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Consequences of Environmental Mercury Exposure for Migratory Fitness and Survival of Passerine Birds

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Abstract

Mercury (Hg) is a global contaminant that persists in the environment. The organic form, methylmercury (MeHg) has been shown to adversely affect bird immune function, foraging behavior, navigation, and flight ability. Many songbirds migrate seasonally, a process that consists of multiple endurance flights and refueling at stopovers. Current knowledge of the effects of MeHg on songbird migration and survival is mostly speculative. In this thesis, I present three studies of MeHg in migratory songbirds. In Chapter 2, I assessed the breeding ground MeHg exposure (inferred from feather Hg) of 15 songbird species captured during fall migration at bird banding stations across Canada, and found exclusive insectivores had the highest feather Hg relative to partial insectivores and non-insectivores. A strong geographical trend showed that birds captured from Eastern Canada had the highest Hg exposure; nearly 2 times and 2.5 times greater than Central and Western Canada, respectively. Analysis of feather hydrogen stable isotopes suggested that birds from the northwest of Canada may experience lower Hg exposure. In Chapter 3, a captive dosing study to investigate the refueling/flight scenario with a small migratory insectivore, the Yellow-rumped Warbler (*Setophaga coronata*) showed that migratory birds that refuel at a highly contaminated stopover site can rapidly bioaccumulate MeHg in blood, muscles and organs within 2-weeks, and MeHg-treated birds had a reduced flight ability in a wind tunnel test. In Chapter 4, in five migratory passerine species, I compared Hg concentrations in tail feathers that were grown at or near breeding grounds prior to autumn migration and retained until the following spring. I predicted a shift in the distribution of species-specific feather Hg values towards lower means in the spring if Hg reduced survival over the migration and

winter periods. The results suggest that MeHg exposure in the breeding areas could have a carry-over effect to reduce migration success and survival of insectivorous songbirds that undergo extensive and demanding migratory journeys. Together, these studies advance our knowledge of the impact of mercury on songbird migration and survival.

Keywords

migration, survival, passerine, contaminants, methylmercury, northern breeding ground, diet, geographical trend, wind tunnel, Yellow-rumped Warbler, hyperphagia, deuterium, Neotropical migrants, Blackpoll Warbler, feather.

Co-Authorship Statement

A version of Chapter 2 will be submitted for publication with Dr. Christopher Guglielmo, Dr. Brian Branfireun and Dr. Keith Hobson. Dr. Guglielmo, Dr. Branfireun and I made substantial contributions to the conception and design of the work. I conducted the mercury analysis and wrote the manuscript, Dr. Hobson conducted deuterium analysis. I analyzed the data. Dr. Hobson, Dr. Branfireun and Dr. Guglielmo provided comments and reviewed the manuscript.

A version of Chapter 3 was published in the journal *Environmental Pollution* (vol. 234, page 894-901, 2018) with Dr. Christopher Guglielmo, Dr. Brian Branfireun and Dr. Cristina Perez as co-authors. Dr. Guglielmo, Dr. Branfireun and I made substantial contributions to the conception and design of the work. Dr. Perez and I conducted experiments. Dr. Guglielmo and I analyzed data and co-wrote the manuscript. Dr. Branfireun, Dr. Guglielmo and Dr. Perez provided comments and reviewed the manuscript.

A version of Chapter 4 was published in the *Journal of Avian Biology* (vol. 49, page 1-8, 2018) with Dr. Christopher Guglielmo, Dr. Brian Branfireun and Dr. Keith Hobson as co-authors. Dr. Branfireun, Dr. Guglielmo and I made substantial contributions to the conception and design of the work. I prepared samples, and conducted mercury analysis. I prepared the samples while Dr. Hobson conducted the deuterium analysis. All authors analyzed and interpreted the data, co-wrote and edited the manuscript.

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List of Abbreviations

| | |
|----------------------|---------------------------------------|
| AFAR | Advanced Facility for Avian Research |
| AMRE | American Redstart |
| BAWW | Black and White Warbler |
| BLPW | Blackpoll Warbler |
| BRCR | Brown Creeper |
| CCS | Calibration Check Standard |
| CMMN | Canadian Migration Monitoring Network |
| COT | Cost of Transport |
| Dw | Dry Weight |
| Fw | Fresh weight |
| $\delta^2\text{H}$ | Deuterium Isotope Ratio |
| $\delta^2\text{H}_f$ | Feather Deuterium Isotope Ratio |
| $\delta^2\text{H}_p$ | Precipitation Deuterium Isotope Ratio |
| HETH | Hermit Thrush |
| Hg | Mercury |
| Hg(II) | Inorganic divalent mercury |
| Hg(P) | Particulate mercury |
| [Hg] | Mercury Concentration |
| LPBO | Long Point Bird Observatory |
| LISP | Lincoln's Sparrow |
| MAWA | Magnolia Warbler |
| MeHg | Methylmercury |
| NOWA | Northern Waterthrush |

| | |
|-------|---------------------------------|
| PPM | Parts per million |
| QMR | Quantitative Magnetic Resonance |
| RCKI | Ruby-crowned Kinglet |
| RH | Relative Humidity |
| SWTH | Swainson's Thrush |
| TEWA | Tennessee Warbler |
| THg | Total Mercury |
| [THg] | Total Mercury Concentration |
| UWO | University of Western Ontario |
| WIWA | Wilson's Warbler |
| WTSP | White-throated Sparrow |
| Ww | Wet Weight |
| YRWA | Yellow-rumped Warbler |
| YWAR | Yellow Warbler |

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Chapter 1

1 General introduction

1.1 Introduction

1.1.1 Mercury as an environmental pollutant

Mercury (Hg) is a natural element that is emitted to the atmosphere from natural sources (e.g., forest fires, volcanoes), anthropogenic activities (e.g., artisanal small scale gold mining, coal burning) and re-emissions of previously deposited Hg (Driscoll et al., 2013). It is estimated that anthropogenic emissions have increased the amount of Hg in the environment by approximately 3-fold since the pre-industrial period (Driscoll et al., 2013; UNEP, 2013). Gaseous Hg has a residence time of up to one year in the atmosphere before depositing to ecosystems as the more soluble inorganic form, divalent Hg (Hg(II)), or particulate Hg (Hg(P)) (Driscoll et al., 2013). Once Hg is deposited to earth surface environments, it can be transformed through microbial processes into the more bioavailable form, methylmercury (MeHg) (Wood et al., 1968; Jensen and Jernelov, 1969).

1.1.2 Effects of mercury on wildlife

Methylmercury bioaccumulates in organisms and biomagnifies through food chains (Borg et al., 1970; Lavoie et al., 2013; Suedel et al., 1994), resulting in concentrations of concern at upper trophic levels. As a potent neurotoxin, MeHg poses a health risk to humans and wildlife (Chan et al., 2003; Mergler et al., 2007; Scheuhammer et al., 2007). A large body of literature detailing exposure to MeHg in vertebrates has revealed adverse impacts via neurotoxicity (e.g., learning, cognitive abilities and memory), embryo

toxicity, deficiency of immune system, impaired motor skills, endocrine disruption and altered reproductive behaviours (Boening, 2000; Wolfe et al., 1998).

1.2 Mercury toxicity in birds

1.2.1 Exposure

Under most conditions, organisms are exposed to Hg primarily as MeHg through diet (Scheuhammer et al., 2007; Wolfe et al., 1998), and the exposure is affected by factors including life stage, age, sex and the amount of Hg in the environment. Of all factors, trophic level is the most commonly accepted control factor that determining MeHg levels among species because MeHg both bioaccumulates and biomagnifies. For example, Hg levels in avian groups generally follow the trend: piscivores > insectivores > granivores (Boening, 2000; Scheuhammer et al., 2007). However, some insectivorous songbirds switch from their exclusive invertebrate-based diet to a mixed diet with plant materials during migration, so even these classifications are not satisfactory. Also, regions with point-source releases of Hg, as well as areas that receive high atmospheric Hg deposition with sensitive characteristics (e.g., adjoining wetlands, low pH), can be potential Hg hotspots for biota.

Recent studies suggest that some passerines may be exposed to MeHg levels as high as large fish-eating species through the consumption of emergent aquatic insects, foraging on higher trophic-level predators (Cristol et al., 2008), and/or living in environments with high MeHg availability (Abeyasinghe et al., 2017; Edmonds et al., 2010; Rimmer et al., 2005; Townsend et al., 2013).

1.2.2 Uptake

Once dietary MeHg enters the bloodstream, it distributes widely into tissues and organs, including the brain, liver, kidney and muscle (Wiener et al., 2003). Blood Hg in birds is reflective of recent dietary MeHg and tends to be correlated with internal tissue Hg (Eagles-Smith et al., 2008), whereas tissue Hg is a reflection of MeHg accumulation in the body pool over months (Bennett et al., 2009; Kenow et al., 2007). Previous studies measured the Hg concentrations in these tissues of dead birds in order to determine whether Hg poisoning was the cause of mortality (Spalding et al., 1994; Sundlof et al., 1994). In tissues, Hg concentrations associated with mortality are generally thought to be about > 5 ppm in brain, and > 20 ppm in liver or in kidney (in wet weight, see reviews in Scheuhammer et al., 2007; Wolfe et al., 1998).

1.2.3 Mercury in feathers

Mercury depuration for birds includes demethylation through the primary detoxification pathways such as in the liver (Scheuhammer et al., 1998; Thompson and Furness, 1989). Female adults transfer Hg into eggs (Ackerman et al., 2017; Brasso et al., 2010). Mercury is also transferred from the body tissues into feathers. Growing feathers are connected to a blood vessel during formation and MeHg is transferred to keratin, which has a high affinity for MeHg. Once the feather matures, blood vessels shrivel and the feather MeHg is stable (Furness et al., 1986). It is commonly accepted that feather molt is an effective way for birds to eliminate at least 50% of their body Hg burden (Bearhop et al., 2000; Furness et al., 1986). Bird feathers are a common tool for biological monitoring for MeHg exposure and feather Hg is highly correlated to Hg in other tissues during the growing period (Braune, 1987; Burger, 1993). With non-

destructive sampling and easy acquisition, feather Hg provides a comprehensive understanding of Hg body exposure for a specific time period and source area. Feather Hg has been widely applied to fish-eating avian species and the findings have indicated that negative effects are linked to feather Hg of > 5 ppm (e.g., Scheuhammer et al., 2007). However, in songbirds, feather Hg has been reported in few species, over restricted spatial scales, or both. In one case, feather Hg concentrations in Carolina Wren (*Thryothorus ludovicianus*) rarely reach 5 ppm, but have been shown to be associated with impaired reproductive success (Jackson et al., 2011).

1.2.4 Effects of mercury on birds

Historically, mercury contamination has been thought of as an aquatic ecosystem issue, because Hg methylation predominantly occurs in anaerobic lake sediments and bottom waters (Gilmour et al., 1992), and bioaccumulation and biomagnification occur in aquatic food webs (Wiener and Spry 1996; Wiener et al., 2003). Concerns about the effects of MeHg have therefore focussed on birds with piscivorous diets including species such as Common Loons (*Gavia immer*) (Evers et al., 2003; Evers et al., 2008), Bald Eagles (*Haliaeetus leucocephalus*) (Rutkiewicz et al., 2011; Scheuhammer et al., 2008) and various seabirds (Bond and Diamond, 2009; Furness et al., 1986; Monteiro and Furness 1997; Thompson et al., 1991). There is mounting evidence that MeHg exposure can cause reductions in avian fitness in laboratory and field studies (see reviews by Scheuhammer et al., 2007; Whitney and Cristol, 2017).

The first reports on Hg and birds were from Sweden in the early 1950s, as high Hg concentrations were found in dead birds contaminated from fungicide-treated seeds (Borg et al., 1969). For over six decades, the majority of published studies have focussed on the

most common endpoint, reproduction, which directly affects population health (Chan et al., 2003; Whitney and Cristol, 2017; Wolfe et al., 1998). At sub-lethal Hg concentrations, effects on reproductive success have been identified across species, including altered pairing (Frederick and Jayasena, 2011), parental behaviors such as laying eggs outside the nest (Heinz, 1979), fewer eggs and fledglings (Heinz, 1979; Brasso and Cristol, 2008; Hallinger et al., 2011; Tartu et al., 2013), eggshell thinning (Heinz, 1979; Olivero-Verbel et al., 2013), as well as decreased egg weight/volume (Olivero-Verbel et al., 2013; Evers et al., 2003). Also, elevated MeHg exposure might have an influence on reproductive decisions, resulting in highly contaminated Arctic seabirds to skip reproduction (Tartu et al., 2013). Consequently, MeHg impaired parenting (Evers et al., 2008) and foraging (Kobiela et al., 2015) behaviours may also lead to poor nutrition or body condition in young birds, which then could have carry-over effects at the individual level, affecting subsequent migration performance (Mitchell et al., 2011) and fitness (Lindström, 1999).

