to toxic amounts of MeHg (Simone, Gencarelli, Hedgecock, & Pirrone, 2016).

Although the mechanisms causing the toxicity of MeHg are not fully understood (Bjørklund, Dadar, Mutter, & Aaseth, 2017), its ability to bioaccumulate can lead to the

tissues of people and wildlife accruing harmful concentrations (Wolfe et al., 1998). The toxic effects of MeHg are rarely lethal, instead toxicity is presented as discrete sublethal effects (for review see Bjørklund, et al., 2017) such as neurotoxicity (Nam et al., 2012), impaired development (O'Kusky, 1983), and reduced immune system function (Becker, Chumchal, Bentz, et al., 2017). Due to biomagnification, organisms at high trophic levels that derive energy from aquatic systems are most at risk of experiencing the toxic effects of MeHg. Organisms exposed to high levels of MeHg include terrestrial animals such as bats (Becker, Chumchal, Broders, et al., 2017) that consume fish and emergent aquatic invertebrates (Cristol et al., 2008).

An animal's exposure to MeHg is dependent on its life history and physiology, in addition to the bioavailability of MeHg in the environment (Lazarus et al., 2018; Scheuhammer et al., 2007). An understanding of how life history and physiology traits can cause variable MeHg concentrations in animal tissues is necessary to determine which species and populations are most at risk. The primary inputs of MeHg include dietary intake (Becker, Chumchal, Broders, et al., 2017), and transfer during ontogenetic development through the fetus/egg (Burgess, Bond, Hebert, Neugebauer, & Champoux, 2013; Lazarus et al., 2018; Lisón, Espín, Aroca, Calvo, & García-Fernández, 2017) and mammary milk (Mansour, Dyer, Hoffman, Schulert, & Brill, 1973). The primary outputs of MeHg include excretion of internally demethylated inorganic Hg (Eagles-Smith, Ackerman, Julie, & Adelsbach, 2009), elimination through keratinized tissues (Wang, Evans, Hickie, Rouvinen-Watt, & Evans, 2014), and maternal transfer to the fetus/egg or milk (Burgess et al., 2013; Lisón et al., 2017).

Assessing the relationship between MeHg concentrations and age can provide insight into an animal's relative balance of MeHg inputs and outputs. In most species of fish and marine mammals, MeHg concentration increases with age due to the affinity MeHg has for muscle protein and a poor ability to eliminate MeHg (Eisler, 1984). In terrestrial and semiaquatic mammals, results from studies examining the relationship between age and concentration of MeHg vary considerably. Rodents and shrews were found to have a higher concentration of MeHg as they grew older (*Mus musculus*: Stern, Cox, Cernichiari, Balys, & Weiss, 2001; *Sorex cinereus*: Tavshunsky, Eggert, & Mitchell, 2017), and in semiaquatic mammals, positive relationships between MeHg and age were found in some studies (*Lontra canadensis*: Ben-David, Duffy, Blundell, & Bowyer, 2001; *Lutra lutra*: Kruuk, Conroy, & Webb, 1997; Lodenius, Skarén, Hellstedt, & Tulisalo, 2014; Dibbern et al., 2021), but not in others (*Lontra canadensis*: Evans, Addison, Villeneuve, MacDonald, & Joachim, 1998; *Mustela vison*, *Lontra canadensis*: Klenavic et al., 2008). Further, there was no relationship detected between MeHg and age in long-lived aquatic birds, even for those that are exposed to large amounts of MeHg (*Larus novaehollandiae scopulinus*: Furness, Lewis, & Mills, 1990; *Pagodroma nivea*: Tartu et al., 2014). This lack of relationship presumably occurs because of their ability to purge large amounts of MeHg when they moult keratinous feathers annually, and lay eggs (Burgess et al., 2013; Furness et al., 1990). Although, MeHg elimination mechanisms similar to birds exist in mammals (Chételat, Ackerman, Eagles-Smith, & Hebert, 2020; Hyvärinen, Tyni, & Nieminen, 2003), the ability of these mechanisms to effectively limit concentrations of MeHg in the tissues of terrestrial and semiaquatic mammals is unknown.

Previous studies on terrestrial and semiaquatic mammals examined carcasses or a single sample from individuals whose age was estimated. Although using carcasses allows for the analysis and comparison of multiple tissue types, there are limitations associated with this methodology. First, only a snapshot of MeHg exposure during the animal's life is provided, such that the relationship between MeHg and age can only be determined at a population level, rather than at an individual level. At a population level, confounding variables are introduced through intraspecific differences, such as diet, that alter MeHg exposure (Chételat et al., 2020). Second, there may be a bias towards sampling carcasses with higher concentrations of MeHg since these individuals may have a higher mortality rate (Mierle, Addison, MacDonald, & Joachim, 2000). These limitations can be avoided by using non-lethal modes of sampling (e.g., fur, claws, feathers) to examine changes in MeHg within an individual's lifespan. Since high concentrations of MeHg can reduce reproductive output of animals (Bianchini, Tozer, Alvo, Bhavsar, & Mallory, 2020; Jackson et al., 2011; Scheuhammer et al., 2007), resolving the relationship between age and MeHg concentrations in mammals is important to help determine how it affects an individual's lifetime fitness.

Insectivorous bats are exposed to high amounts of MeHg (Zukal, Pikula, & Bandouchova, 2015) because they occupy a high trophic position, regularly consume large amounts of insects relative to their mass, and are long lived (Kurta, Bell, Nagy, & Kunz, 1989; Wilkinson & South, 2002). The species used in this study, *Myotis lucifugus* (little brown myotis), is considered a generalist that forages over aquatic and terrestrial environments where it primarily consumes Diptera, Ephemeroptera, Chironomidae, Trichoptera, and Lepidoptera, among others (Belwood & Fenton, 1976; Clare, Barber,

Sweeney, Hebert, & Fenton, 2011). In regions with high MeHg availability, *M. lucifugus* (Little, Burgess, Broders, & Campbell, 2015a), among other bat species (Chételat et al., 2018; Yates et al., 2014), have been found with concentrations of MeHg in their fur greater than those found to cause neurochemical changes in *M. lucifugus* (Nam et al., 2012) and sublethal effects in other mammals (10 μg/g; *Peromyscus maniculatus*: Burton et al., 1977; *Mustela vison*: Wobeser, Nielsen, & Schiefer, 1976).

Within bat populations a relatively large amount of variation in THg concentrations among adult individuals exists, the sources of which are unknown (Little et al., 2015a). Given the long lifespan of bats, with some *M. lucifugus* living beyond 30 years in the wild (Keen & Hitchcock, 1980), one potential source of variation in MeHg concentrations is age. Adult bats have been reported to have higher mean MeHg concentrations and higher variation than conspecific sympatric juveniles (Heiker, Adams, & Ramos, 2018; Yates et al., 2014). Since methods to determine the age of an adult bat are still in development (Wilkinson et al., 2020), the changes to MeHg concentrations in the tissues of an adult bat as it ages is currently unknown. Capture-mark-recapture techniques combined with fur sampling, provide a way to assess MeHg concentrations at multiple ages of one individual.

Fur has been used as a matrix to monitor heavy metal exposure in a variety of mammalian taxa, including bats (Becker, Chumchal, Bentz, et al., 2017; Klenavic et al., 2008; Mina et al., 2019; Wiig, Renzoni, & Gjertz, 1999). Mammalian fur is a keratinous tissue with a high affinity for MeHg and provides an elimination pathway for MeHg (Chételat et al., 2020). Previous studies on bats have found 71–95% of total mercury (THg) in fur to be MeHg (Yates et al., 2014). Mammalian fur has also been shown to have THg

concentrations that are proportional to THg concentrations in other tissues (brain, muscle, blood, and liver) (Malvandi, Ghasempouri, Esmaili-Sari, & Bahramifar, 2010; Yates et al., 2014). These relationships make the use of THg in fur an effective proxy and cost-effective alternative to assess MeHg concentration in the internal tissues of bats (Yates et al., 2014).

A consideration regarding the use of fur for determination of MeHg concentrations in bats is their ambiguous moult cycle (Fraser, Longstaffe, & Fenton, 2013). Hair follicles are in contact with the blood such that the concentration of MeHg in the fur is related to the concentration of MeHg in the blood at the time of fur growth (Hernout et al., 2016). Once the fur is no longer in contact with the blood, it is inert, and the concentration of MeHg does not change. Therefore, newly grown fur in mammals will share a stronger relationship to the current state of MeHg in tissue than older fur (Hernout et al., 2016). In mammals that moult annually or biannually, the time of fur collection is therefore expected to have an effect on the recorded concentration of MeHg, depending on whether fur was collected before or after the moult (Hyvärinen et al., 2003). Although the timing is not well defined, moulting in bats is presumed to occur once a year during the summer or early autumn, and varies depending on species and sex (Fraser et al., 2013).

The goal of this project was to quantify the relationship between age and MeHg in the tissues of a long-lived terrestrial mammal to make inference on patterns of MeHg concentrations in tissues. Specifically, I analysed Hg concentrations in the fur of tagged female *M. lucifugus* captured in Salmonier Nature Park (SNP), Newfoundland and Labrador (NL), Canada with two objectives.

1) Quantify inter-annual variation in the THg concentration of fur of the study population.
2) Use samples collected from the same individual over multiple years to quantitatively characterise patterns of fur THg concentrations in individuals as they age.