Several studies have focused on the effects of Hg on reproduction of non-migratory small songbirds. For example, one laboratory study using a model songbird species the Zebra Finch (*Taeniopygia guttata*), found that elevated dietary MeHg reduced reproductive success up to 50% and increased the latency to re-nest after a clutch loss (Varian-Ramos et al., 2014), both of which could result in reduced populations. Consistent with these findings, Jackson et al., (2011) found that elevated Hg burden in female Carolina Wrens reduced nest success at a contaminated breeding site, with a 3-fold higher nest abandonment rate compared to a reference site. Bird song is essential during reproduction since it is used in territory defense and mate selection, which are

related to breeding success and Hg has been found to alter singing behavior in songbirds (Hallinger et al., 2010). Overall, these changes to reproductive performance could potentially result in reduced songbird reproduction.

1.3 Songbird migration

Of the approximately 10 000 bird species on Earth, 60% belong to the most widely distributed order of Passerines (songbirds) (Boles, 1995). For many small migratory songbirds (less than 15 grams, e.g., warblers, kinglets, sparrows and thrushes), life cycles are dynamic (breeding, molting, migrating and overwintering) and spatially extensive, as the birds make regular, seasonal movements between breeding and wintering areas, encountering various environmental conditions and threats throughout the year. Many long-distance migratory songbirds, such as the American Redstart (*Setophaga ruticilla*), spend 3 to 5 months in their northern breeding grounds, 1 to 2 months in autumn migration, 6 to 7 months in tropical wintering areas, and another month in spring migration (Sherry et al., 2016). Migration generally includes an autumn phase (southward) and a spring (northward) phase. The distance of migration varies among passerine species. For long distance migrants, a journey can be two endpoints between Argentina and the northern boreal forest in Canada.

A full migration journey typically consists of many long flights (several hours, even days, in duration), interspersed by periods of rest and fueling. Such flights require not only extraordinary navigational skills, but also large energy reserves as well as a high capacity for flight. For example, some individuals cross the Gulf of Mexico or the Sahara Desert-distances in excess of thousands of kilometers (Adamík et al., 2016; Williams et al., 1978). One of the most exciting migrations is by a tiny songbird species the Blackpoll

Warbler (*Setophaga striata*), that is able to migrate in excess of 2770 km directly over a stretch of the Atlantic Ocean, non-stop (DeLuca et al., 2015). Therefore, there is no doubt that any unexpected extra costs during this critical migration period could have direct negative effects on migration success and even subsequent overwintering or breeding.

1.4 Potential effects of mercury on migratory songbirds

1.4.1 Pre-migration

For small passerines, the energy needed to travel the distance between breeding and wintering grounds generally exceeds the amount they can store and carry. Thus, they make several stopovers to replenish fuel stores for the next bout of flight. To accomplish a long non-stop flight successfully (e.g., over water), individuals need an adequate supply of fuel, not just for the journey, but also for any emergencies like unfavorable weather that might arise. Thus, prior to migratory flights, birds undergo physiological adjustments to maintain a much higher metabolic rate for flight, thermoregulation and recovery (Newton, 2008). One of the strategies is that migrants increase their food intake. They forage intensively and increase body mass through the deposition of fat and lean mass (Kvist et al., 2001). Passerines generally spend about 75-90% of the journey duration at stopovers (Newton, 2008), typically take 1 to 3 weeks to replenish their fuel stores, and may double their body weight prior to crossing large ecological barriers (Biebach et al., 1986).

For hyperphagic migrants, the main exposure to MeHg is through food ingested to fuel subsequent flights, and they may rapidly accumulate this toxicant at stopovers such as wet and acidic habitats (e.g., boreal forest, St. Louis et al., 1994), industrialized areas, gold mining regions (Li et al., 2009; Turner and Southworth, 1999), or some rice-based

agricultural areas near abandoned mines (Qiu et al., 2008). Whether there is a link between increased food intake and Hg accumulation, and their effects on subsequent flight is unknown.

1.4.2 Migratory fitness

In non-avian migratory species, Hg can negatively affect vision (Ventura et al., 2005; Cavalleri et al., 1995), as well as learning and spatial memory (Falluel-Morel et al., 2007; Farina et al., 2011; Swaddle et. al, 2017), and therefore could disrupt an association between the pattern of sensory input produced by the magnetic field and the visual surrounding. Snapping Turtle (*Chelydra serpentina*) hatchlings with high levels of maternally-inherited Hg failed to show consistent magnetic alignment (Landler et al., 2017), indicating Hg could disrupt orientation and spatial abilities, which are essential for migration success. Homing Pigeons (*Columba livia*) that were exposed to MeHg prenatally and continually after hatch exhibited flight impairment manifested by less efficient homing, reluctance to fly, and slower flight speed on initial flights (Moye et al., 2016). The flight phase of migration requires fasting songbirds to exercise continuously at an intensity of about 12 times the basal metabolic rate (Alexander, 1998) for several hours to a few days (Berthold, 2001). Thus, any factor that inhibits the ability to complete long endurance flights will increase the risk of migration failure. Indeed, laboratory studies on Zebra Finches have showed some indication of negative effect of Hg on migration. For example, fasting MeHg treated birds increased their Hg levels in blood (Seewagen et al., 2016), while long-term MeHg dosed individuals showed impaired spatial memory and other cognitive effects (Swaddle et. al, 2017). However, we lack

direct evidence of negative effects of MeHg exposure on endurance flights in migratory passerines.

1.4.3 Survival

Even though an abundance of studies have shown that Hg can negatively affect some important population parameters, including reduced reproductive success and nestling growth, there is no strong evidence that Hg negatively affects overall individual survivorship of piscivorous avian, or other avian species in the field (Whitney and Cristol, 2017). Common Loon, which is the most studied species with reduced reproduction and early development in the field, did not exhibit differences in apparent individual survival (Mitro et al., 2008). The same trend was found in young Snowy Egrets (*Egretta thula*; Henny et al., 2017). For large, long-lived seabirds, no apparent change in return rate or annual survival was related to Hg body burden (Goutte et al., 2014; Thompson et al., 1991). The only field study on Hg-related survivorship of a migratory songbird, failed to detect the significant difference of the annual survival of Tree Swallows (*Tachycineta bicolor*) between Hg-contaminated sites and reference sites (Hallinger et al., 2011).

The negative effects caused by environmental Hg such as impaired immune function, foraging behaviour and navigation, can individually or together affect songbirds during migration and potentially their ultimate survival. Songbirds may be more sensitive to Hg toxicity than larger avian species. For example in mammals, small carnivorous minks (*Mustela vison*), have fewer survival days compared to larger carnivores otters (*Lutra canadensis*) (Wren et al., 1988). Thus, compared to larger piscivorous avian predators, migratory passerines have a smaller tissue compartments (lower detoxification capacity),

and a higher metabolic rate (higher food intake and a quick accumulation for toxins).

Migratory songbirds may experience culling due to an additional stressor, however there is currently no information on these potential effects of environmental MeHg.

1.5 Objectives

The overall objective of my doctoral research is to better understand the pattern of Hg exposure in migratory songbirds across breeding grounds, and assess the impacts of Hg on songbird migratory fitness and survivorship. To address this, this thesis is structured in three research chapters addressing a set of sub-objectives.

- 1) Examine the patterns of Hg exposure across a wide range of passerine species, accounting for diet, age, sex and geographic range using feathers (Chapter 2).

- 2) Determine the effects of dietary MeHg exposure on migratory flight performance (Chapter 3).

- 3) Evaluate migratory survival of passerine species as a function of Hg body burden and migration distance (Chapter 4).

The thesis concludes with a general discussion to synthesize the findings of individual chapters and to present a generalized model of Hg exposure and its effects in migratory birds (Chapter 5).

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Chapter 2

2 Spatial patterns of feather mercury exposure in migrant songbirds across breeding grounds in Canada

2.1 Introduction

2.1.1 Mercury and its impact on birds

Mercury is a global contaminant that can travel long distances atmospherically then deposit to the Earth's surface as wet and dry deposition (Driscoll et al., 2013).

Atmospheric deposition of Hg has increased at least 3-fold compared to pre-industrial period, due to human activities such as the burning of coal for power generation, and this has led to contamination in even remote areas distant from emission sources (Driscoll et al., 2013; Fitzgerald et al, 1998; Pacyna et al., 2010), including northern boreal forest.

For Hg to enter the food chain, it must first be converted to methylmercury (MeHg) in anaerobic environments like lake sediments and wetlands, where it then may be bioaccumulated and trophically biomagnified (Gilmour et al., 1992).

The major route of exposure to MeHg for birds is through their diets (Eagles-Smith et al., 2009; Scheuhammer et al., 2007). Terrestrial-feeding songbirds can be exposed to elevated MeHg in diet by consuming aquatic emergent insects (Tsui et al., 2012) or their predators such as spiders (Cristol et al., 2008). A higher proportion of invertebrates in diet is generally linked to higher MeHg exposure in songbirds (Cristol et al., 2008; Jackson et al., 2015). They may also obtain elevated MeHg via seeds that have grown near highly contaminated locations, such as rice paddies near Hg mining sites (Abeysinghe et al., 2017), however this is less of a widespread concern. For birds, exposure to MeHg has been shown to cause impaired learning/cognitive abilities and

memory, embryo toxicity, deficiency of the immune system, endocrine disruption, impaired motor skills and altered reproductive behaviours (see review in Whitney and Cristol, 2017).

In North America, billions of birds, including many migratory passerine birds, rely on Canada's northern forests as their critical breeding grounds (Wells and Blancher, 2011). Dietary MeHg exposure has been recognized with reduced songbird reproductive success in both the manipulative laboratory and field study settings. The recorded effects of Hg on songbird reproduction include modified singing behavior (Hallinger et al., 2010) and decreased nesting success (Jackson et al., 2011). However, the concentrations of mercury exposure in many migratory songbird species at their breeding grounds has not been reported.

The work on MeHg exposure to date tends to be species limited, spatially restricted, or both. Specifically, the studies refer to MeHg exposure at the breeding stage have been limited to specific species that live in aquatic relevant habitats like wetlands/estuaries (e.g., Saltmarsh Sparrows *Ammodramus caudacutus*, Lane et al., 2011; Marsh Wrens *Cistothorus palustris*, Hartman et al., 2013) and isolated montane forested areas that receive direct atmospheric Hg deposition (e.g., Bicknell's Thrush *Catharus bicknelli*, Rimmer et al., 2005; *Catharus* Thrush, Townsend et al., 2014). Mercury monitoring in a variety of songbird species was conducted on one of the mercury hotspots-Northeastern region of North America (Jackson et. al, 2015).

2.1.2 Factors affecting mercury exposure

In addition to the dietary controls discussed above, the amount of Hg in the environment also appears to control Hg levels in higher organisms including birds. Spatial patterns of Hg in piscivorous fish, bats, Common Loon (*Gavia immer*) and Rusty Blackbird (*Euphagus carolinus*) have all shown a west to east increase of Hg body burden, that is roughly consistent with patterns of atmospheric Hg deposition (Chételat et al., 2018; Depew et al., 2013; Edmonds et al., 2010; Evers et al., 1998). In birds, other studies also reported that age and sex play a role in Hg exposure (Evers et al., 2008; Thompson et al., 1991). In general, adults are proposed to be exposed to higher Hg level comparing to juveniles because of the accumulation effect, while male are likely to accumulate relatively high Hg level as females may deposit Hg into eggs but this pattern varied among species (Ackerman et al., 2016, 2017; Agusa et al., 2005).

2.1.3 Use of feathers for monitoring bird mercury exposure

Although the negative effects of Hg effects on reproduction have been reported, it is often hard to estimate the Hg exposure/body burden in migratory songbirds at their breeding sites. Most of them are breeding at remote northern areas that are hard to access, and some species show a shift in diet selection during migration or wintering and therefore their Hg body burden at breeding ground might reflect the diets in different life stages (Pyle, 1997).

Nestlings grow feathers at their natal site, and in North America many adult passerines undergo a complete feather molt prior to autumn migration at or near their breeding location (Pyle, 1997; Rohwer et al., 2005). Body MeHg is redistributed and transferred from tissues and organs into growing feathers, and remains stable following feather

growth. As flight feathers are retained in most species over the non-breeding season until molt in the following summer, tail feathers can be used at other times and places as indicators of Hg body burden on the breeding grounds (Appelquist et al., 1984; Rohwer et al., 2005).

In addition, the stable isotopes of hydrogen (feather deuterium, $\delta^2\text{H}_f$) have been widely used as a valuable tracer for geographic origins (breeding grounds) in North America because of the known pattern of $\delta^2\text{H}$ in precipitation ($\delta^2\text{H}_p$) (Hobson and Wassenaar, 1996). The latitudinal gradient of $\delta^2\text{H}_p$ across North America is reflected in local food webs and is ultimately incorporated in bird feathers (Hobson and Wassenaar, 1996). In general, more negative $\delta^2\text{H}_f$ corresponds to higher latitude. For feathers grown by nestlings and fledglings or by adults that molt during or immediately following breeding, including most boreal passerines (Pyle, 1997), such assignments reflect the natal or breeding area, respectively. The values of $\delta^2\text{H}_f$ can be assigned to the regions where those feathers could possibly have grown (Hobson et al., 2015).