Previous Hg analysis in SNP performed by Little et al., (2015a) found the fur of 15 *M. lucifugus* to have a mean THg of 10.9 μg/g (SD ± 3.96), which is above the threshold of 10 μg/g where physiological effects are expected to occur (Nam et al., 2012). Given the relatively large variation of THg concentrations observed in *M. lucifugus* adults in SNP, it is expected that part of this variation can be explained by age. I expect the bioaccumulation of MeHg in the internal tissues will lead to increasing MeHg concentrations in the fur with age. Alternatively, if elimination pathways remove enough MeHg to equal or exceed the inputs, MeHg concentrations will not increase with age and will instead reach a steady or declining state as an adult.

2.3 Methods

2.3.1 Study Area

The fur specimens used in this study were collected between 2012 and 2017 in and adjacent to SNP, NL, Canada (Lat: 47.3º, Long: -53.3º). Salmonier Nature Park is a 1455 ha nature reserve that is part of the Avalon Forest Ecoregion. Surface waters in SNP exhibit low pH due to the low acid neutralization ability of the soil and rock (Clair, Dennis, Scruton, & Gilliss, 2007). Low pH is correlated with increased methylation of Hg (Zhu et al., 2018); and

wildlife in SNP are thus susceptible to accumulating harmful concentrations of MeHg (Little et al., 2015a).

2.3.2 Sample Collection

Myotis lucifugus were captured in SNP using mist nets (Avinet, Dryden, New York, USA) and harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia). Age class (adult or juvenile) was determined based on the degree of ossification of the third metacarpal-phalangeal joint (Kunz & Anthony, 1982). Captured bats had a passive integrated transponder (PIT) tag (0.09 g; EID-ID100 implantable transponders, EIDAPInc, Sherwood Park, Alberta, Canada and Trovan Electronic Identification Systems, UK) subdermally injected between the scapulae. Recaptured bats already carrying a PIT tag were identified using a handheld PIT tag reader, providing data on time between captures. Individuals first captured as adults were assigned an age of 'at least' 1. In the subsequent captures of these individuals, they were assigned an 'at least' age which corresponded to the number of years since their first capture. Fur samples were taken from between the scapulae using cuticle scissors. To prevent cross contamination of fur, scissors were cleaned between individuals by washing in bleach, then water, followed by ethanol. Fur was placed in polypropylene vials and frozen at -20 and/or -80^{o}C. Differences in storage temperature were a result of space limitations; however, given the stability of Hg in fur, these differences are not expected to not change the THg content (Phelps, Clarkson, Kershaw, & Wheatley, 1980). Forearm length and mass were measured, and sex was determined. Reproductive status (pregnant, lactating, post-lactating, not obviously pregnant, or non-reproductive) was determined by palpitation of the abdomen and visual inspection of the nipples for signs of lactation (Racey & Swift,

1985). Following processing, all animals were released at the site of capture. All animal handling and sample transport was done in accordance with the protocol approved by St. Mary's University Animal Care Committee and the Government of Newfoundland and Labrador (SMU-MSVU protocol 11-18**,** 12-17, 13-15, 14-10A, 15-12, 16-12; Government of Newfoundland and Labrador protocol IW2011-14, IW2012- 26, IW2013-10, IW2014-31, IW2015-52, IW-2015-11, WLR2016-12, WLR2017-16, WLR2017-17).

2.3.3 Mercury Analysis

Due to a limited sample size of male bats, only fur from adult female bats was analysed. Prior to Hg analysis, fur samples were visually examined for external sources of contamination such as dirt. Fur with visible contamination was cleaned with a Kim wipe. To avoid loss of sample mass, fur was not washed to remove other potential sources of exogenous contamination. Chételat et al. (2018) and Little et al. (2015b) both found that washing bat fur does not alter THg content. Thus, I assumed any exogenous contamination was negligible.

Fur samples were analysed for THg content using a direct mercury analyser in accordance with Environmental Protection Agency (EPA) method 7473 in the Biotron Centre for Experimental Climate Change Research at Western University, London, Ontario (Milestone Tricell DMA 80, Milestone, Inc., Shelton, CT). For quality control, IAEA-086 human hair was used as an external certified reference material (CRM) to determine total mercury recovery (average relative percent recovery = 101 %). Analytical precision was determined using sample duplicates (average relative percent difference = 5%). Detection limit for THg was 0.07 ng. THg concentrations are reported in µg/g dry weight (dw).

Methylmercury was analysed using a modified version of EPA method 1630 in an ISO 17025 accredited laboratory in the Biotron Centre for Experimental Climate Change Research at Western University using a Tekran® 2700 automated methylmercury analyser. Percent MeHg recovery was determined using a human hair CRM (IAEA-086) (average relative percent recovery = 110 %). Analytical precision was determined using sample duplicates (average relative percent difference = 2%). Detection limit for MeHg was 0.051 ng/g. MeHg concentrations are reported in µg/g dw.

2.3.4 Statistical Analysis

I used THg concentrations in 163 fur samples, 9 of which were subsampled and analysed for MeHg concentration. A regression analysis was used to estimate the proportion of THg in the fur comprised of MeHg.

A trend analysis was performed on the THg content on the fur of 60 randomly selected (10 samples/year) adult females to identify any trends in annual THg concentrations in bat fur. To avoid sample bias towards older individuals, only bats that had an 'at least' age of 1, and thus of unknown age, were selected. To limit the possibility of THg variation within years due to factors such as moulting and insect availability, samples were only collected from adult females captured from July 28 – August 5. These dates were selected based on the coincidence of sampling periods in all years. Due to heteroscedasticity in THg concentrations among years, a Mann-Kendall non-parametric trend test (Mann, 1945; Kendall, 1955) was performed using the R package, *Kendall* (McLeod & McLeod, 2015).

I used the fur from 47 adult female bats captured ≥ 2 times over the study period to investigate changes in fur THg concentrations from subsequent captures of the same

individual. Using the R package *lme4* (Bates, Mächler, Bolker, & Walker, 2015), I created multiple linear mixed-effects models from three *a priori* selected parameters (Burnham & Anderson, 2002) including *at least age*, *Julian day*, and *year*. *Julian day* is the day of year the fur sample was taken (day 1 to day 365) and was included to account for annual moulting which has been suggested to occur during the summer months (Fraser et al., 2013). Bats were caught between May 15 (day 135) to August 13 (day 225). If moulting occurs in the summer months, individuals captured earlier in the year may not have moulted at the time of capture. If THg increases with age, I would thus expect earlier captures to have a lower THg relative to captures later in the year. *Year* was included to determine the importance of year-to-year trends in THg exposure. There was no evidence of multicollinearity among the parameters (variance inflation factor < 5) as determined using the *vif* function in the R package, *car* (Fox & Weisberg, 2019).

I compared seven linear mixed-effects models and one random effects model (Table 1.2.) using Akaike's information criterion corrected for small sample size (AIC_c) (Burnham & Anderson, 2002) with the *MuMIn* package in R (Bartoń, 2019). Following the procedure in Zuur et al. (2007), and using AIC values, the optimal random effects structure was identified to be a random intercept and slope structure (Appendix A: Table 2). Thus, each mixed-effects model included random intercepts and slopes with individual as the random effect. Using multimodel inference, I calculated the unconditional parameter estimates for the parameters in the models making up ≥ 95 % of the confidence set.

All data analysis was done in R version 4.0.3 (R Core Team, 2020).

2.4 Results

Total mercury concentrations in all analysed fur of adult female *M. lucifugus* ranged from 2.692 µg/g dw to 30.888 µg/g dw (mean = 11.809; SD = 4.520). In 9 of these samples, fur was subsampled and analysed for MeHg concentration. I found a strong relationship between MeHg and THg, with MeHg making up 69.4 % - 95.8 % ($F = 33.08$; d.f. = 7; $R^2 = 0.800$; $P < 0.001$) of the THg content in fur (Figure 1.1.), supporting THg as an effective proxy for MeHg. From 2012 to 2017 the annual mean concentration of THg in the fur of sampled individuals did not show any significant trend ($\tau = -0.6$; $P = 0.133$; Figure 1.2.; Table 1.1.)

The THg concentration of 103 fur samples from 47 female *M. lucifugus* who had fur samples taken multiple (2-3) times throughout the study period were analysed. Three models were included in the 95% confidence set of models to explain variation in THg concentration in the fur of individuals and each had one fixed effect variable (Table 1.2.). The three models in the 95% confidence set included the parameters *at least age* and *year*. Multimodel inference suggests there was a negative relationship between *at least age* and THg concentration with an estimated rate of change -0.476 (95 % CI = -0.898, -0.053) µg/g/year. *Year* shared a similar negative relationship with THg, with a rate of change of -0.455 (95 % CI = -0.869, -0.042) µg/g/year. Given that the confidence interval associated with the estimates for *at least age* and *year* did not cross 0, the confidence in the direction of these estimates is high. *Julian day* was not in any of the top models and it did not appear to be an important predictor of THg concentration.

Model 1, which included *at least age* as the fixed effect, had the strongest support with a model probability of 45.1 % and explained 26.0 % of the variance (Figure 1.3.). This model also had a strong correlation (r = -0.58) between the intercept and the slope within the random effects (Figure 1.4.). This correlation indicates that individuals who were first caught with a high THg concentration in their fur were more likely to have a decreasing THg concentration with age relative to individuals who were first captured with a low THg concentration. The full list of fur THg concentrations used to identify trends over the study period and to identify age dependent patterns is provided in Appendix A: Table 1.

Table 1.1. Yearly mean (±SD), minimum, and maximum of total Hg concentrations in fur taken from 60 adult females captured between July 29 – August 7 from 2012 to 2017 in Salmonier Nature Park, NL, Canada. Each year had an equal sample size of 10. These samples were taken from individuals who had an 'at least' age of 1. THg concentrations are reported in dry weight (dw).

		THg (µg/g; dw)	
Year	**Sample period**	**Mean (±SD)**	**Min, Max**
2012	August 5	13.485 (2.784)	9.931, 18.780
2013	July 29	15.024 (8.585)	2.692, 30.888
2014	July 31 – August 2	10.582 (2.807)	7.371, 15.418
2015	August 6 – August 7	11.291 (4.785)	6.856, 20.759
2016	July 29 – August 3	8.980 (3.392)	3.232, 14.898
2017	July 31 – August 2	9.104 (3.705)	4.490, 14.594

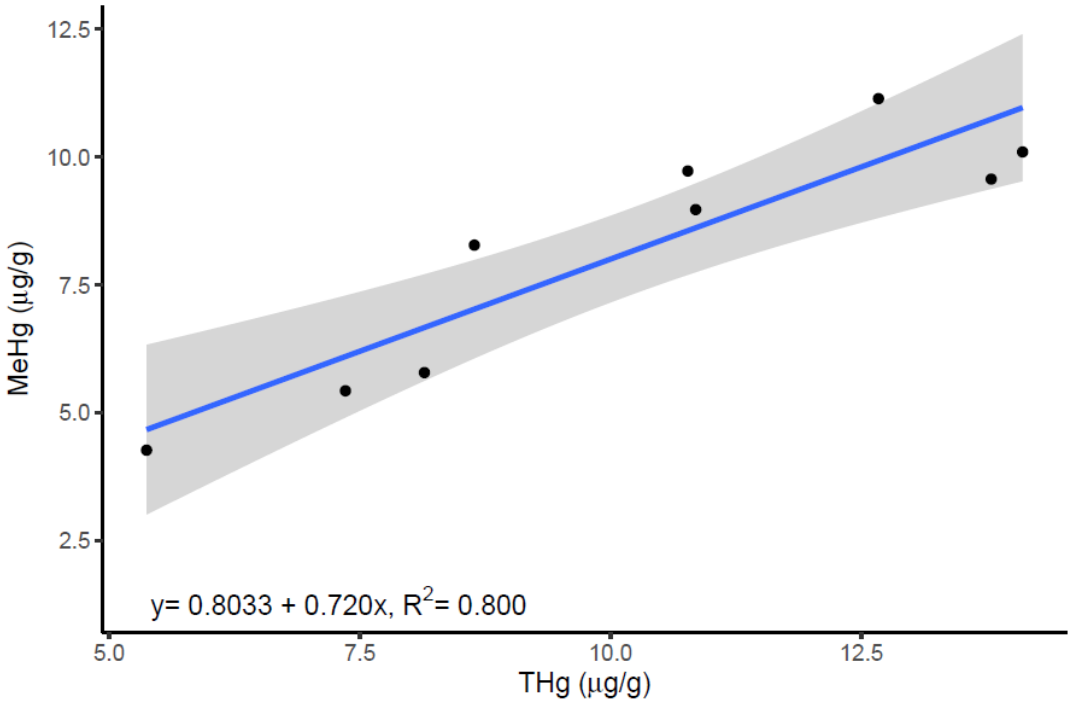

Figure 1.1. Linear regression between the concentration of MeHg (µg/g; dw) and concentration of THg (µg/g; dw) in the fur of adult female bats captured from 2012-2017 in Salmonier Nature Park, NL, Canada (n = 9). There is a strong correlation between the variables as indicated by the blue line ($P < 0.001$) and its 95 % confidence interval (grey area).

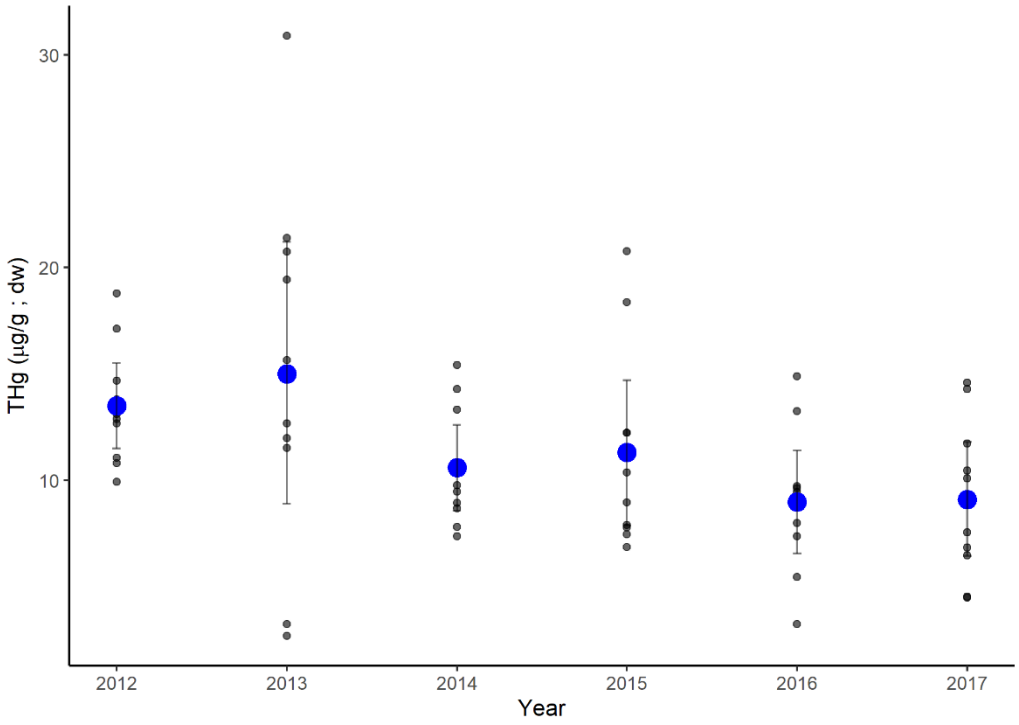

Figure 1.2. Total Hg (µg/g; dw) in fur from 60 adult females (grey dots) captured between July 29 – August 7 from 2012 to 2017 in Salmonier Nature Park, NL, Canada. These samples were taken from first time captures, so the 'at least' age of all individuals is 1. The mean of each year is given (blue circles) along with the corresponding 95% confidence interval (bars). The Mann-Kendall trend analysis indicated no evidence of a trend in THg concentrations over the study period (τ = -0.6, P = 0.133).

Table 1.2. List of *a priori* models used to explain THg concentrations of adult female bats in Salmonier Nature Park, NL, Canada, and ranked by AIC_c. There are seven random intercept and slope models with individual as a random effect, and one null random effects model with individual as the random effect. The number of parameters (k), difference between the top-ranked model and the i^{th} model (Δi), Akaike weight (w_i), cumulative Akaike weight ($\sum w_i$), and R^2 are reported for all models. Models above the line (model 1-3) make up ≥ 95% confidence set. All models used individual as a random effect.

Model rank	**Fixed Effect(s)**	**Random effect**	**k**	**Δi**	**w_i**	**$\sum w_i$**	**R^2**
1	at least age	Individual	6	0	0.451	0.451	0.260
2	year	Individual	6	0.371	0.374	0.825	0.258
3	at least age + year	Individual	7	2.163	0.153	0.977	0.261
4	null	Individual	7	7.823	0.009	0.987	0.137
5	at least age + Julian day	Individual	7	9.032	0.005	0.991	0.262
6	year + Julian day	Individual	6	9.545	0.004	0.995	0.259
7	Julian day	Individual	8	9.990	0.003	0.998	0.229
8	at least age + year + Julian day	Individual	3	11.294	0.002	1.000	0.264