Thus, the information about Hg body burden as well as the latitudes of breeding grounds are archived in metabolically inert tissues (i.e., tail feathers here). Although bio monitoring has been widely used feather as a tool in fish-eating birds (e.g., Nisbet et al., 2002), in songbirds, the tissues are still limited in blood and eggs (Ackerman et al., 2017; Jackson et al., 2015).

2.1.4 Objectives

Given the lack of continent-wide information about Hg in migratory songbirds, the overall goal of this study was to assess Hg exposure across a range of bird species use

feather samples from national bird survey across Canada. Specifically, I aimed to examine the relationships among MeHg exposure, diet structure, age, sex and geographic origin using feathers.

2.2 Methods and materials

2.2.1 Sampling locations

In the 2007 fall migration season, 15 songbird species were sampled by staff and volunteers at 15 bird banding station members of the Canadian Migration Monitoring Network (CMMN), which consists of standardized observation and migration count stations across Canada (Figure 2.1). It is not possible to directly identify the place of origin for all species and individuals from each sampling location; however, $\delta^2\text{H}_f$ isoscapes (see maps in Hobson and Wilgenburg, 1996) and band recovery data (see Hobson et al., 2015) were used to cluster the breeding grounds of birds captured at the CMMN stations into three broad geographic regions (Western, Central and Eastern Canada). Based on the above information, I assumed that birds of all species captured at stations 1 to 4 had a high likelihood of originating in the green shaded area (Western), while individuals captured at stations 5 to 12 were grouped to the blue shaded area (Central), then birds captured from stations 13 to 15 were assigned to the red shaded area (Eastern) (see Figure 2.1).

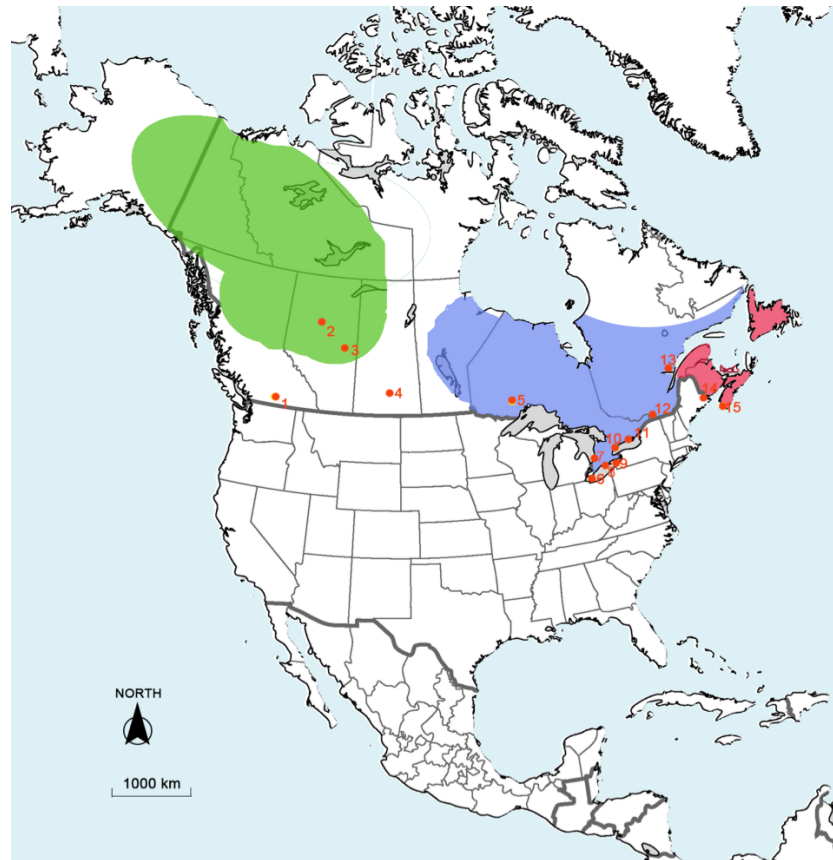


Figure 2.1 Estimated passerine breeding grounds and the associated sampling stations in Canadian Migration Monitoring Network (CMMN) that feathers were collected in 2007 fall. The shaded areas represent estimated breeding grounds (green: Western; blue: Central; red: Eastern) for migratory songbirds. The red dots and numbers represent sampling stations: 1) Vaseaux Lake 2) Lesser Slave Lake; 3) Beaverhill; 4) Last Mountain; 5) Thunder Cape; 6) Tommy Thompson Park; 7) Pelee Island; 8) Long Point; 9) Haldimand; 10) Ausable; 11) Prince Edward Point; 12) McGill; 13) Observatoire d'oiseaux de Tadoussac; 14) St. Andrew's and 15) Atlantic.

2.2.2 Study species

Fifteen songbird species were selected based on their broad breeding distributions across Canada and their diet preferences (Rodewald, 2015). For a list of scientific names, species codes, foraging guild and diet structures see Table 2.1. The traditional classification of birds by trophic level is strongly governed by both life history and life stage (De Graaf et al., 1985). In terms of small migrant songbirds, many of them show seasonal shifts in diet selection during migration or wintering. For example, some species change from eating primarily invertebrates to a mixed diet with fruits, (e.g., Yellow-rumped Warbler *Setophaga coronate*, Hunt and Flaspohler, 1998). Others feed selectively on plant materials (fruits or seeds) during migration, such as the White-throated Sparrow (*Zonotrichia albicollis*; Falls and Kopachena, 2010). As the focus of this study was on MeHg, and MeHg in diet is strongly associated with bioaccumulated and biomagnified MeHg in invertebrate food webs, here I more broadly considered the proportion of invertebrates in diet through the whole life cycle based on published species information (Rodewald, 2015). Species were classified into 3 broad groups: 1) exclusively insectivorous (invertebrate diets only); 2) partially insectivorous (invertebrate diet during breeding; some plant materials during migration/wintering); 3) non-insectivorous (predominantly plant materials in diet). The term “non-insectivorous” is an overgeneralization as all birds will consume invertebrates from time to time; however, it is used as an exclusive category here for clarity.

Table 2.1 Songbird species sampled and classification of foraging guild, proportion of invertebrates in diet in this study.

| Species | Code | Foraging guild (De Graaf et al., 1985) | Proportion of invertebrates in diet (Rodewald, 2015) |
|--|-------------|---|---|
| American Redstart (<i>Setophaga ruticilla</i>) | AMRE | lower-canopy gleaner/forager | partially insectivorous |
| Blackpoll Warbler (<i>Setophaga striata</i>) | BLPW | upper-canopy gleaner/forager | partially insectivorous |
| Brown Creeper (<i>Certhia americana</i>) | BRCR | bark-gleaner | exclusively insectivorous |
| Black and White Warbler (<i>Mniotilta varia</i>) | BAWW | bark-gleaner | exclusively insectivorous |
| Hermit Thrush (<i>Catharus guttatus</i>) | HETH | ground gleaner/forager | non-insectivorous |
| Lincoln's Sparrow (<i>Melospiza lincolni</i>) | LISP | ground gleaner/forager | non-insectivorous |
| Magnolia Warbler (<i>Setophaga magnolia</i>) | MAWA | lower-canopy gleaner/forager | partially insectivorous |
| Yellow-rumped Warbler (<i>Setophaga coronata</i>) | YRWA | lower-canopy gleaner/forager | partially insectivorous |
| Northern Waterthrush (<i>Parkesia noveboracensis</i>) | NOWA | ground gleaner/forager | exclusively insectivorous |
| Ruby-crowned Kinglet (<i>Regulus calendula</i>) | RCKI | lower-canopy gleaner/forager | partially insectivorous |
| Swainson's Thrush (<i>Catharus ustulatus</i>) | SWTH | ground gleaner/forager | non-insectivorous |
| Tennessee Warbler (<i>Oreothlypis peregrina</i>) | TEWA | upper-canopy gleaner/forager | partially insectivorous |
| Wilson's Warbler (<i>Cardellina pusilla</i>) | WIWA | lower-canopy gleaner/forager | partially insectivorous |
| White-throated Sparrow (<i>Zonotrichia albicollis</i>) | WTSP | ground gleaner/forager | non-insectivorous |
| Yellow Warbler (<i>Setophaga petechia</i>) | YWAR | lower-canopy gleaner/forager | partially insectivorous |

2.2.3 Feather collection

As indicators of breeding ground MeHg exposure, archived tail feathers from 1946 individual birds collected as part of the national bird survey described above were provided in labeled paper envelopes by Bird Studies Canada. Details of feather collection can be found in Hobson et al. (2015). As part of the CMMN program, the sex and age classes (juvenile: hatch year; adult: after hatch-year) were determined for each individual through plumage, examination of pneumatization, and other features (Pyle, 1997).

2.2.4 Mercury analysis

Total mercury (hereafter THg) in feathers has been shown to comprise > 95% MeHg (Edmonds et al., 2010; Rimmer et al., 2005). Thus, feather THg concentration ([THg]) was determined instead of the direct analyses of MeHg because the analysis of THg by pyrolysis and atomic absorption detection is dramatically faster and significantly less involved. Prior to analysis, the whole feathers were cleaned with 1% acetone and deionized water then dried at ambient temperature overnight. Total Hg concentration was determined using a Direct Mercury Analyzer (DMA 80, Milestone Inc., USA), following US Environmental Protection Agency Method 7473 (EPA, 1998). Whole feathers were weighed on a clean nickel sample boat, and covered by aluminum foil in order to keep the very light samples in place during analyses. Blank checks and tests with certified reference materials Human Hair (IAEA-86) confirmed that this approach ensured complete combustion of the sample, and did not contribute measurable contamination. Laboratory quality control samples included a method blank (empty nickel boats with aluminum foil), calibration check standard (CCS), and a duplicate sample with each batch of 20 or fewer samples. All Hg analyses met measurement quality objectives in

accordance with the certified methods. I reported [THg] as parts per million (ppm, fresh weight, fw). The quality assurance was presented as mean \pm S.E.; CCS: $101.39 \pm 1.05\%$ ($n = 105$) and mean relative percent difference in duplicates: $14.27 \pm 0.84\%$ ($n = 116$). The difference among duplicates were within the acceptable range. Mean recovery of certified reference materials was $105.40 \pm 0.99\%$ ($n = 100$). All samples had concentrations greater than 10 times the minimum detection limit of the method. Mercury analyses were undertaken in the Biotron Centre for Experimental Climate Change Research at Western University in an ISO 17025 accredited trace metal laboratory.

2.2.5 Feather deuterium analysis

Individuals ($n = 388$) of 5 selected songbird species (HETH, LISP, MAWA, RCKI, YWAR) that co-occurred across Canada, were sampled evenly in 3 regions to evaluate general latitudinal origins in feather Hg exposure.

A subset of feathers were analyzed for $\delta^2\text{H}_f$ using the comparative equilibration method described by Wassenaar and Hobson (2003), at the Stable Isotope Hydrology and Ecology Laboratory of Environment Canada in Saskatoon, Canada and a detailed description of the feather deuterium analysis can be found in Hobson et al. (2015). I only consider the deuterium values at range from -90‰ to -190‰ , which correspond to the general northern breeding ground ranges (Figure 2 in Hobson et. al, 2015). In general, more negative values correspond to more northern geographic origin.

2.2.6 Statistical procedures

General linear models (GLM) for each species were used to predict the [THg] based on region (Western, Central or Eastern Canada), sex (male or female) and age class

(juvenile or adult). Where age and sex data were missing in some species, only the regional effect was assessed. I reported sample size (n), F, p value and estimate parameters in the Table 2.2. The relationship among MeHg in feathers, diet structure and region were evaluated when considering all species as a whole passerine order, using two-way ANOVA with Tukey's HSD post hoc tests. Linear regressions were used to examine the relationship between \ln [THg] and $\delta^2\text{H}_f$ in each region to see if this pattern varied among regions. The level of statistical significance was defined as an alpha value of 0.05. The [THg] was presented as mean \pm standard deviation, if not otherwise stated. For statistical analysis, [THg] was natural log transformed to meet the assumption of normality and fit linear trend lines. R version 3.3.2 was used for data analysis and visualization (R Core Team, 2016).

2.3 Results

2.3.1 Age and sex effects on feather mercury

Age did not influence feather [THg] in 8 of 10 species (Table 2.2), excepting BRRCR ($F_{1,115} = 25.19$; $P < 0.001$) had approximately 60% higher [THg] in adults, whereas WTSP ($F_{1,163} = 9.20$; $P = 0.003$), had about 36% lower [THg] in adults. There was no statistical difference in [THg] based on sex for the 5 species that had completed data on sex (Table 2.2).