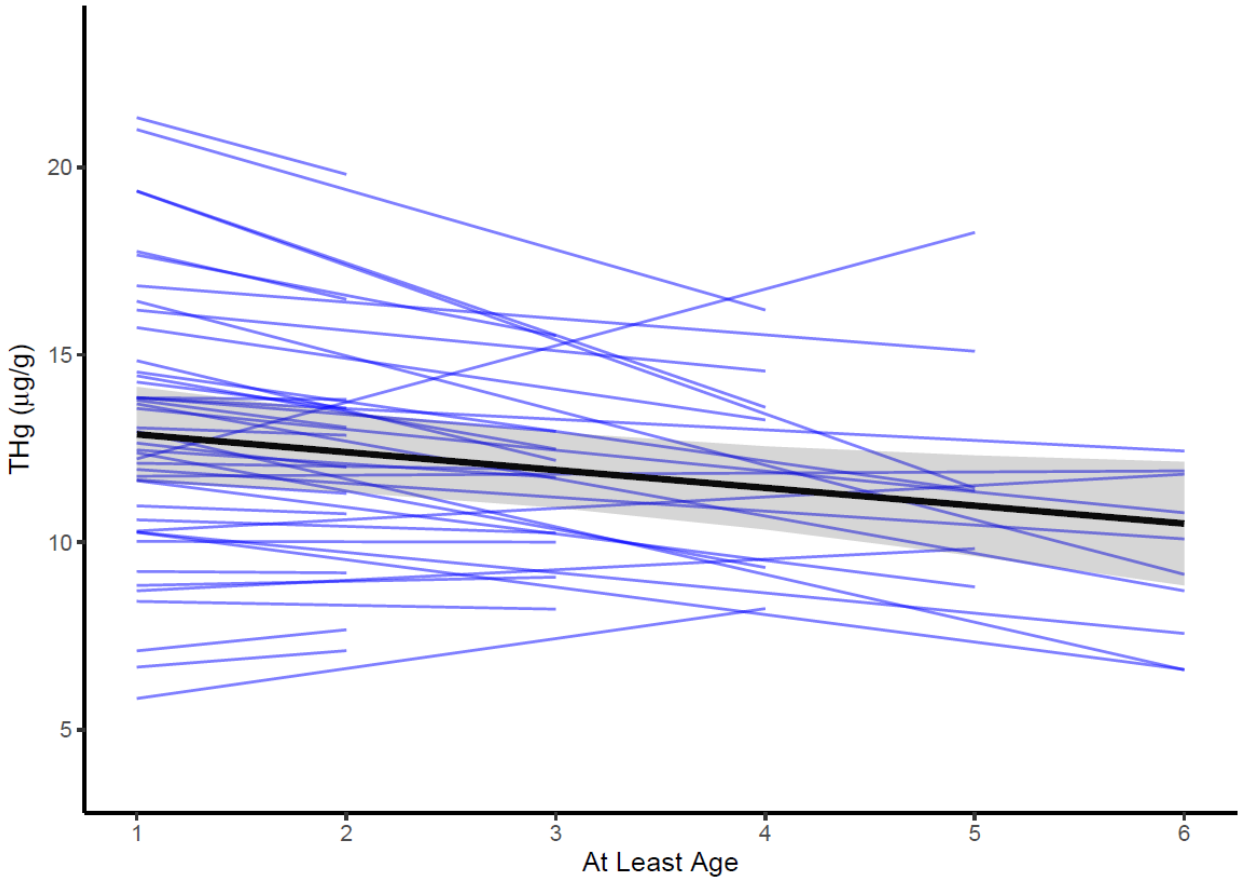

Figure 1.3. Linear mixed-effects model which best explains THg concentrations in the fur of adult female bats captured in Salmonier Nature Park, NL, Canada, including *at least age* as a fixed effect and individual as a random effect. Blue lines give the estimated change in THg over age for individuals. Individuals were captured two or three times throughout the study and the rates of change and intercepts are estimates given by the model. The black line gives the full model estimate for the change in THg over age with the 95 % confidence interval (grey area).

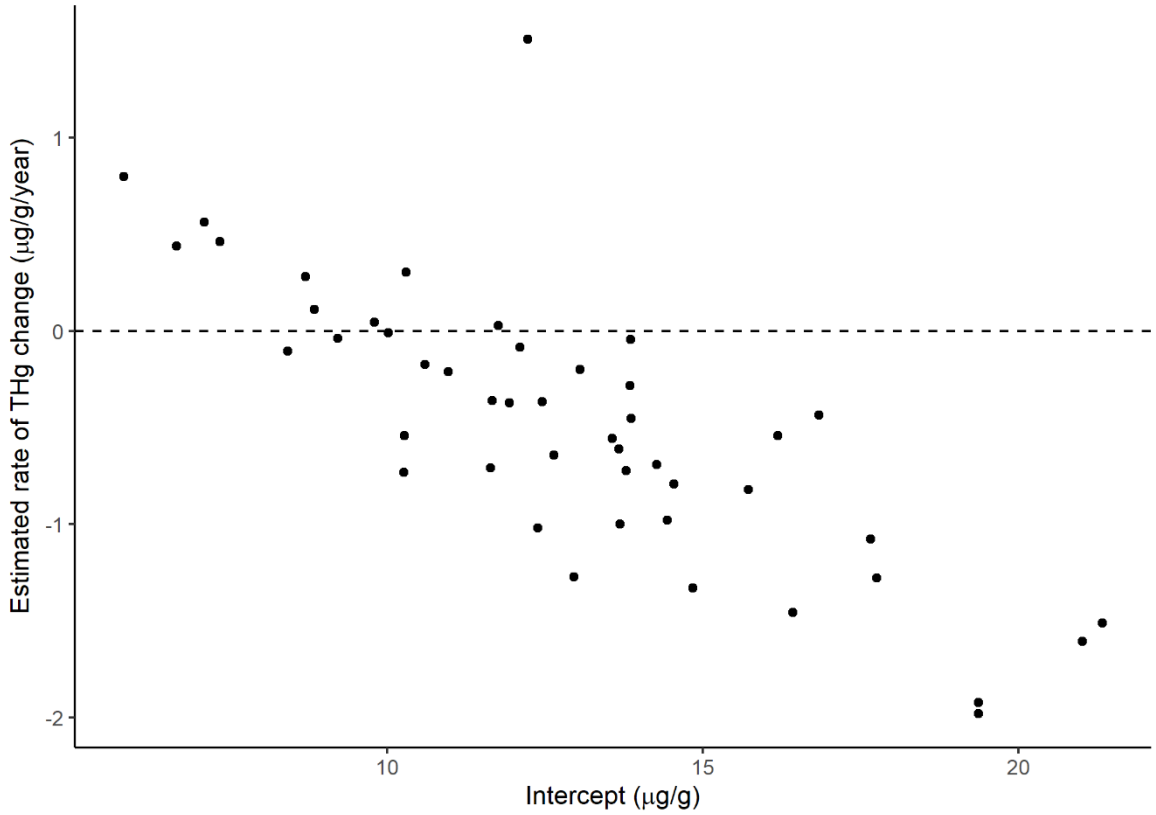

Figure 1.4. The estimated rate of change in THg concentration with *at least age* in relation to the estimated intercept for each individual. These estimates were obtained from the linear mixed-effects model which had *at least age* as a fixed effect and individual as a random effect. Points falling above the dashed line had an increasing rate of change and points falling below the dashed line had a decreasing rate of change. There was a strong negative correlation between the intercept and rate of change (r = -0.58).

2.5 Discussion

In the majority of individuals used in this study, THg concentrations in fur decreased with age. To my knowledge, this is the first study on terrestrial mammals assessing changing THg concentrations with adult age using samples taken at multiple points during an

individual's life. It is thus unknown if declining THg concentrations with adult age is unique to bats or is a more general phenomenon among long-lived terrestrial mammals. In any case, this decline with age is supportive of the contention that adult female bats possess the mechanisms to eliminate enough MeHg to prevent a rise in concentrations with age. Since THg concentrations in adult bats have been shown to be higher than in sympatric conspecific juveniles (Heiker, Adams, & Ramos, 2018; Yates et al., 2014), MeHg concentrations appear to increase sharply after parturition up until early adulthood, followed by a slow decline through adulthood.

The estimates for the parameters that appeared in the three top models, *at least age* and *year*, indicate that fur THg concentrations declined with age in the study population. The top model, which included *at least age* as a fixed effect and individual as a random effect showed an overall decline in THg concentrations with age, with 37 individuals estimated to have a negative rate of change and 10 individuals estimated to have a positive rate of change. In the top model, there was also a strong correlation between the estimated rate of change in THg concentration and the estimated intercept. This correlation means that individuals that were initially caught with a lower THg concentration were estimated to have a more positive change in THg concentrations with age than individuals initially caught with a higher THg concentration. This pattern implies that THg concentrations in the fur of individuals is converging as individuals age. It is possible that the individuals initially caught with lower THg concentrations were young adults whose THg concentrations had yet to peak.

Reproductive processes may provide a significant MeHg elimination route such that the input of MeHg into non-breeding juveniles, who are not using these pathways, exceeds

the outputs. If true, this process explains why THg concentrations rise in tissues following parturition up until early adulthood and then reach steady or declining levels. The use of reproductive mechanisms as a MeHg elimination route in bats has also been found by previous studies on bats and other mammals. In Schreiber's bent-winged bats (*Miniopterus schreibersii*), high concentrations of THg have been found in the fetal brain relative to the brain of the mother (Lisón et al., 2017). In polar bears (*Ursus maritimus*), the THg concentration in the tissues of offspring were found to be positively related with the THg concentrations of maternal tissues (Bechshoft, Derocher, Richardson, Lunn, & St Louis, 2016), and in many marine mammals placenta and milk have high THg concentrations (e.g., *Mirounga angustirostris*: Habran, Debier, Crocker, Houser, & Das, 2011; *Delphinus delphis*: Lahaye, Bustamante, Dabin, Churlaud, & Caurant, 2007).

In addition to reproductive processes, the initial increase in THg concentration as a juvenile followed by the declining concentrations as an adult, may be due to dietary shifts over the course of an individual's lifetime. There is some evidence that resource use between *M. lucifugus* juveniles and adults differ (Adams, 1996; Adams, 1997). If juveniles shift their diet to insects containing less MeHg as they age, their overall exposure would decrease, and MeHg concentrations in their tissues could stagnate or decline.

There was no trend detected in THg concentrations over the study period with the Mann-Kendall trend analysis. The estimate for the parameter *year* was negative however, and there may have been a decline in MeHg exposure over the study period. Although, anthropogenic inputs of Hg into the atmosphere have declined in North America resulting in declines in atmospheric Hg concentrations (Zhang et al., 2016), the relatively long residence

time of Hg in soil and the biota (decades) compared to atmosphere (years) causes a substantial time lag for declines in atmospheric Hg concentrations to be reflected in wildlife (Wang et al., 2019). Given that *year* and *at least age* were colinear, I expect the magnitude of the estimate of *year* is due in part to this collinearity.

The parameter *Julian day* was not included in any of the top models. Decreasing THg concentrations in the tissues of animals over time should mean the concentration in the fur is higher before an individual moults than after the moult. There are two likely reasons why this change was not detected. First, the seasonal timing and inter-individual differences in the moult timing are not well defined, so it is possible moulting occurs before or after the sampling season. Alternatively, there may be low synchrony in the timing of moulting among individuals in the population. Either case would prevent detection of intra-annual trends in fur THg concentrations. Second, the average change in THg concentration before and after the moult may be small enough such that any patterns were masked by the measurement error associated with THg analysis.

The results from this study have important implications regarding wildlife exposed to toxic levels of MeHg. Foremost, terrestrial mammalian females in early adulthood who are exposed to sublethal amounts of MeHg are likely at the greatest risk of experiencing the adverse effects of MeHg, such as decreased reproductive capacity. Further, offspring produced immediately following the onset of sexual maturity of the mother may receive higher amounts of MeHg than offspring produced by mothers in later life stages. Young adults already face the challenge of inexperience when reproducing (Culina et al., 2019; Postma, Bester, & de Bruyn, 2013) and the added impacts of high MeHg concentrations on

the probability of offspring success (Ceccatelli, Bose, Edoff, Onishchenko, & Spulber, 2013) may further inhibit reproductive output.

This study did not include any males and the differences of MeHg accumulation between sexes demand research attention. Similar to shrews (*Crocidura russula*: Sánchez-Chardi, López-Fuster, & Nadal, 2007), patterns of MeHg accumulation with age may be different in male bats who do not possess the same reproductive elimination routes as females. Determining the pattern of MeHg accumulation with age in males would offer some insight into the mechanisms causing the decrease in fur THg concentrations with age observed in the adult females of this study. For example, if male bats display increasing THg concentrations with age, it would provide evidence that reproductive pathways are a primary MeHg elimination route for females, and vice versa.

The mean THg concentration in the fur analysed was greater than the concentration which has been proposed as the threshold for which individuals are expected to experience sub-lethal effects (10 µg/g; Nam et al., 2012). Moreover, eight individuals were observed to have at least double the threshold, and one of those individuals had a THg concentration triple the threshold. Many bat species in North America are already of serious conservation concern as a result from the fungus *Psudeogymnoascus destructans*, which causes white-nose syndrome (WNS) (Frick et al., 2010). The disease caused mass mortality events in affected populations and the fungus was recently discovered on the island of Newfoundland (Canadian Wildlife Health Cooperative, 2020). One of the sub-lethal effects of MeHg is immuno-suppression (Becker, Chumchal, Bentz, et al., 2017) and interactions between WNS and high levels of MeHg may exacerbate the effects of WNS. In addition, if the bat

population's reproductive capacity is compromised by high levels of MeHg, the ability for the population to recover from a WNS induced mortality event may be inhibited. Attempts to characterise the interactive effects of WNS and MeHg should be taken to understand the differential regional impacts WNS would have as the fungus continues to spread through North America.

2.5.1 Conclusion

In this study, I observed decreasing concentrations of THg in the fur of adult female bats. Although this trend may be partially driven by changing levels of MeHg in the environment, it appears females have the capacity to eliminate MeHg at a rate that keeps tissue concentrations at a steady or decreasing level. Since THg concentrations in bats and other mammals have previously been shown to be lower in juveniles than adults (Dibbern et al., 2021; Heiker et al., 2018), wildlife managers should be aware that adult females in the early stages of life may face additional survival and reproductive challenges presented by high concentrations of MeHg in tissues. The results of this study could be clarified if long-term sampling continues in SNP and by identifying any dietary changes that occur within an individual's life that could affect their level of exposure to MeHg. Finally, identifying age dependent changes in MeHg concentration in other K-selected groups of terrestrial mammals who derive energy from aquatic systems could determine if the decreasing trend I observed is unique to bats, or a more general phenomenon among terrestrial mammals.

Chapter 3

An analysis of morphometric measurement error of wild bats

3.1 Overview

Morphometric data is critical to the advancement of many disciplines of biology. Accurate and reliable inferences based on morphometric data are dependent on the measurement error (ME) and observer bias (OB). Therefore, quantifying ME and OB is necessary for researchers to understand the limitations of a given morphometric. In this study, I quantify the ME and OB associated with forearm measurements in small insectivorous bats using field data collected by multiple research teams over a 7-year period. I separated the error into three classes (intra-observer, intra-team, inter-team) and calculated the percent ME and mean absolute deviation for each class. For intra-observer, intra-team, and inter-team ME, there was a 1.17 %, 12.35 %, and 10.54 % error, respectively. To quantify OB, I calculated the difference between repeated forearm length measurements from the same bat made by different teams of research personnel. I performed 10 paired *t*-tests comparing forearm length measurements of the same individuals made by different teams and found that 4 of the 10 comparisons were significantly different. These results suggest that when effect sizes are small ME could add enough variation to mask relationships of interest between variables and that OB can cause regular type I errors when comparing populations measured by different observers. I recommend researchers using morphometrics carefully consider the ME and OB associated with the morphometric variable being used when designing studies to increase confidence in inferences.

3.2 Introduction

The collection of morphometric data from wild specimens is common and essential for zoologists and ecologists. Due to high natural variance and unidentified confounding variables, conclusions of studies using morphometrics are often drawn based on weak relationships among variables (Toft & Shea, 1983). Reducing and quantifying the variation created by artifacts, such as measurement error (ME) and observer bias (OB), is important to detect relationships.

Morphometric ME is the variation associated with multiple measurements of an unchanging character taken from the same individual (Baily & Burnes, 1990). Error in morphometric measurements is related to many factors such as precision of the measuring device, observer experience, measurement conditions, and consistency in measurement technique. As ME increases, precision decreases, thereby inflating actual variation. The additional variation can mask relationships between variables and lead to a type II error by incorrectly failing to reject the null hypothesis (Toft & Shea, 1983).

Observer bias occurs when differences in measurement technique between observers cause the ME to skew in a consistent direction. Two potential errors can occur when biased observers take measurements from multiple populations, including the false conclusion that the populations are different (type I) or the same (type II) with respect to the measured variable (Simpson, Roe, & Lewontin, 1960). The importance of the ME and OB is relative to the variation found among individuals in the population. Therefore, ME is frequently represented as a percent (%ME) which is calculated using the variance of measurements within individuals relative to the variance among individuals (Bailey & Byrnes, 1990).

It is common for ME and OB to be quantified for wildlife morphometrics (Muñoz-Muñoz & Perpiñán, 2009; Waite & Mellish, 2009; Watters et al., 2016; Palmeirim, 1998). Since the effectiveness and reliability of statistical tests depend on the precision and accuracy of measurements, estimating ME and OB is crucial to study design. If the morphometric ME cannot be lowered, then the anticipated sample size should be increased accordingly, such that there is confidence in assessing relationships.

The forearm is among the most commonly measured morphometric in bat research. Forearm length (FAL) is often used for species identification (Rodhouse, Scott, Ormsbee, & Zinck, 2009), post-natal age estimation (Wilkinson & Brunet-Rossinni, 2009), and as a proxy for differences in body size within (Yue et al., 2019) and among species (Thiagavel, Santana, & Ratcliffe, 2017). The forearm is the preferred morphometric variable to use because measurement can be done quickly in the field and the landmarks for measurement are well-defined. Nevertheless, the efficacy of using FAL as a proxy for intraspecific body size has recently come under scrutiny. McGuire et al. (2018) found that using body mass as the sole predictor of body fat appeared to be just as effective as using a body condition index (ratio of body mass to FAL). The inability of FAL in combination with body mass to predict body fat appears to occur due to a lack of intraspecific covariation between FAL and body size (McGuire et al., 2018). Conversely, other studies indicate that there is intraspecific covariation of FAL to other body size metrics in bats (tibia and digits: Storz et al., 2001; cranium: Bornholdt, Oliveira, & Fabian, 2008). Seemingly contradictory results highlight the need to quantify ME, since it may add sufficient variation to mask relationships between variables.

Palmeirim (1998) assessed the intra- and inter-observer error in cranial measurements of bats performed with calipers. It was concluded that using cranial measurements from multiple observers for a single analysis could lead to type I or type II errors. Data collection in this study, which used skeletal specimens, was performed under better conditions than is common for field work. Conditions in the field should be conducive to larger error because the animal is alive, there is tissue over the bone, and researchers are often under time constraints to process the animal. It is therefore likely that the intra- and inter-observer error of FAL measurements made in the field is higher than that for cranial measurements found by Palmeirim (1998). Further, the precision of FAL measurements is certainly above the vernier caliper resolution of 0.01 mm that is commonly reported as the precision.

The goal of this study was to quantitatively characterise the ME and OB from FAL measurements made in the field. I analysed FAL measurements of *Myotis lucifugus* (little brown myotis) and *Myotis septentrionalis* (northern myotis) with two objectives.

1) Quantify the intra- and inter-observer measurement error.
2) Determine if the inter-observer bias is large enough to cause type I errors.

From these analyses, I provide recommendations for researchers using morphometrics to consider for a more robust study design.

3.3 Methods

3.3.1 Data Collection

Myotis lucifugus and *M. septentrionalis* were captured in Salmonier Nature Park (Lat: 47.3º, Long: -53.3º) and Pynn's Brook (Lat: 49.1º, Long: -57.5º), Newfoundland and Labrador, during May to August in 2011 – 2017 using mist-nets (Avinet, Dryden, New York, USA) and harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia). Age class (adult or juvenile) was determined based on the degree of ossification of the third metacarpal-phalangeal joint (Kunz & Anthony, 1982). Forearm length was measured in triplicate using vernier calipers and sex was determined. Captured bats had a passive integrated transponder (PIT) tag (0.09 g; EID-ID100 implantable transponders, EIDAPInc, Sherwood Park, Alberta, Canada and Trovan Electronic Identification Systems, UK) subdermally injected between the scapulae. Recaptured bats already carrying a PIT tag were identified using a handheld PIT tag reader. Individuals first captured as adults were assigned an 'at least' age of 1. In subsequent captures, individuals were assigned an 'at least' age corresponding to the number of years since last capture. Following processing all animals were released at the site of capture.