Table 2.2 Results from the General linear model (GLM) that examined the effects of region, age class and sex on mercury concentrations in individual songbird species. Natural log-transformed mercury concentration was the dependent variable while age class, sex and region were the predictors ^a. The reference predictors were represented in the blanket. The sample size (n), F, p value and estimate parameters were reported.

| Species | Region (reference: West) | | Age class (reference: Juvenile) | | Sex (reference: Female) | | Statistical variables | | | |
|---------|--------------------------|---|---------------------------------|------------------|---|-------------------|--------------------------------------|------------------|----------------|--------|
| | n ^b | F, P | Estimates (Central) | Estimates (East) | F, P | Estimates (Adult) | F, P | Estimates (Male) | R ² | P |
| AMRE | 151 | F_{2,135} = 19.50; P<0.001 | 0.519 | 1.008 | F _{1,135} = 1.41; P = 0.237 | 0.124 | F _{1,135} =1.09; P =0.298 | -0.098 | 0.20 | <0.001 |
| BAWW | 155 | F_{2,148} = 56.74; P<0.001 | 0.677 | 1.781 | F _{1,149} = 1.39; P = 0.241 | 0.187 | F _{2,149} =1.86; P= 0.175 | -0.148 | 0.45 | <0.001 |
| BLPW | 151 | F_{2,147} =22.54; P<0.001 | 0.803 | 1.260 | F _{1,147} =3.69; P = 0.056 | 0.231 | NA | NA | 0.27 | <0.001 |
| BRCR | 125 | F_{1,115} =22.87; P <0.001 | NA | 0.543 | F_{1,115} = 25.19; P <0.001 | 0.599 | NA | NA | 0.25 | <0.001 |
| RCKI | 81 | F_{2,78} = 7.53; P= 0.001 | 0.567 | 0.563 | NA | NA | NA | NA | 0.14 | 0.001 |
| NOWA | 172 | F_{2,164} = 13.92; P <0.001 | 0.210 | 0.695 | F _{1,164} = 0.01; P = 0.905 | 0.017 | NA | NA | 0.13 | <0.001 |
| YWAR | 101 | F_{2,98} = 18.42; P <0.001 | 0.876 | 0.760 | NA | NA | NA | NA | 0.26 | <0.001 |
| WIWA | 139 | F _{2,133} = 2.96; P = 0.055 | 0.065 | 0.454 | F _{1,133} = 0.73; P= 0.395 | 0.097 | F _{1,133} =0.05; P=0.824 | 0.025 | 0.02 | 0.179 |
| YRWA | 160 | F_{2,133} = 22.69; P <0.001 | 0.499 | 1.200 | F _{1,133} =3.41; P =0.067 | 0.232 | F _{1,133} = 0.38; P = 0.538 | -0.072 | 0.26 | <0.001 |
| HETH | 85 | F_{2,82} = 5.73; P = 0.005 | 0.485 | 0.745 | NA | NA | NA | NA | 0.10 | 0.005 |
| MAWA | 98 | F_{2,95} = 16.86; P <0.001 | 0.714 | 1.098 | NA | NA | NA | NA | 0.25 | <0.001 |
| SWTH | 156 | F_{2,152} = 5.30; P = 0.006 | 0.301 | 1.038 | F _{1,152} =1.16; P = 0.283 | 0.138 | NA | NA | 0.05 | <0.001 |
| TEWA | 122 | F _{1,76} =3.51; P= 0.064 | -0.364 | NA | F _{1,75} = 0.67; P = 0.387 | 0.415 | F _{1,75} =1.44; P = 0.235 | -0.168 | 0.04 | 0.132 |
| WTSP | 168 | F_{2,163}=6.53; P = 0.002 | 0.493 | 0.595 | F_{1,163}= 9.20; P = 0.003 | -0.361 | NA | NA | 0.09 | 0.000 |
| LISP | 82 | F _{2,79} = 0.36; P = 0.698 | 0.092 | 0.175 | NA | NA | NA | NA | -0.02 | 0.698 |

^a Bold font indicated statistical significance in predictors. ^b n representd sample size. NA means no observed values or missing data.

2.3.2 Effects of diet on feather mercury

The overall mean feather [THg] for all individuals sampled ($n = 1946$) was 1.49 ± 1.54 ppm (range 0.13 to 25.19 ppm). The effect of diet structure was significant ($F_{2,1937} = 12.31$, $P < 0.0001$) (Figure 2.2). Generally, feather [THg] was highest in exclusively insectivorous species, followed by partial insectivores then non-insectivorous species without considering regional effects. The means of [THg] in three exclusively invertebrate-eaters (BRCC, NOWA, BAWW) ranged from 1.34 to 3.04 ppm (BRCC was not sampled in the Western region and it had a mean of 3.04); the mean of [THg] in partial insectivores ranged from 0.41 to 2.36 ppm (TEWA was not sampled in Eastern region and it had the lowest mean of 0.41 ppm), while the four non-insectivorous species (LISP, HETH, WTSP and SWTH) had the lowest mean [THg] (ranged from 0.62 to 1.43 ppm).

2.3.3 Differences in feather mercury among regions

When diet was controlled for, the regional effect on [THg] was significant ($F_{2,1937} = 16.34$, $P < 0.00001$). Among 15 species, 11 species showed a strong regional (Western < Central < Eastern) difference in [THg] (BRCC showed Central < Eastern as no BRCC population was distributed in the Western region). Instead of strong regional effects, WIWA showed a marginal but not statistically significant difference in [THg] among regions while LISP showed in [THg] no difference among regions. Species TEWA was not sampled in the Eastern region and showed no difference between the Central and the Western regions. Mean [THg] in feather was 2.45 ± 2.48 ppm in the Eastern region, being about 1.8 times greater than the Central (1.38 ± 1.19 ppm) and at least 2.5 times

higher than the Western Canada (0.94 ± 0.90 ppm) (Figure 2.2). Certain species had notably greater differences in mean [THg] among regions. Among all species, BRCR had the highest mean [THg] (5.13 ± 3.66 ppm) in the Eastern region, which was 2 times greater than in the Central region (2.39 ± 1.34 ppm). The second highest group in [THg] was from YRWA in Eastern region (3.82 ± 4.41 ppm), which had 2 times greater than in Central region (1.70 ± 0.96 ppm) and 3.5 times greater than in Western region (1.13 ± 0.83 ppm). The third highest group was in Eastern NOWA, with an average [THg] of 3.72 ± 2.17 ppm. Comparing to Eastern group and Central group, the Eastern NOWA was about 1.5 times and 1.8 times higher.

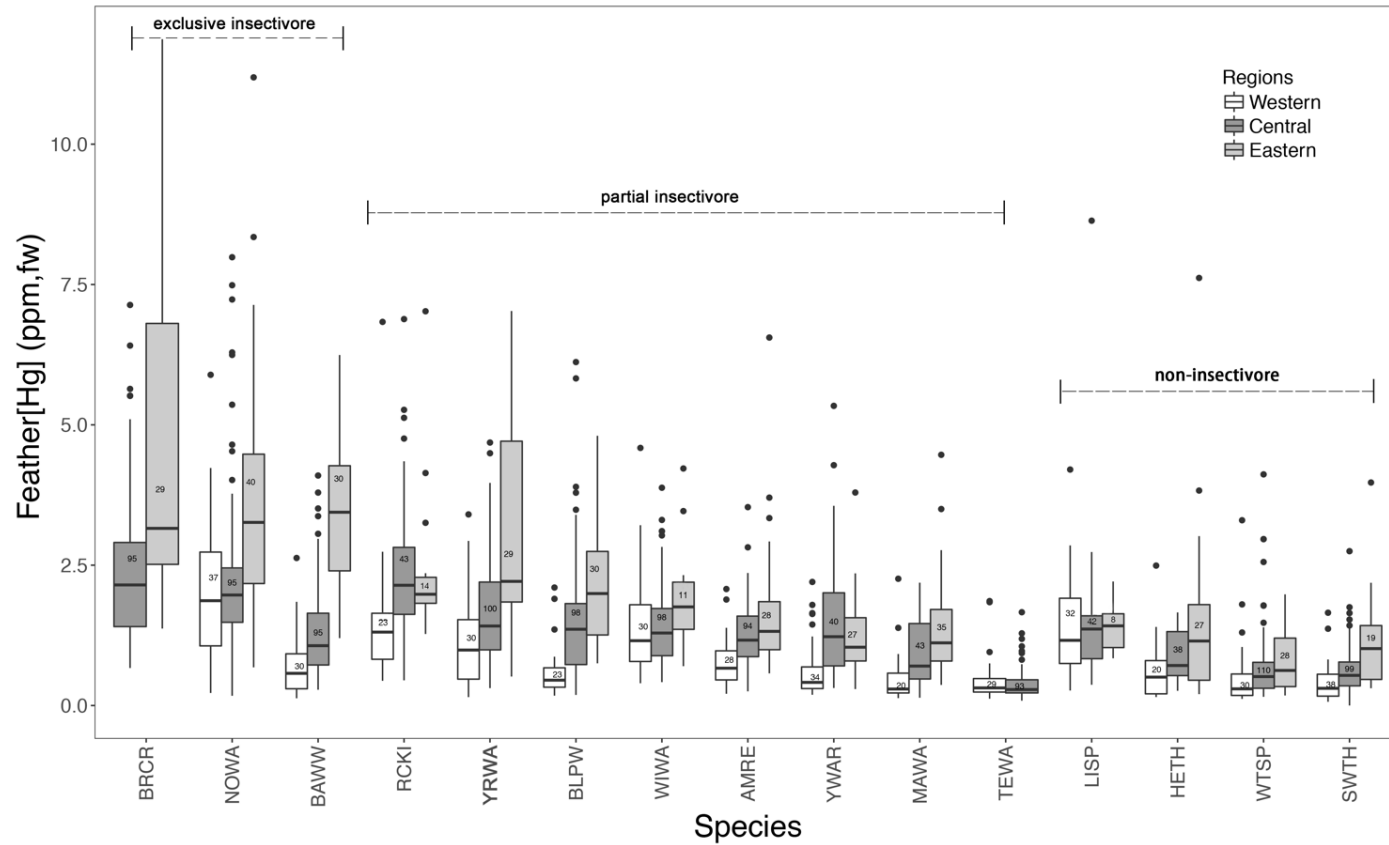


Figure 2.2 Feather mercury concentrations among songbird species with varied proportions of invertebrate-based diets on a whole life cycle. The sample size is shown within each individual boxplot. The line in the middle of the box represents the median, and the lower and upper ends of the box are the 25% and 75% quartiles, respectively. Individual points are outliers. The y-axis is truncated at 12 ppm for clarity; however, there are individual data points that are higher than this value (AMRE: 13.31 ppm; Eastern. BRCR: 14.07 ppm; Eastern. RCKI: 15.83 ppm; Central. YRWA: 25.19 ppm, Eastern). BRCR was not sampled in the Western region, and TEWA was not sampled in the Eastern region.

2.3.4 Differences in feather mercury with latitude

Based on the broad interpretation of the δ^2H_f results, there is higher [THg] in feathers from birds originating from more southerly breeding grounds ($\ln [\text{THg}] = 0.01 \times \delta^2H_f + 1.06$, $F_{1,386} = 24.84$, $P < 0.001$; adjusted $R^2 = 0.06$, Figure 2.3). This overall pattern is strongly influenced by the increase in [THg] from North to South in the Western region ($\ln [\text{THg}] = 0.01 \times \delta^2H_f + 1.67$, $F_{1,131} = 10.81$, $P = 0.001$, adjusted $R^2 = 0.07$). No trend was found either in the Central region ($\ln [\text{THg}] = -0.00005 \times \delta^2H_f + 0.15$, $F_{1,187} = 0.0003$, $P = 0.99$, adjusted $R^2 = -0.005$) or in the East region ($\ln [\text{THg}] = -0.006 \times \delta^2H_f - 0.61$, $F_{1,64} = 2.23$, $P = 0.14$, adjusted $R^2 = 0.02$). The discrepancy between Figure 2.2 and Figure 2.3 is a function of species sampled. In Figure 2.2, a strong regional difference is noted in individual species, with the Eastern region being highest, but these species were not included in the δ^2H_f analyses because they were not ubiquitous across Canada.