All animal handling and sample transport was done in accordance with the protocol approved by St. Mary's University Animal Care Committee and the Government of Newfoundland (SMU-MSVU protocol 11-18, 12-17, 13-15, 14-10A, 15-12, 16-12; Government of Newfoundland protocol IW2011-14, IW2012- 26, IW2013-10, IW2014-31, IW2015-52, IW-2015-11, WLR2016-12, WLR2017-16, WLR2017-17).

3.3.2 Statistical Analysis

Sex and species were assumed to have no impact on ME so all male and female *M. septentrionalis* and *M. lucifugus* data were pooled. Previous literature only verifies the assumption that bone length remains static during adulthood in mammals other than bats (for review see Kilborn, Trudel, & Uhthoff, 2002). I therefore tested this assumption to ensure any variation seen between repeated measurements of the same individual was due to ME, and not bone growth. To test this assumption, I regressed difference in FAL with time between captures.

To address objective 1 and quantify intra-observer and inter-observer error, I separated ME into three classes:

a) Intra-observer error - the variation in the triplicate measurements of FAL made by the same observer during each capture.
b) Intra-team error - the variation within individual bats captured at different times by the same research team.
c) Inter-team error - the variation within individual bats captured at different times by different research teams.

This classification scheme was used because, although the personnel present on the night of capture was recorded, the specific observer who measured FAL was not recorded for each capture. I was thus unable to assign observers to each measurement, but instead assigned measurements to a research team.

For each class of ME, I followed the procedure outlined by Bailey & Byrnes (1990) and partitioned the total variance into within individual variance (s^2_{wi}) and among individual

variance (s^2_a) using a model II ANOVA. I estimated s^2_{wi} using the mean squared deviation within individuals (MS_{wi}) and estimated s^2_a using the formula (Bailey & Byrnes, 1990)

$$s^2_{wi} = \frac{MS_a - MS_{wi}}{m} \quad (1)$$

where MS_a is the mean squared deviation among individuals and m is the mean number of repeated measurements per individual. I then calculated the %ME using the formula (Bailey & Byrnes, 1990)

$$\%ME = 100\% \frac{s^2_{wi}}{s^2_{wi} + s^2_a} \quad (2)$$

The ME for each class was also represented using the mean absolute deviation from the within individual mean.

To quantify how OB can influence FAL measurements, I calculated the difference between repeated FAL measurements from the same individual made by different research teams. In cases where an individual was caught multiple times by the same team, I used the mean FAL of measurements made by that team. There were 6 teams that collected FAL measurements over the study period. I ran 10 paired *t*-tests comparing FAL measurements and used a Bonferroni correction to adjust for multiple comparisons. Sample size varies for each comparison because only individuals who were caught by both teams being compared were included.

All data analysis was done in R version 4.0.3 (R Core Team, 2020). Data was tested for normality with a Shapiro-Wilk test, quantile-quantile plots, and random dispersion in the residuals.

3.4 Results

In total, 1872 forearm measurements were collected from 289 unique adult individuals captured 2-6 times over the study period. Of all captured individuals, 230 were captured by two or more teams, and 81 were captured two or more times by the same team. Among individuals, bats had forearms ranging from 35.61 mm to 41.74 mm in length. I did not find a significant trend between the difference in FAL measurements and time between captures, supporting the assumption that the forearms of individuals are not changing size as they age (Figure 2.1. $F = 2.178$; d.f. = 287; $P = 0.141$; $R^2 = 0.004$).

The intra-capture, intra-team, and inter-team ME accounted for 1.17 %, 12.35 %, and 10.54 % of the total population variance, respectively (Table 2.1.). The mean absolute deviation was a mean of 0.06 mm for intra-observer error, 0.18 mm for intra-team error, and 0.19 mm for inter-team error (Figure 2.2.).

Table 2.1. Error classes and associated percent measurement error (%ME). Mean squared error (*MS*), both for within and among individuals, were found using a model II ANOVA. The mean number of repeated measurements per individual (*m)* was used to calculate %ME.

Error type	***MS***		***m***	**%ME**
	Within	Among		
Intra-observer	0.01	3.06	3	1.17
Intra-team	0.14	2.14	2.08	12.35
Inter-team	0.10	1.95	2.10	10.54

I ran 10 paired *t*-tests to assess inter-team bias. Team 1 was only compared to team 3 because individuals captured by team 1 were not recaptured by any team other than team 3. Also, I did not compare team 4 to team 5 because only two individuals were caught by both teams. Significant differences were found in 4 of the 10 comparisons ($P < 0.05$; Figure 2.3.). The largest difference between groups occurred between teams 5 and 6, with measurements by team 6 being, on average, 0.41 mm larger than team 5. The mean difference of all significant comparisons was larger than the level of uncertainty of ± 0.084 mm given by the law of propagation of error and calculated using the root-mean-square of the intra-observer measurement error (Birge, 1939). A full list of comparisons and the associated P values are reported in Appendix B.

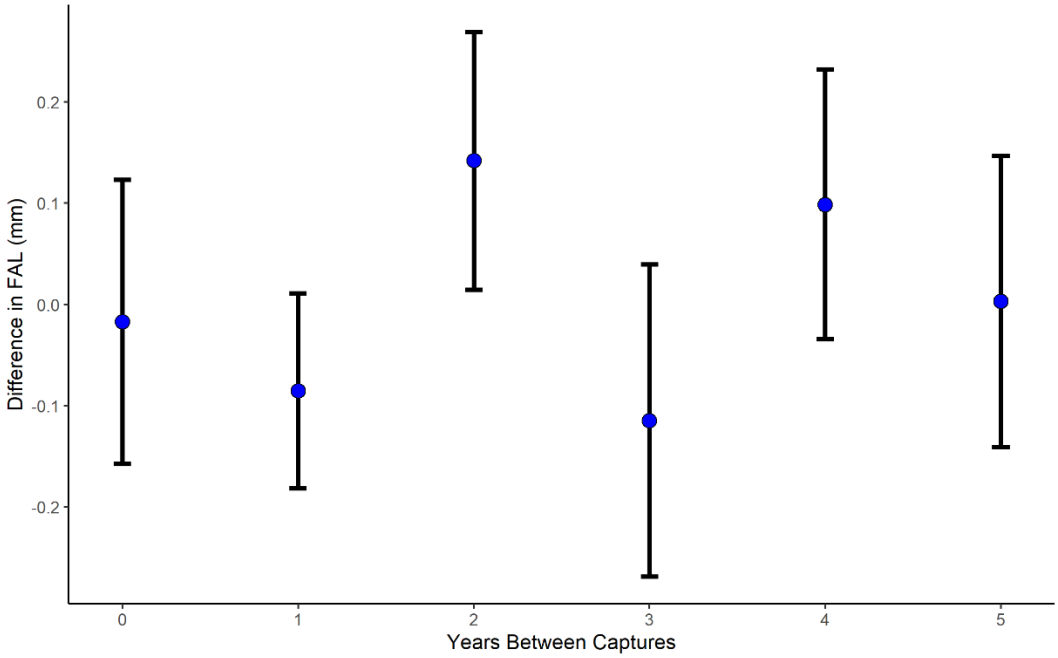

Figure 2.1. Years between captures of individuals compared to difference in forearm length (FAL). The mean of each time interval is given (blue circles) and the corresponding 95 % confidence interval (bars). There is no evidence of a relationship between the variables ($F = 2.178$; d.f. = 287; $P = 0.141$; $R^2 = 0.004$), supporting the assumption that FAL does not change with increasing age in adult bats.

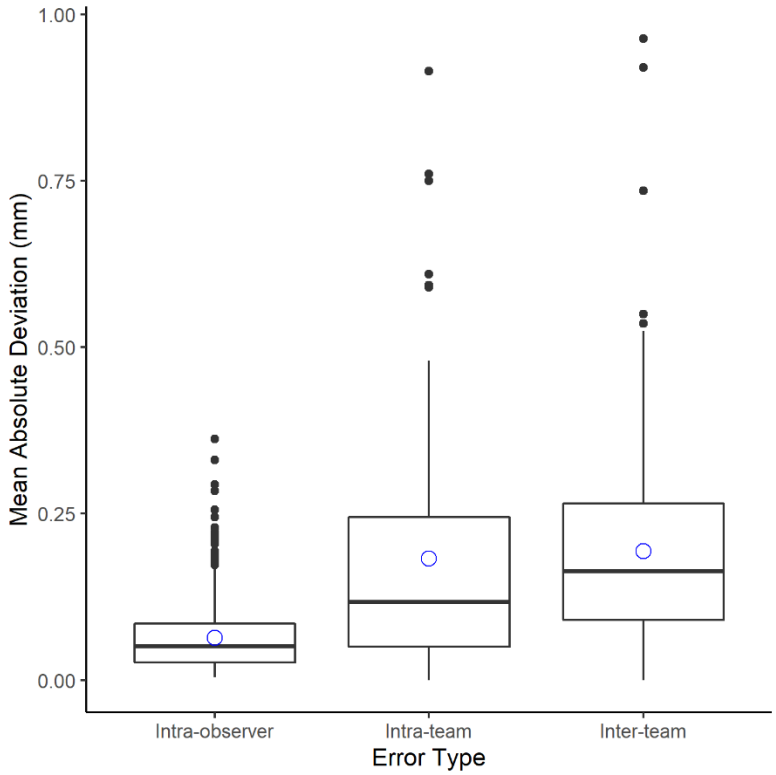

Figure 2.2. Mean absolute deviation in forearm length for the three classes of error. For intra-observer error, each point represents the mean absolute deviation from the mean of the triplicate measurements made within the capture. For intra- and inter-team error, each point represents the mean absolute deviation from the within individual mean. Boxes show mean (blue circles), median (line), 25th and 75th percentiles (end of boxes), 5th and 95th percentiles (whiskers), and outliers (dots).