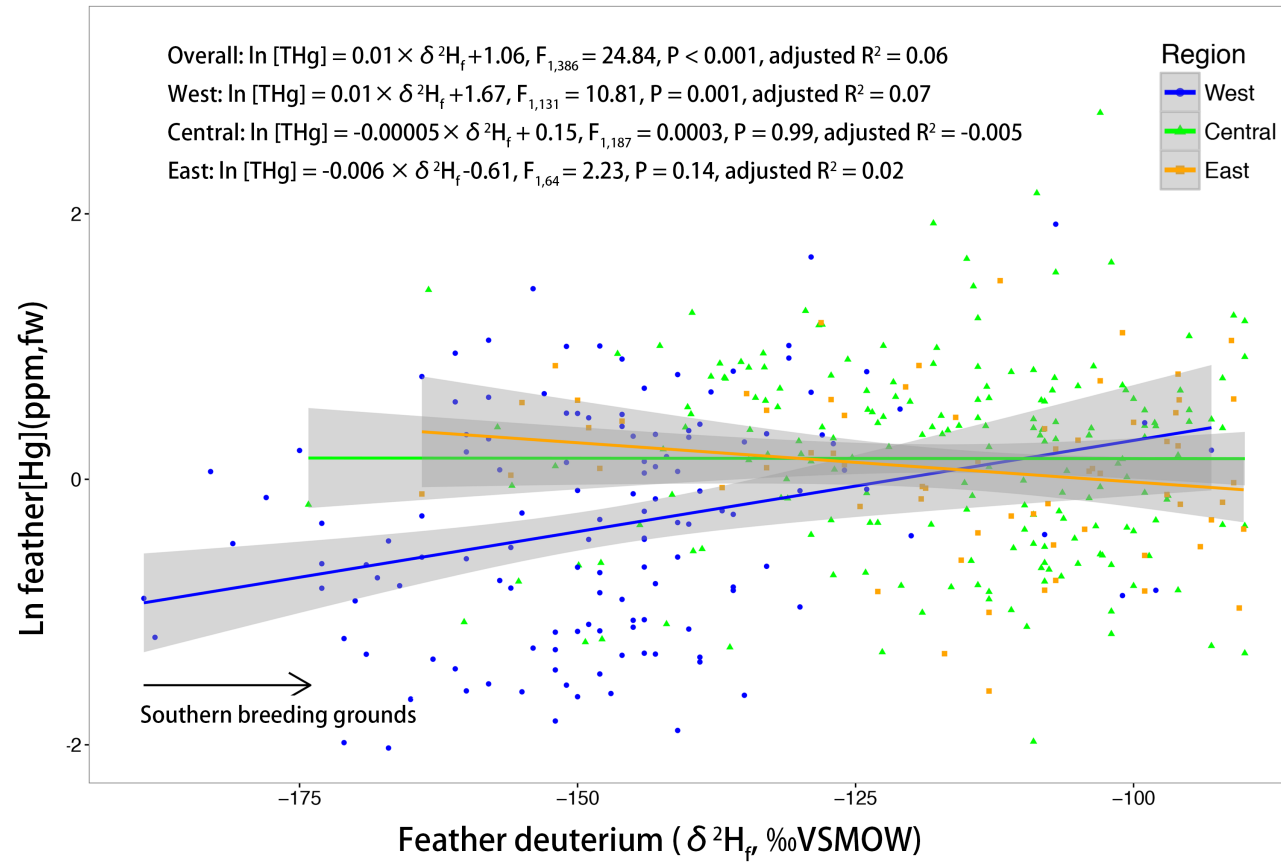


Figure 2.3 Individual feather deuterium value ($\delta^2\text{H}_f$, ‰VSMOW) plotted against natural log-transformed Hg with regression lines shown. The color lines represent the grouped breeding regions that individuals come from. The more positive deuterium value here indicated more southern breeding grounds.

Overall, there was a statistically significant interaction between diet structure and region on feather [THg] ($F_{4, 1937} = 4.74$, $P = 0.001$, Figure 2.4). Western non-insectivores had 0.57 ppm lower [THg] than those from the East, and there was no difference from Central birds. Western partial insectivores, had 0.45 ppm lower [THg] than the Central group and 0.92 ppm lower than the Eastern region. In the mainly invertebrate diet group, the differences were greatest, with Western birds having 0.53 and 1.26 ppm lower average [THg] than the Central and Eastern birds, respectively.

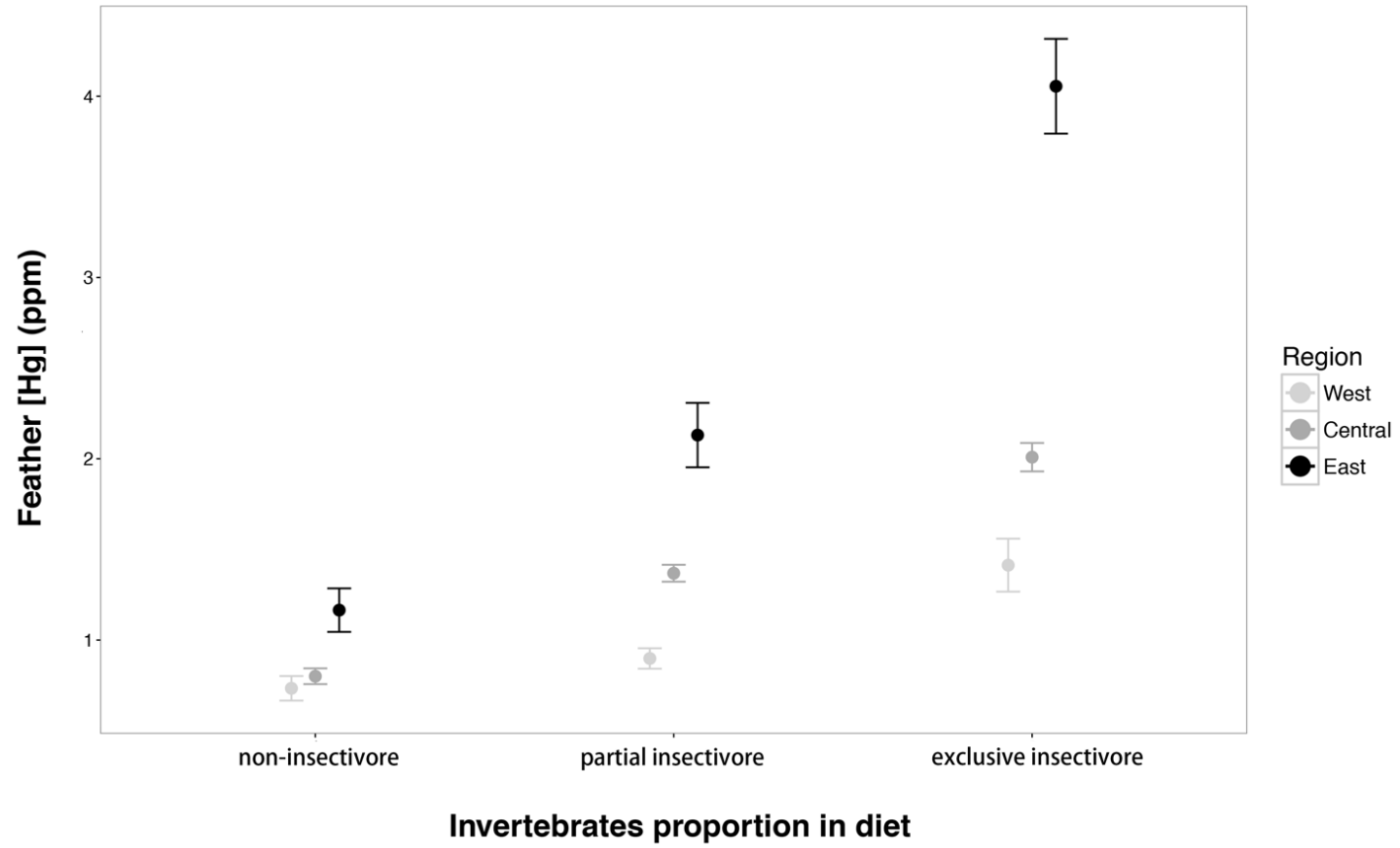


Figure 2.4 Interaction plot for a two-way ANOVA testing dietary and regional effects on feather [THg] in songbirds that breeding in North America. Points represent means for groups, and error bars indicate standard errors of the mean.

2.4 Discussion

2.4.1 Age/sex factors

No apparent age or sex effects on [THg] were found in most of the studied species here. This is not consistent with Common Loon study which found the mean [THg] in male was significantly higher than in female (Evers et al., 1998); but is consistent with the other investigation in seabirds (Becker et al., 2002; Thompson et al., 1991). As females depurate Hg into the eggs (Ackerman et al., 2017), this implies that in songbirds, the amount of Hg lost from the body into eggs might be lower compared with the elimination into feathers during molt. This is consistent with Agusa et al. (2005) who found that the elimination of Hg in gulls was low in maternal transfer and high in feathers. Age or sex does not help explain variation in individual species, suggesting that combining feather [THg] into a pooled dataset for future songbird monitoring projects is possible.

2.4.2 Dietary controls on feather mercury

2.4.2.1 Exclusive insectivores

The exclusive insectivore group has the highest feather [THg] compared to the partial insectivore and non-insectivore groups. Brown Creepers had a highest mean [THg] at 3.05 ppm, which is similar to Rusty Blackbirds (*Euphagus carolinus*) reported elsewhere (Edmonds et al., 2010). The Brown Creeper is an insectivorous bark gleaner, widely distributed in forests in North America and forages primarily on predatory arthropods, including spiders and pseudo scorpions (Poulin et al., 2013), which potentially extend the length of food chain and thus Hg biomagnification. Northern Waterthrushes had the

second higher mean feather [THg] at 2.56 ppm among the species examined and also had been found to have high blood [THg] on both breeding and wintering grounds (Evers et al., 2012). The Northern Waterthrush is a large wood warbler that forages at ground level near wet habitats like bogs, swamps, rivers and streams (Whitaker and Eaton, 2014), which are often considered mercury methylation hot spots (St. Louis et al., 1994, 1996). The high Hg exposure in this species could be the interactive effects of their exclusive insectivorous diets and habitat preference. The Black and White Warbler generally bark forages from the canopy to the ground, and is the only North American wood-warbler that regularly forages along large limbs and trunks of trees. The major food items are lepidopterans, as well as various arthropods, which are predatory invertebrates (Kricher, 2014).

2.4.2.2 Partial insectivores

The wide variation in feather Hg concentrations among the 8 partial insectivorous species might be related to their dynamic dietary selections based on a whole life cycle (Bairlein, 2002; Both, 2010; Studds and Marra, 2011). During migration and winter, they occupy a variety of habitats due to migration schedules and routes. Therefore, individuals may be flexible to food availability (invertebrates, seeds, fruits and other vegetable matter) in response to environmental conditions during migration and overwintering. Thus, their Hg levels in diet may be highly variable, leading to high variation in feather Hg concentrations.

2.4.2.3 Non-insectivores

Most of the non-insectivorous species sampled had the lowest feather [THg] concentrations measured. One exception was the Lincoln Sparrow which is a wetland species that is found mainly in boggy habitats (Ammon, 1995). As they tend to inhabit ecosystems that are promote Hg methylation, their diet which includes only very small proportions of invertebrates may be relatively higher in MeHg than more recognizably insectivorous species.

2.4.3 Geographic patterns

The data collected in this chapter have provided a unique opportunity to evaluate MeHg exposure in migratory songbirds on a national scale. The geographic pattern (increasing from west to east) of feather [THg] in North American passerines was similar to that of the Common Loons, piscivorous fish and bats (Chételat et al., 2018; Depew et al., 2013; Evers et al., 1998). The high [THg] found in songbirds from the Eastern region is consistent with the identification of biological Hg hotspots in northeastern North America (Evers et al., 2007; Jackson et al., 2015). A clear example of this is that the highest [THg] was recorded in an adult female Yellow-rumped Warbler, an insectivore, sampled in Nova Scotia, Canada. This banding station is located near Kejimikujik National Park, one of the biological hotspots identified in Evers et al., (2007), and corresponds to the location of the highest [THg] reported for Rusty Blackbird (*Euphagus carolinus*) feather at 52 ppm (Edmonds et al., 2010). Seven other individuals from this location had elevated mercury ranging from 10.65 to 14.27 ppm. Additionally, my findings a latitudinal difference are consistent with the pattern of total annual Hg deposition that shows the northwestern region of Canada having the lowest total Hg

deposition in North America (Figure 4.2 of Environment and Climate Change Canada., 2016). This suggests that atmospheric Hg deposition may play an important role on MeHg exposure at songbird breeding grounds, as has been found for bats (Chételat et al., 2018).

2.4.4 Conservation implications

No individual bird exceeded the critical [THg] feather of 40 ppm for adverse effects on the piscivorous Common Loon (Evers et al., 2008). However, according to Jackson et al. (2011), 3 ppm in feather has been linked to impaired nesting success, suggesting that potentially 10% of the songbird population may at the very least have impaired nest success due to Hg contamination. In the East region, around 30% of individuals exceeded this threshold, including long distance insectivores such as Black and White Warbler, Brown Creeper, Yellow-rumped Warbler and Northern Waterthrush.

2.5 Conclusions and future directions

The data reported here offer a unique opportunity to evaluate continental-scale patterns (longitudinal: West < Central < East and latitudinal: North < South in the Western region) of feather Hg exposure in a variety of songbird species across their breeding grounds. The findings reinforced the importance of trophic position (the food they eat) as well as the geographic origins (the region they live). This study reveals that the proportion of invertebrates in diet are reflected in Hg concentrations in feathers and a more refined assessment of trophic position for different passerine species would be useful.

These findings do not provide any information on the impacts of Hg exposure on songbird fitness. To more clearly evaluate the risk to the passerine populations, research priority should be given to exclusive/partial insectivores breeding from eastern North American breeding grounds.

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Chapter 3

3 Dietary exposure to methylmercury affects flight endurance in a migratory songbird

3.1 Introduction

Mercury (Hg), a well-known and potent neurotoxin, is one of the most widespread pollutants threatening human and wildlife health (Driscoll et al., 2013). The most bioavailable and bioaccumulative form, methylmercury (MeHg) is of great concern in aquatic (Boening, 2000; Jackson, 1998; Wolfe et al., 1998) as well as terrestrial ecosystems (Cristol et al., 2008). The negative effects of MeHg on avian species of relatively higher trophic positions have been well documented (Evers et al., 2008; Fimreite, 1974; Frederick and Jayasena, 2011), and MeHg exposure has been linked to impaired motor skills and cognitive behaviors in piscivorous birds (Bennett et al., 2009; Kenow et al., 2010; Wolfe et al., 1998). Recent studies suggest that songbirds, particularly insectivores, may experience lower reproductive success (Brasso and Cristol, 2008; Jackson et al., 2011; Varian-Ramos et al., 2014) and disrupted immune function when exposed to MeHg (Hawley et al., 2009; Lewis et al., 2013). Flight performance could also be affected. For example, chronically MeHg-treated Zebra Finches hesitated to forage under high predation risk, suggesting impaired escape flight ability (Kobiela et al., 2015). European Starlings (*Sturnus vulgaris*) exposed to chronic sub-lethal MeHg over 36 weeks molted wing primary feathers more quickly than controls, which may lead to poor feather quality, and these birds also had weaker take-off flight performance (Carlson et al., 2014). Homing Pigeons (*Columba livia*) that were exposed to MeHg prenatally and continually after hatch exhibited flight impairment manifested by less efficient homing,

reluctance to fly, slower flight speed on initial flights (Moye et al., 2016). These findings suggest that MeHg exposure could negatively affect the migration ability of songbirds.

Every year, billions of birds migrate on regional and continental scales between their breeding and wintering grounds, and migration is part of the life cycle of over half of the avian biodiversity in North America (Berthold, 2001). Migration is challenging for birds not only because it requires a large expenditure of energy and is dangerous (unpredictable weather, habitats, disease and predation risk), but also because it requires dramatic changes in behaviors (e.g., nocturnality, diet preference, orientation and navigation) and physiology (body composition, muscle metabolism, digestive morphology and function) (Alerstam and Lindström, 1990; Berthold, 2001). Prior to and during migration, birds are hyperphagic, leading to rapid body mass gain through the deposition of fat and lean mass (Lindström and Piersma, 1993). Hyperphagic migrants could be especially vulnerable to MeHg in sites or regions where forage contains elevated concentrations of MeHg, such as wet and acidic habitats in the boreal forests (St. Louis et al., 1994), industrialized sites (Li et al., 2009; Turner and Southworth, 1999), gold mining areas (Telmer and Veiga, 2009) and agricultural regions (Abeysinghe et al., 2017).

Migration is an energetically demanding component of a bird's life history (Wikelski et al., 2003). The flight phase requires songbirds to exercise continuously at an intensity of about 12 times the basal metabolic rate (Alexander, 1998) for several hours to a few days (Berthold, 2001). Between flights, small passerines generally rest and refuel at stopover sites, and approximately 90% of the migratory period (Newton, 2008) and almost 70% of the total energy required for migration are spent at stopovers (Wikelski et al., 2003). Any factor that inhibits the ability to take off at a steep angle to escape

predators (Williams and Swaddle, 2003) at stopover, or to complete long endurance flights will increase the risk of migration failure. Here, I sought to understand how MeHg bioaccumulation in the hyperphagic state may affect flight performance of small songbirds, a question of vital importance to bird conservation (Seewagen, 2010).

I performed two studies to investigate the effects of MeHg on the Yellow-rumped Warbler (*Setophaga coronata*). In Study 1, I fed diets containing MeHg at concentrations that may be found at contaminated sites to songbirds in a photo-stimulated migratory state, and measured changes in body weight, body composition, and total Hg concentrations (hereafter [THg]) in key tissues. In Study 2, I tested the hypothesis that as a neurotoxin, MeHg would negatively affect burst and endurance flight performance. I predicted that MeHg treated birds would have reduced vertical takeoff speed, and would fly poorly and expend more energy in 2-h wind tunnel tests.

3.2 Materials and methods

3.2.1 Animal care

The Yellow-rumped Warbler is one of the most abundant and widespread songbird species in North America (Hunt and Flaspohler, 1998). It breeds in the boreal and western montane forests of Canada and the United States, and winters in mangroves, scrub, forests, and coffee plantations across a wide geographical range extending from the southern United States to Neotropics. The diet includes small invertebrates and fruits. Yellow-rumped warblers will readily eat formulated diets in captivity and can be flown for many hours in wind tunnels (Guglielmo et al., 2017). Birds (n = 51) were caught by mist nets at Long Point, Ontario, Canada (42°34' 58" N, 80°23'53" W) from 19 September to 10 October 2014, and transported by vehicle to the Advanced Facility for

Avian Research (AFAR), University of Western Ontario (UWO), London, Canada. Birds were collected under a scientific collection permit from the Canadian Wildlife Service (SA-0208), and under Animal Ethics Protocol 2010-216 from the University of Western Ontario Animal Care Committee (Appendix A). They were housed in four large indoor aviaries (2.3W × 2.4H × 3.5L m) until experiments began. All birds were kept healthy under constant environmental conditions at approximately 20°C, 47% of relative humidity (RH), with *ad libitum* access to MeHg-free synthetic diet and water until trials started. The synthetic agar-based mash diet contained 60.2 % carbohydrate, 13.4% protein, and 10.7% lipid (dry mass basis, hereafter dw).

I manipulated the light cycles to simulate seasonal overwintering and migration conditions. In first two months of captivity, birds were kept under a 12 h light:12 h dark (12L:12D) fall migration photoperiod. Then, the photoperiod was switched to a winter photoperiod (9L:15D) in November to break photo refractoriness (Nicholls et al., 1988). By increasing the day length, I stimulated the spring migration phase. Specifically, before each experiment started, birds were changed to a long-day photo cycle (16L:8D) to initiate migratory condition (fattening and restlessness, see below).

3.2.2 Wind tunnel

The hypobaric climatic wind tunnel with adjustable air pressure, temperature and humidity can simulate environmental conditions that birds would experience in flight. It is a specialized tool designed for flying animals in which warblers and other birds will fly individually for many hours (Gerson and Guglielmo, 2011; Guglielmo et al., 2017; Maggini et al., 2017). The wind tunnel has a recirculating design with the test section enclosed in a plenum (about 4W × 2.5H × 5 m). The closed octagonal test section is

1.5W × 1H × 2L m and an open section allows birds and investigators easy access to the closed test section without disturbing the flow (Gerson and Guglielmo, 2011). Birds in this study were flown in dim light conditions and a net was installed at the rear of the open test section to catch any birds that lost control. In the first month of captivity, birds were habituated to the wind tunnel at 15°C, 70% RH, air speed range between 7 and 10 m s⁻¹ for two 15 minute-flights to screen their baseline flight performance. Birds (n = 24) that flew without encouragement with higher flight scores (see Appendix B Table B.1) were assigned to Study 2, the remaining (n = 27) with lower flight scores were assigned to Study 1.

3.2.3 Study 1 Dietary methylmercury dosing

Two dosing concentrations were chosen to simulate relevant environmental MeHg (MeHgCl) diets that migrants may encounter at contaminated stopover sites. A 0.5 ppm (unit: µg/g, hereafter ppm) MeHg wet weight (ww) (validated as 1.56 ppm dw as [THg]) dose was close to the concentrations found in invertebrates (e.g., spiders) at contaminated sites in the United States (Cocking et al., 1991; Cristol et al., 2008; Ortiz et al., 2015), while a 1 ppm wet weight (2.73 ppm dw) dose was related to the most heavily contaminated mining site in China (Abeyasinghe et al., 2017) and industrial terrestrial sites in United States (Talmage and Walton, 1993; Zhang et al., 2012; Zhou et al., 2016). Diet preparation is described in the Appendix B Table B.2.

In February 2015, 27 birds were randomly assigned to Control, 0.5 and 1 ppm groups (9 per group). Birds were housed separately or in pairs from the same diet treatment in cages (70 W × 50 L × 50 H cm). The light cycle was switched from the short-day winter photoperiod (9L:15D) to long-day photoperiod (16L:8D) to initiate migratory condition 1

week before dosing. Control birds were fed a MeHg free diet with validated background [THg] of 0.005 ppm (ww), while other two groups were fed nominal dietary concentrations of 0.5 and 1 ppm MeHg diet, respectively.

I measured body mass and body composition as well as blood [THg], at the start of the experiment (day 0) and then weekly (day 7 and day 14). Body mass was measured with a balance (± 0.001 g) while body composition (fat and lean mass) was measured with a quantitative magnetic resonance body composition analyzer (QMR; Echo Medical Systems) (Guglielmo et al., 2011). About 35 μ L whole blood was taken from the brachial vein using a 26-gauge needle and heparinized capillary tube for [THg] measurement. On day 14 the birds were anesthetized using isoflurane (Baxter, Mississauga, Canada), and decapitated once unresponsive. Kidney, liver, brain, and pectoralis muscle were collected in cryogenic tubes, immediately frozen in liquid nitrogen, and stored at -80° C until analysis. Simple flow chart of experimental design of Study 1 can be found in the Appendix B Figure B.1.

3.2.4 Study 2 methylmercury and flight ability

Based on the results of Study 1, I chose a dose of 0.5 ppm MeHg for Study 2 because the 0.5 ppm dose and the blood Hg levels in this dosing group both represent more realistic songbird MeHg levels in the ecosystems. Beginning on 3 March 2015, each day, two individuals (one Control, one MeHg group) were haphazardly selected and moved from 9L:15D photoperiod into 16L:8D photoperiod (light on at 07:00 am). In total, each of the Control or MeHg groups contained 6 cages of birds (a total of 12 birds per group, maximum two birds per cage). After 13 days of long-day photoperiod adjustment, each bird was measured for baseline takeoff performance. After a 14-day exposure to a long-

day photoperiod, each bird was blood sampled and the Control bird continued to feed on a MeHg-free diet while the MeHg bird was switched to the 0.5 ppm MeHg diet for another 14 days. On day 0, 7 and 14 of diet manipulation the birds were weighed and scanned by QMR for body composition. I re-tested vertical takeoff flight ability on day 14 of dosing in the morning at about 08:00 am. Immediately after the takeoff test, I flew one Control or MeHg bird in the wind tunnel at approximately 10:00 am and the other bird from the pair was rescanned by QMR and flown at about 13:30 pm. For simulating natural migration behavior, the birds were food-deprived at least 1 h before the wind tunnel test. By rotating two birds per day into the treatments I ensured that all birds were exposed to the long day photoperiod and test diets for the same amount of time. A flow chart of experimental design of Study 2 can be found in the Figure B.2 of Appendix B.

3.2.4.1 Vertical takeoff flight

I measured takeoff using a vertical flight chamber (0.4W × 1.8H × 0.4L m) consisting of a metal frame enclosed with white opaque board at the back and side walls and a clear plastic sheet with a grid of squares permitting observation through the front (based on designs of Kullberg et al., 2002 and Chin et al., 2009). Vertical takeoff flight performance was recorded by using a high-speed Motion Pro X4 Plus camera (model No. X4MP-G-4, Integrated Design Tools, Inc., 200 fps) facing the front of the chamber. Each bird was introduced by hand into the chamber from the bottom and then released in a standardized way inside the chamber. After a 10-min rest interval, each bird was tested a second time.

Using the tail tip as a reference point, I assessed two parameters of takeoff flight capacity: 1) vertical flight speed (V , m s^{-1}) and 2) energy expenditure (E , J), which were

estimated as the bird flew 0.5 meter between 50 cm to 100 cm above the bottom of the chamber. I stopped measurement 50 cm from the top of the chamber to ensure that birds were not decelerating at the end of flight. Each bird performed two takeoff flights, and the data from the fastest flight were used for analysis. As one frame covered 1/200 second, $V \text{ (m s}^{-1}\text{)} = \frac{0.5}{\text{number of frames} \times (1/200)}$. To assess energy used, I applied a modified equation according to Williams and Swaddle (2003), mechanical energy change per unit mass $e \text{ (J kg}^{-1}\text{)}$, $e = 1/2 V^2 + gz$, where g is acceleration due to gravity (9.81 m s^{-2}), and z is height (m), here it is 0.5 m. I then multiplied change in instantaneous energy by individual mass (kg) to obtain E , a measure of Joules (J). From Williams and Swaddle (2003), it was suggested a greater E indicates greater levels of mechanical energy gain, indicating stronger takeoff flight performance. When the videos were analyzed, the analysis was blind to dietary treatment types.

3.2.4.2 Wind tunnel flight

Endurance flights were conducted by an independent observer who was unaware of the dosing treatments. Once the wind tunnel reached the desired settings (approximately 8 m s^{-1} , 15°C , 70% RH), the bird was introduced into the test section and the start time was recorded. Air speed was slightly adjusted to maintain a comfortable flight for individual birds. If a bird landed on the floor or was blown backwards, the wind speed was decreased to assist the bird back to flight. True air speed was continuously logged in one-second intervals. After the first 30-min of adjustment, the flight was terminated if the bird could not maintain flight for a continuous 5-min period, otherwise birds were removed 2 h after the flight initiation. All birds were weighed, scanned by QMR, blood sampled and euthanized immediately following the flight.