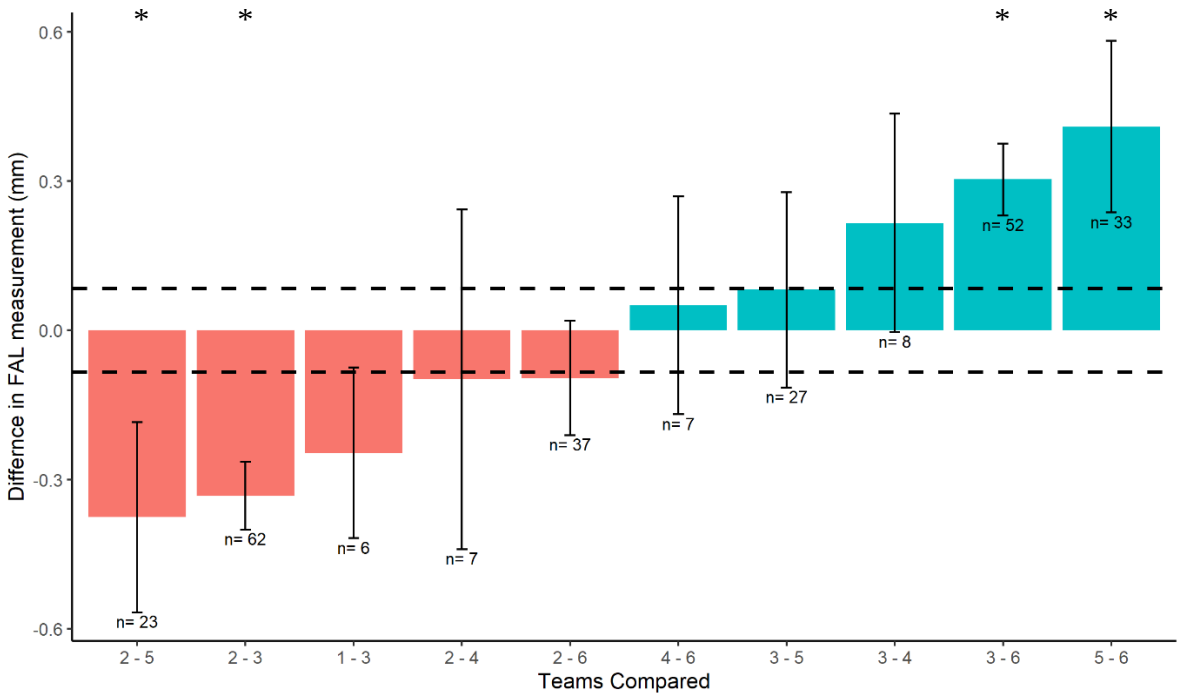

Figure 2.3. Bar plot comparing measurement error in forearm length between pairs of teams. Bars show mean measurement error of teams being compared. (e.g., Forearms measured made by team 5 were a mean of 0.37 mm smaller than the same forearms measured by team 2). Error bars indicate the 95 % confidence interval around the means. Numbers below the error bar indicate the respective sample size in each comparison. P values were adjusted for multiple comparisons using the Bonferroni correction. The area between the dashed lines represents the area of uncertainty due to the intra-observer measurement error determined for objective 1 (± 0.084 mm).

* $P < 0.05$

3.5 Discussion

The ME associated with FAL is much higher than the vernier caliper resolution of 0.01 mm that is often reported as the precision when measuring FAL (Thiagavel et al., 2017;

Verde Arregoitia et al., 2018). This imprecision could add enough variation to data with small effect sizes such that the relationship between a morphometric measured with calipers and a covariate are masked.

The assumption that FAL does not change as an adult was supported since the FAL of individuals did not change predictably with time between captures; therefore, any difference between repeated measurements of the same individual is due to ME. This result aligns with the bone growth patterns of other mammals, where bone growth halts near the time sexual maturity is reached (Kilborn, Trudel, & Uhthoff, 2002). Although small changes in FAL over time may have been undetected due to ME, it is unlikely these changes will have impacted the results in any meaningful way. The inability to make inferences on small bone length changes over time highlights the importance of knowing ME, since false conclusions might be made when the ME is unknown.

The %ME observed within (12.35 %) and between (10.54 %) research teams were larger than the intra-observer %ME (1.17 %). Notably, there was very little difference in the intra-team %ME compared to the inter-team %ME. This lack of difference may have occurred for two reasons. First, the inter-observer ME between repeated captures is just as large as the intra-observer ME between captures. Second, multiple FAL measurements of the same bat were made by different observers as often within a team as between teams. In this case, both intra-team and inter-team ME would have been subject to the same amount of inter-observer ME. Given the consistent group of researchers who were unique to each team, the former is more likely. A more complete understanding of the result necessitates further inquiry with a more rigorous study design.

I observed significant differences in the FAL of the same individuals measured by different teams. These differences indicate that FAL measurements taken by different research teams are subject to bias. Even though the real difference in FAL between each of these comparisons is assumed to be 0.00 mm, 4 of 10 comparisons showed a significant difference. If it is assumed other observers have the same level of bias as in this study, there is a 40 % chance that a type I error will be made when comparing small differences in FAL between populations measured by different research teams. The maximum significant difference between teams of 0.41 mm accounts for 6.7 % of the total 6.13 mm range of the study population. The observed probability of bias causing type I errors should incite increased skepticism when reviewing literature that finds significant differences in FAL, or other similarly sized morphometrics, between populations of the same species (Solick & Barclay, 2006; Wu et al., 2014; Yue et al., 2019). If significant FAL differences between populations are observed to be smaller than the maximum mean bias of 0.41 mm observed, researchers should strongly consider if this difference in FAL could be due to bias. Conversely, in other situations such as species ID or differences in body size between species, a bias of 0.41 mm will likely not influence conclusions.

Given the results, I recommend that researchers measuring morphometrics take precautions to limit inter- and intra-observer ME. The most effective method to limit bias is to ensure only one observer measures all individuals being used in the study. I realise that given time constraints, having only one observer may not always be feasible. If more than one person is required to measure a morphometric, it is recommended the observers calibrate their measurements at the start, and throughout any study.

Further potential to increase the accuracy and precision of morphometrics can be found in photogrammetric methods, where the size of a character is estimated using images. The accessibility of small high-quality cameras with the capacity to capture high quality photos, including at night, is increasing (Mungee & Athreya, 2020). These developments in camera technology give researchers the opportunity to use photogrammetry as a less biased method to measure FAL and other morphometrics. If calibrated and used systematically, photogrammetry allows for increased comparability of morphometric measurements within studies and across studies (Mungee & Athreya, 2020; Waite & Mellish, 2009). The precision of photogrammetric methods also allows studies assessing covariates of morphometrics to have more confidence that weak or absent relationships are based on reality, and not due to lack of precision.

Morphometrics are an important and necessary tool for ecologists, however, I have shown these metrics are susceptible to error and biases which could lead to false conclusions. When reviewing literature which has found small but significant differences between populations, it is important to view results under a skeptical lens. Further, when designing morphometric studies, researchers need to recognise the associated ME and bias relative to the predicted trends and effect sizes. Thinking critically about ME and bias can help assess the power of statistical inferences and prevent erroneous conclusions.

Chapter 4
Synthesis

Using archives from a long-term capture-mark-recapture (CMR) project, I examined age dependent trends of fur mercury concentrations in bats and the error associated with bat forearm length (FAL) measurements. In Chapter 2, I determined that the THg concentrations, and thus MeHg concentrations, in the fur of adult female bats were declining with age. Since MeHg bioaccumulates (Wolfe et al., 1998), this decline with age may seem counterintuitive. Nonetheless, the mechanisms that eliminate MeHg from tissues seemingly reduce the load of MeHg in an adult female bat's body over time. Although this is the first study to observe a decline throughout the life of long-lived terrestrial mammals, similar patterns exist in long-lived aquatic birds, with trends observed in the wandering albatross (*Diomedea exulans*) being the most comparable (Tavares, Xavier, Phillips, Pereira, & Pardal, 2013). Like the wandering albatross, previous studies (Heiker et al., 2018; Yates et al., 2014) and Chapter 2 of this thesis indicate a sharp increase in MeHg concentrations after parturition, a peak in concentrations in early adulthood, followed by a decline throughout adulthood. There is likely inter-individual variation in the timing of peak THg concentrations that are dependent on life history characteristics such as breeding status and dietary preferences.

Although many temperate hibernating bats reach sexual maturity by their first spring, individuals often skip breeding in their first year to allocate limited energy stores towards survival, rather than reproduction (Culina et al., 2019). Since maternal transfer of MeHg may be a major elimination route (Lisón et al., 2017), female individuals who skip breeding in early adulthood may continue to have rising MeHg levels in their tissues until their first pup

rearing. This peak in MeHg concentrations places the highest concern of sub-lethal effects on new breeders since their capacity to reproduce successfully may be compromised.