I assessed five parameters of wind tunnel flight performance: 1) total number of strikes during flight; 2) number of strikes per min in first 30 mins; 3) strike duration and flight duration; 4) average flight speed and 5) energy expenditure. Strikes were defined as mistakes when a bird lost control during flight, meaning that it was blown to the back net or landed on the floor of the test section. Strike duration (min) was the time period when birds were grounded or blown to back nets. Flight duration (min) was calculated by using total period in the wind tunnel minus strike duration. Average flight speed was calculated from the logged air speed during times when the bird was in flight. Total energy during flight (kJ) was measured by summing the loss of fat (g) $\times 39.6$ kJ g⁻¹ and the loss of wet lean mass (g) $\times 5.3$ kJ g⁻¹ measured by QMR. Average rate of energy expenditure/flight power (Watts) was calculated from total energy expenditure and the flight duration (total energy expenditure/flight duration). Cost of transport (COT, kJ km⁻¹) was calculated from the total energy used and flight distance (individual flight speed \times flight duration). Here I only analyzed and reported energy costs for birds that were QMR scanned immediately within 5 min before and after wind tunnel flight. Because the first 30 mins were the most difficult time for birds to adjust themselves in the wind tunnel, I used the median number of strikes per min in first 30 mins to assess their adjustment ability in the wind tunnel.

Prior to Study 2, two birds from the Control group were excluded from flight tests because they accidentally lost tail feathers during routine handling. In total, 10 Control birds and 12 MeHg-dosed birds were included in this takeoff experiment. Data from the first individual tested in the wind tunnel (a Control bird) was excluded because it landed repeatedly on a net I had positioned at the front of the wind tunnel test section. I removed the front net for rest of the wind tunnel flights. I failed to activate the continuous record

of the wind tunnel air speed for one Control bird and one MeHg treated bird, thus, the sample sizes varied based on the specific analyses reported below.

3.2.5 Laboratory analysis

Previous studies show that [THg] in some avian tissues (e.g., blood, egg) is over 90% in the methylated form (Rimmer et al., 2005). Though demethylation may vary among tissues (muscle, kidney and liver) in aquatic species (Kim et al., 1996), generally over 80% in internal tissues of [THg] in Zebra Finch is MeHg (Varian-Ramos et al., 2017). Therefore, I measured [THg] as an index of [MeHg] in blood, tissues in the Biotron, UWO, Ontario, Canada using a Direct Mercury Analyzer (DMA-80, Milestone Inc., Shelton, USA) following US EPA Method 7473 (EPA, 1998). Blood samples were thawed and analyzed directly, while tissue samples were first lyophilized at 40°C for 24 h to reach a consistent weight (Lyph-Lock 6, Labconco, Kansas, USA). I report blood concentrations as a liquid concentration (ww), and tissues on a dw basis. Instrument calibration used certified reference materials (Human Hair IAEA-86 for tissues, Caprine Blood SRM 955c for blood) were used as standards. Laboratory quality control samples included a method blank, certified concentration standard (CCS) and a duplicate sample with each batch of 20 or fewer samples. Quality assurance was presented as mean \pm S.E. Mean percent recovery was $87.63 \pm 0.78\%$ (IAEA-86; n = 3), $104.33 \pm 3.37\%$ (SRM 955c; n = 8), $92.15 \pm 0.98\%$ (DORM-2; n = 3) and $97.85 \pm 0.81\%$ (CCS; n = 36) during the running of the samples reported here. The relative percent difference between duplicates (n = 10 pairs) was $6.84 \pm 1.40\%$ for all samples with concentrations greater than 10 times the minimum detection limit.

3.2.6 Statistical analysis

I used the `ez ANOVA` function from the `ez` package in R statistical software for 2-way repeated measures ANOVA with Type III sum of squares to test for changes in blood [THg] among groups and among treatment periods. Linear regression models were used to examine the relationships between blood [THg] and [THg] of other tissues. One-way ANOVA was applied for each tissue [THg] among treatments on day14 and Tukey's post hoc tests were used to assess differences among groups.

Due to hyperphagia induced by long-day photoperiod, birds significantly increased body mass between the initial and final takeoff flight tests, which could affect (reduce) takeoff performance. Thus, I used ANCOVA with Type III SS using change in body mass as a covariate to determine whether there was a difference in the pre to post dosing change in speed and energy expenditure between treatment groups.

For wind tunnel flights, I used a non-parametric test (Wilcoxon rank-sum test) to test for differences between dosing groups in flight duration and strike duration. As the pre-flight body mass did not affect ($P > 0.1$) measures of energy expenditure and flight speed in ANCOVA, I dropped this variable and tested for treatment differences with a Welch two-sample t-test. To estimate the effect threshold of blood [THg] on the number of strikes in first 30 min in the 0.5 ppm treatment I used the `drc` package in R (`drc ()` function, I chose 3 parameter model function `LL.3()` for the fitted model since the lower limit in my data was 0 for no strikes) for dose-response analysis.

All data were analyzed using R Version 3.3.1. Sex and age were not considered, as most of individuals were females and juveniles. Results were represented as mean \pm S.E.

I interpreted a P value < 0.05 in Study 1 because I did not have a priori expectations of tissue differences and P value < 0.1 as statistical significance in Study 2, since I predicted MeHg would negatively affect flight performance.

3.3 Results

3.3.1 Body mass and body composition

In Study 1, there was no significant difference in the rate of body mass gain ($F_{2,24} = 0.03$, $P = 0.97$) or body composition change (Fat: $F_{2,24} = 0.28$, $P = 0.76$; Lean: $F_{2,24} = 2.07$, $P = 0.15$) among groups (Table 3.1). The same pattern was found in Study 2 (Body mass: $F_{1,22} = 0.23$, $P = 0.64$; Fat: $F_{1,22} = 0.49$, $P = 0.49$; Lean: $F_{1,22} = 0.01$, $P = 0.94$).

Table 3.1 Body mass and body composition of yellow-rumped warblers over two weeks feeding on Control, 0.5 ppm and 1.0 ppm MeHg diets (Mean \pm S.E., unit: gram). No significant difference was detected in the rate of body mass gain ($F_{2,24} = 0.03$, $P = 0.97$ for Study1; $F_{1,22} = 0.23$, $P = 0.64$ for Study2) or body composition change (Fat: $F_{2,24} = 0.28$, $P = 0.76$, Lean: $F_{2,24} = 2.07$, $P = 0.15$ for Study 1; Fat: $F_{1,22} = 0.49$, $P = 0.49$, Lean: $F_{1,22} = 0.01$, $P = 0.94$ for Study 2) among groups.

| | Day 0 | | | Day 7 | | | Day 14 | | |
|---------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | 0ppm Diet | 0.5ppm Diet | 1ppm Diet | 0ppm Diet | 0.5ppm Diet | 1ppm Diet | 0ppm Diet | 0.5ppm Diet | 1ppm Diet |
| Study1 | | | | | | | | | |
| Mass | 10.8 \pm 0.2 | 10.9 \pm 0.2 | 10.8 \pm 0.3 | 11.5 \pm 0.4 | 11.7 \pm 0.4 | 12.0 \pm 0.6 | 13.6 \pm 0.2 | 13.2 \pm 0.5 | 13.3 \pm 0.4 |
| Fat | 0.8 \pm 0.1 | 0.8 \pm 0.2 | 0.8 \pm 0.1 | 1.5 \pm 0.4 | 1.1 \pm 0.3 | 1.5 \pm 0.4 | 3.0 \pm 0.2 | 2.5 \pm 0.4 | 2.6 \pm 0.4 |
| Lean | 7.7 \pm 0.1 | 8.0 \pm 0.1 | 8.0 \pm 0.2 | 7.8 \pm 0.1 | 8.6 \pm 0.1 | 8.4 \pm 0.2 | 8.6 \pm 0.2 | 8.6 \pm 0.1 | 8.6 \pm 0.2 |
| Study2 | | | | | | | | | |
| Mass | 12.1 \pm 0.4 | 11.7 \pm 0.3 | NA | 13.3 \pm 0.4 | 13.0 \pm 0.3 | NA | 13.0 \pm 0.4 | 13.1 \pm 0.3 | NA |
| Fat | 1.9 \pm 0.3 | 1.6 \pm 0.3 | NA | 3.1 \pm 0.3 | 2.8 \pm 0.2 | NA | 2.8 \pm 0.3 | 2.9 \pm 0.2 | NA |
| Lean | 8.3 \pm 0.2 | 8.2 \pm 0.2 | NA | 8.3 \pm 0.1 | 8.4 \pm 0.1 | NA | 8.3 \pm 0.2 | 8.4 \pm 0.1 | NA |

NA represents “not applicable” here.

3.3.2 Blood and tissue total mercury

Blood [THg] was affected by diet treatment ($F_{2,24} = 372.87$, $P < 0.0001$) and dosage period ($F_{2,48} = 889.12$, $P < 0.0001$), and there was a significant interaction between treatment group and dosage period ($F_{4,48} = 286.10$, $P < 0.0001$). Blood [THg] was close to zero on day 0 in all groups, and remained consistent in Controls throughout the experiment. After 7 days of feeding on MeHg diets, blood [THg] in 0.5 ppm group was 4.9 ± 0.3 ppm while in 1 ppm group blood [THg] was 10.8 ± 0.4 ppm. On day 14, blood [THg] in 0.5 ppm group was 9.4 ± 0.3 ppm and in 1ppm group [THg] was 17.6 ± 0.8 ppm (Figure 3.1). On day14 of Study 1, all tissues showed significant differences among treatment groups (Table 3.1). Patterns of Hg bioaccumulation in key organs and muscles were similar to that observed in blood, but [THg] was much greater (about 24-fold) after 14 days in liver, over 10-fold in brain and muscle, and over 38-fold in kidneys comparing to the diet (dw to dw). Also, I found strong correlations between blood and tissue [THg] (Table 3.2).

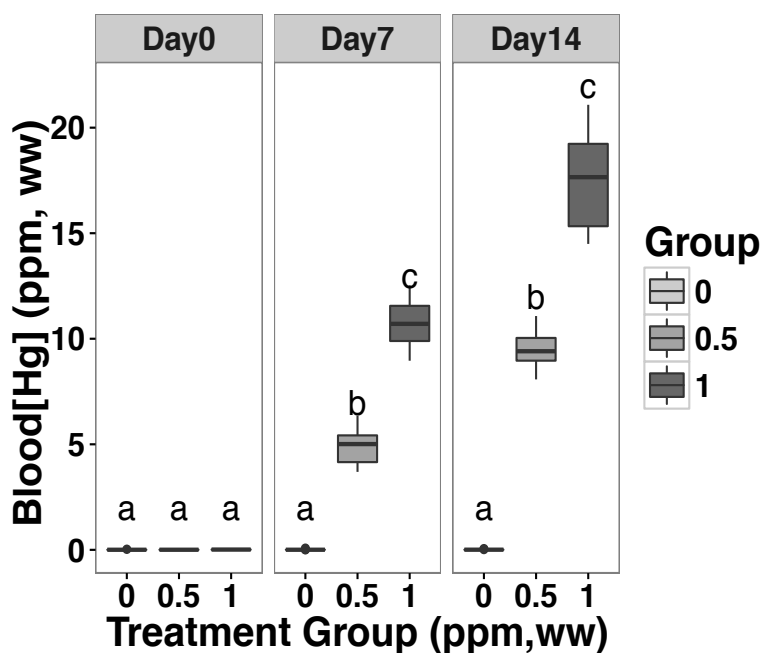


Figure 3.1 Total blood mercury concentration ([THg] ww) of Yellow-rumped Warblers over two weeks of dietary dosing with MeHg. Box plots show the distribution of blood [THg] from Control (0), 0.5, 1 ppm treatments on day 0, 7, and 14 of dosing (n = 9 per group). The line in the middle of the box represents the median, and the lower and upper ends of the box are the 25% and 75% quartiles, respectively. The lines indicate 1.5 times the size of the hinge, which is the 75% minus 25% quartiles. Different letters indicate statistically significant Tukey's post hoc test differences among groups.

Table 3.2 Total mercury concentration ([THg], Mean \pm S.E., unit: $\mu\text{g/g}$, ppm, dw) of Yellow-rumped Warbler tissues after 14 days feeding on 0, 0.5 and 1.0 ppm MeHg diets (ppm, ww). Equations to predict individual tissue [THg] (y, dw) from blood [THg] (x, ww) are provided. Linear regression models were used to examine the relationships between blood [THg] and tissue [THg]s. One-way ANOVA was applied for each tissue[THg] among groups and Tukey's post hoc tests were used to assess difference among groups.

| Tissue[Hg] | 0 ppm Diet | 0.5 ppm Diet | 1 ppm Diet | One-way Anova | Equation | R ² |
|------------|----------------------------|-----------------------------|------------------------------|---------------------------|------------|----------------|
| Kidney | 0.1 \pm 0.0 ^c | 59.6 \pm 2.8 ^b | 111.9 \pm 5.3 ^a | F _{2,24} = 262.6 | y = 6.30 x | 0.98 |
| Liver | 0.0 \pm 0.0 ^c | 38.6 \pm 2.9 ^b | 64.9 \pm 6.0 ^a | F _{2,22} = 71.2 | y = 3.78 x | 0.97 |
| Brain | 0.0 \pm 0.0 ^c | 20.3 \pm 1.4 ^b | 40.2 \pm 2.3 ^a | F _{2,24} = 170.6 | y = 2.23 x | 0.97 |
| Muscle | 0.0 \pm 0.0 ^c | 19.9 \pm 1.3 ^b | 38.4 \pm 2.3 ^a | F _{2,24} = 153.0 | y = 2.17 x | 0.99 |
| Blood | 0.0 \pm 0.0 ^c | 9.4 \pm 0.3 ^b | 17.6 \pm 0.8 ^a | F _{2,24} = 330.9 | NA | NA |

Because the intercepts of equations were not significantly different from zero, I reported equations without intercepts. All p values of regressions, one-way ANOVA and post doc tests were < 0.0001 . All treatments differed from each other within a tissue type as indicated by different superscripts. NA represents "not applicable" here.