Another advantage of CMR data is not just to look at trends in individual health, but also variation in sampling methods across years. In Chapter 3, I defined the measurement error (ME) and observer bias (OB) of forearm length, a commonly used morphometric in bat research. The size of the ME and OB associated with FAL measurements may lower the confidence of inferences made from statistical analyses when the strength of a relationship between FAL and a covariate is low. In instances such as intraspecific differences in body condition where the effect size is small, any effects that exist may be masked by the error. Conversely, a large effect size mitigates the influence of ME and OB, lending confidence to the inference of results. Researchers must carefully consider their scientific question and estimated effect size to determine if the ME and OB could lead to statistical errors.

Long-term CMR projects provide invaluable opportunities to research and monitor wildlife while gaining information that may be critical to supporting the health of the study population. Even though questions may arise that are outside the scope of the original purpose of the study, archives can provide insight into emerging issues that were not relevant when the project was initiated. For example, it was unknown that the bat population in SNP was being exposed to sub-lethal amounts of MeHg until Little et al. (2015a), examined archived fur from that region. Following this study, I have assessed the risk of MeHg toxicity this population faces throughout their lives. In the face of extreme climate and ecosystem change, it is imperative to establish and continue long-term CMR projects that will inform future research, potentially revealing threats to wildlife that could not be captured with short-

term monitoring. The benefits of early threat detection, especially for endangered and at-risk species, is paramount to current and future conservation efforts in Canada and abroad.

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Appendix A

Table 1. Raw total mercury data for Chapter 2 demonstrating decreasing concentrations with age. Individuals captured and sampled multiple time are noted as recaptured = "y". Individuals with an at least age of 1 and a Julian day between 210-218 were used to determine THg trends over the study period.

Individual	At least age	Year	Julian day	THg (µg/g)	Recaptured
10111	1	2012	136	9.492	y
10111	6	2017	161	12.417	y
10133	1	2012	136	13.573	y
10133	4	2015	159	14.108	y
10133	6	2017	187	12.399	y
10145	1	2012	136	14.719	y
10145	2	2013	172	12.251	y
10153	1	2012	136	14.508	y
10153	2	2013	201	13.762	y
10160	1	2012	136	11.612	y
10160	5	2016	183	7.835	y
10208	1	2012	136	17.436	y
10208	5	2016	198	16.665	y
10209	1	2012	136	16.316	y
10209	4	2015	223	13.669	y
10238	1	2012	136	9.729	y
10238	6	2017	214	5.374	y
10264	1	2012	137	13.155	y
10264	4	2015	159	8.667	y
10264	4	2015	202	8.144	y
10297	1	2012	137	11.646	y
10297	3	2014	214	11.285	y
10297	6	2017	196	9.96	y
10315	1	2012	137	9.683	y
10315	6	2017	187	6.673	y
10320	1	2012	137	13.952	y
10320	5	2016	215	10.041	y
10320	6	2017	187	7.756	y

10322	1	2012	137	11.993	y
10322	2	2013	168	10.503	y
10748	1	2012	218	12.671	n
10754	1	2012	218	13.109	y
10756	1	2012	218	9.931	n
10758	1	2012	218	13.795	n
10764	1	2012	218	10.809	n
10768	1	2012	218	17.136	n
10771	1	2012	218	11.059	n
10774	1	2012	218	14.687	n
10779	1	2012	218	18.78	n
10788	1	2012	218	12.87	n
10805	1	2012	219	11.366	y
10805	6	2017	168	12.458	y
10853	1	2012	219	13.75	y
10853	6	2017	178	10.851	y
10873	1	2012	219	17.77	y
10873	3	2014	217	13.092	y
10873	6	2016	190	8.644	y
10914	1	2012	221	21.668	y
10914	4	2015	179	12.586	y
10940	1	2012	222	13.187	y
10940	6	2017	173	5.216	y
10974	1	2012	223	21.448	y
10974	5	2016	163	10.555	y
11873	1	2013	172	10.716	y
11873	5	2017	174	22.393	y
11883	1	2013	172	8.012	y
11883	3	2015	186	8.642	y
11899	1	2013	191	14.084	y
11899	2	2014	207	10.194	y
11939	1	2013	192	13.577	y
11939	3	2015	139	13.803	y
11965	1	2013	194	22.902	y
11965	4	2016	163	16.584	y
11965	4	2016	199	16.545	y
12057	1	2013	201	16.902	y
12057	3	2015	204	9.892	y
12063	1	2013	202	14.947	y
12063	3	2015	179	19.457	y

12063	4	2016	199	13.125	y
12086	1	2013	204	7.512	y
12086	5	2017	163	9.937	y
12088	1	2013	204	11.763	y
12088	2	2014	216	10.769	y
12088	3	2015	178	13.715	y
12120	1	2013	205	15.027	y
12120	5	2017	174	11.3	y
12145	1	2013	207	10.213	y
12145	3	2015	140	9.876	y
12201	1	2013	210	2.692	n
12202	1	2013	210	11.529	n
12213	1	2013	210	21.403	n
12214	5	2017	163	12.471	y
12214	5	2017	174	15.1	y
12216	1	2013	210	3.229	n
12218	1	2013	210	20.758	n
12219	1	2013	210	19.433	n
12224	1	2013	210	12.667	n
12229	1	2013	210	11.986	n
12233	1	2013	210	15.655	n
12244	1	2013	210	30.888	n
12573	1	2014	209	3.763	y
12573	4	2017	212	8.464	y
12602	1	2014	212	8.671	n
12603	1	2014	212	9.755	n
12605	1	2014	212	13.322	n
12606	1	2014	212	10.77	n
12610	1	2014	212	8.938	n
12615	1	2014	214	7.814	n
12616	3	2016	211	10.849	y
12616	4	2017	161	15.568	y
12621	1	2014	214	14.287	n
12623	1	2014	214	9.476	n
12624	1	2014	214	15.418	n
12628	1	2014	214	7.371	n
13168	1	2015	139	9.282	y
13168	3	2017	174	9.926	y
13189	1	2015	140	7.842	y
13189	1	2015	204	5.333	y

13232	1	2015	169	5.068	y
13232	2	2016	195	8.187	y
13274	1	2015	179	15.563	y
13274	3	2017	214	11.445	y
13281	1	2015	179	10.273	y
13281	1	2015	204	8.476	y
13291	1	2015	185	9.538	y
13291	2	2016	176	7.581	y
13300	1	2015	218	7.774	y
13300	1	2015	190	8.952	y
13300	3	2017	163	6.528	y
13317	1	2015	196	18.911	y
13317	2	2016	157	16.722	y
13318	1	2015	196	10.872	y
13318	2	2016	157	10.242	y
13364	1	2015	218	7.905	n
13365	1	2015	218	12.227	y
13365	3	2017	199	11.908	y
13368	1	2015	218	10.376	n
13382	1	2015	219	7.457	n
13383	1	2015	219	12.228	n
13386	1	2015	219	6.856	n
13387	1	2015	219	8.952	n
13389	1	2015	219	18.376	y
13389	3	2017	208	16.44	y
13391	1	2015	219	20.759	y
13391	2	2016	198	23.32	y
13786	1	2016	198	5.962	y
13786	2	2017	154	5.886	y
13806	1	2016	211	7.357	n
13807	1	2016	211	13.249	n
13808	1	2016	211	9.709	n
13814	1	2016	214	9.621	n
13815	1	2016	214	8.856	n
13816	1	2016	214	3.232	n
13823	1	2016	214	5.448	n
13841	1	2016	216	14.898	n
13844	1	2016	216	7.984	n
13853	1	2016	216	9.446	n
13871	1	2016	226	9.746	y

13871	2	2017	198	19.08	y
14022	1	2017	212	6.83	n
14023	1	2017	212	6.466	n
14025	1	2017	212	14.594	n
14026	1	2017	212	10.45	n
14030	1	2017	213	4.538	n
14031	1	2017	213	10.095	n
14032	1	2017	213	4.48	n
14033	1	2017	213	14.297	n
14050	1	2017	214	11.731	n
14052	1	2017	214	7.554	n

Table 2. Comparison of random effects structure for the linear mixed-effect models explaining THg concentrations in bat fur. All models used *at least age*, *year*, and *Julian day* as fixed effects. The random intercept model, and the random intercept and slope model used individual as the random effect. The number of parameters (k), AIC values, difference between the top-ranked model and the i^{th} model (Δi), and log-likelihood (LL) are reported for each model. The optimal random effects structure, random slope and intercept, was selected based on the model with the lowest AIC value.

Random effect structure	**k**	**AIC**	**Δi**	**LL**
No random effect	5	591.61	16.27	-290.81
Random intercept	6	575.34	3.02	-281.67
Random intercept and slope	8	572.32	0	-278.16

Appendix B

Table 1. Significance testing for paired *t*-tests comparing forearm length (FAL) measurements of the same individuals made by different teams. The mean difference in FAL is a measure of the direction and magnitude of bias. For example, the forearm length of individuals measured by team 3 were a mean of 0.247 mm smaller than the same forearms measured by team 1. The 95% confidence interval (± CI) around the mean is also reported. The reported P values were adjusted for multiple comparisons using the Bonferroni correction.

Teams compared	Sample size	Mean difference in FAL	± CI	P value
1-3	6	-0.247	0.172	0.141
2-3	62	-0.333	0.068	< 0.001
2-4	7	-0.099	0.342	1
2-5	23	-0.376	0.191	0.005
2-6	37	-0.096	0.116	1
3-4	8	0.216	0.220	0.529
3-5	27	0.082	0.196	1
3-6	52	0.304	0.072	< 0.001
4-6	7	0.050	0.219	1
5-6	33	0.409	0.173	< 0.001