3.3.3 Vertical takeoff performance

The means of changes in speed (post minus pre) were $-0.09 \pm 0.14 \text{ m s}^{-1}$ in the Control group (range: -0.63 to 0.94) and $-0.29 \pm 0.06 \text{ m s}^{-1}$ in the MeHg group (range: -0.67 to 0.08). There was no effect of the MeHg ($F_{1,19} = 1.43$, $P = 0.25$) on speed change after controlling for the effect of body weight change ($F_{1,19} = 5.10$, $P = 0.04$). Changes of energy expenditure (E) were significantly related to the change in mass ($F_{1,19} = 10.12$, $P = 0.01$), but did not differ between treatments ($F_{1,19} = 1.30$, $P = 0.27$): range: -0.01 to 0.02 J in the Control group and 0.01 to 0.02 J in the MeHg group.

3.3.4 Endurance flight performance

Birds fed the 0.5 ppm MeHg diet for 14 days during migratory hyperphagia had a significantly shorter flight duration (Control: 118.81 ± 0.86 mins, range: 111.97 to 119.99 ; MeHg: 98.41 ± 11.87 mins, range: 8.08 to 120.00 ; $W = 80$, $P = 0.04$) and longer strike duration (Control: 0.86 ± 0.57 mins, range 0 to 5.40 ; MeHg 3.99 ± 1.69 minutes, range: 0 to 21.15 mins; $W = 20$, $P = 0.04$). There was no effect of MeHg on flight speed (Control $8.14 \pm 0.01 \text{ m s}^{-1}$, $n = 8$; MeHg $8.11 \pm 0.01 \text{ m s}^{-1}$, $t = 1.54$, $df = 15.15$, $P = 0.14$, $n = 11$). Three birds from MeHg group could not complete a full 2-h flight because they had many strikes in the first 30 mins, causing me to stop the test to prevent injury; while all 9 of 9 control birds finished their flights. Thus, I did not report the total strikes during flight here but instead, to standardize the strikes in all birds, I assessed the median number of strikes per min in first 30 min, and I found a significant difference between treatments ($W = 20$, $P = 0.02$; Figure 3.2). The mean of the Control group was 0.04 ± 0.02 (range: 0 to 0.17), while in MeHg group was 0.33 ± 0.12 (range: 0 to 1.17) strikes

per min. Number of strikes per min during first 30 min of flight in MeHg group was non-linearly related to [THg] in blood, suggesting a threshold effect near 11 ppm (Figure 3.3).

I only used the individuals (6 for Control and 4 for MeHg) that flew over 90 minutes with QMR scans made immediately before and after flights (afternoon only). With limited sample sizes, I did not detect any effect of MeHg on energy expenditure calculated as flight power ($t = -1.31$, $df = 4.04$, $P = 0.26$) or COT ($t = -1.23$, $df = 4.09$, $P = 0.28$). Yet, the mean COT in Control ($0.19 \pm 0.02 \text{ kJ km}^{-1}$, range: 0.15 to 0.26) tended to be lower than in the MeHg group $0.25 \pm 0.04 \text{ kJ km}^{-1}$ (range: 0.16 to 0.36). In the Control group the mean flight power was $1.57 \pm 0.14 \text{ Watts}$ (range: 1.24 to 2.10), while the mean of MeHg group was $2.06 \pm 0.35 \text{ Watts}$ (range: 1.30 to 2.90).

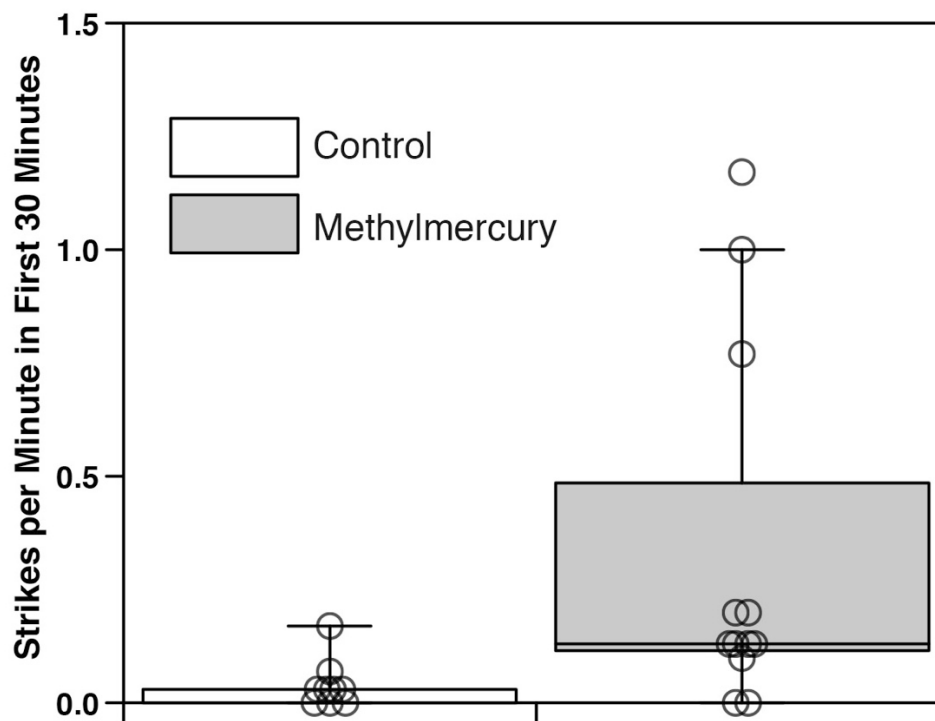


Figure 3.2 Strikes per minute (in first 30 mins) of Yellow-rumped Warblers flying in a wind tunnel after feeding for 14 days on Control and MeHg diets. A significant difference between treatments ($W = 20$, $P = 0.02$; Figure 3.2) was found: the mean of the Control group (0 ppm ww) was 0.04 ± 0.02 ($n = 9$, range: 0 to 0.17), while in MeHg group (0.5 ppm ww) was 0.33 ± 0.12 ($n = 12$, range: 0 to 1.17) strikes per min. Boxes are 25th and 75th percentiles with median indicate by the line. Whiskers are 5th and 95th percentiles. Open circles are individual data points, in some cases offset for clarity where there are multiple points with the same value.

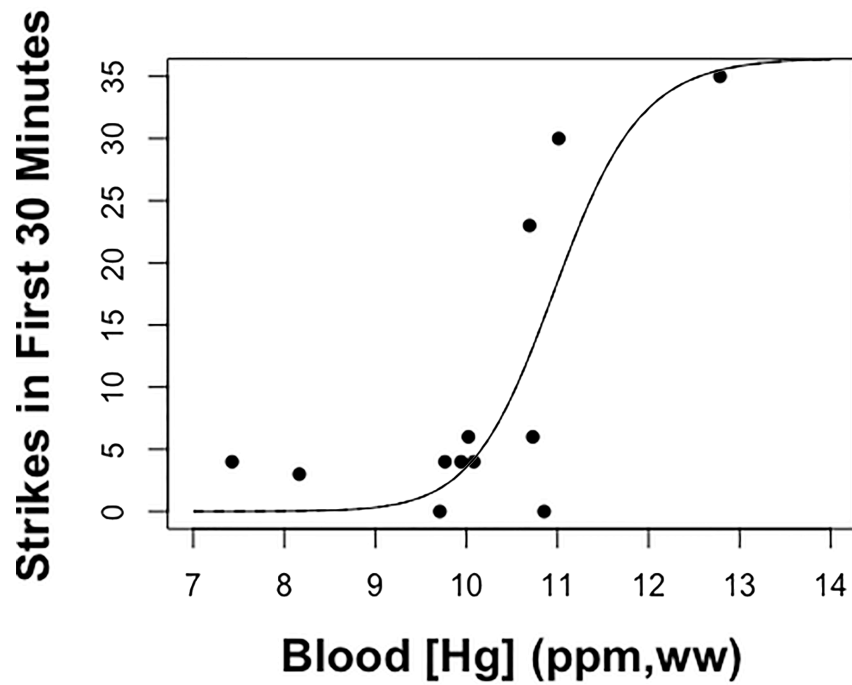


Figure 3.3 A non-linear regression between blood total mercury [THg] and strikes per minute (in first 30 mins) during flight in a wind tunnel of Yellow-rumped Warblers fed for 14 days on a MeHg diet. Black dots are individual data points in MeHg group (0.5 ppm ww, n = 12). The predicted number of strikes

$$= \frac{36.50}{(1 + e^{-23.61 \times (\ln(\text{blood [THg]}) - 10.99)})}$$

3.4 Discussion

These experiments showed that in the migratory state, hyperphagic warblers exposed to MeHg rapidly accumulated Hg to extremely high concentrations in blood, muscles, and organs. I also demonstrated experimentally that MeHg-treated birds had more difficulty flying in a wind tunnel, reduced ability to complete an endurance flight, and tend to have increased energy costs. These results suggest that songbirds undergoing premigratory mass gain, or stopping to refuel in MeHg contaminated sites, may potentially bioaccumulate MeHg in tissues to levels that will negatively affect their ability to migrate successfully.

Methylmercury bioaccumulation rates vary among avian species (Bennett et al., 2009; Carlson et al., 2014; Kenow et al., 2007b; Varian-Ramos et al., 2014). The piscivorous species that have been studied have generally been measured at chick or juvenile stages (Ackerman et al., 2011, Kenow et al., 2007b) where feather molt (the main pathway for MeHg depuration) was involved during exposure. This makes the bioaccumulation rate difficult to compare with my study. In general, compared to my hyperphagic warblers, MeHg accumulation is much slower in species with larger body size (indicating larger body pool compartments) and lower metabolism rate. For example, captive adult American Kestrels (*Falco sparverius*) (about 100 g in body mass) accumulated Hg in blood to about two times greater than its dosage concentration (3 ppm, dw) through diet in the first two weeks of dosing (Bennett et al., 2009). Other songbird species in a non-migratory state took months to reach a 10-fold MeHg accumulation in blood (Carlson et al., 2014; Varian-Ramos et al., 2014). Conversely, my migratory warblers accumulated Hg close to 10 times in blood in only one week and then approximately 20 times in the

second week of dosing. The extremely high Hg level accumulated in a short time period observed in my study may be due to an increased food intake and a high metabolic rate in these small migrants while in a migratory state. Also, the feather molting process has been suggested as an important way to excrete MeHg as MeHg binds tightly and stably with feather keratin (Braune and Gaskin, 1987; Lewis and Furness, 1991; Thompson and Furness, 1989). My birds did not molt during the period of this study; thus they did not lose any MeHg through this process. In the same scenario, migratory birds generally finish their molt before they begin migration (Barta et al., 2008). If a bird is on migration and becomes exposed to MeHg at a stopover site, it might keep its mercury burden until migration is over, possibly passing MeHg on to offspring through eggs (Ackerman et al., 2017) before it molts again. Thus, when migrants encounter toxicants at their stopover sites, they may not be able to eliminate Hg through this mechanism.

Blood [THg] is a commonly used indicator of Hg burden in avian species (Evers et al., 2005; Jackson et al., 2015). Previous field studies suggest that blood [THg] is highly correlated to other tissues in several piscivorous bird species (Kenow et al., 2007a; Eagles-Smith et al., 2008). However, little is known about how blood mercury is correlated to tissues in migratory passerines. My study showed blood [THg] linearly predicted muscle and organ [THg], and my estimated equations will provide insights to estimate songbird Hg body burden in the field. The results of my study are consistent with previous studies showed that [THg] in captive Great Egret (*Ardea alba*) nestlings (Spalding et al., 2000) was greatest in the kidney and the liver, followed by brain and muscle. This similar trend was consistently found in four other wild water bird species (Eagles-Smith et al., 2008). In general, the liver is a potential detoxification organ and the

kidney is a major reservoir of inorganic Hg in birds. A conservative estimate of the suggested threshold of liver Hg concentrations to maintain healthy aquatic avian species is below 10 ppm, ww (Wolfe et al., 1998). The [THg] in my warblers was much higher than this. The high internal tissue concentrations recorded in the warblers in this study have been found to cause mortality in other avian species (Finley et al., 1979; Scheuhammer, 1988; Spalding et al., 1994; Wiemeyer et al., 1987). My warblers did not show any clinical signs of toxicity during the two-week dosing. Other vertebrates, such as mink (*Neovison vison*) (Aulerich et al., 1974) and Red-tailed Hawk (*Buteo jamaicensis*) (Fimreite and Karstad, 1971) did not show any signs of Hg toxicity until after three weeks. Even Zebra Finches dosed for 2 months at the same dosing level I used showed no mortality (Scheuhammer, 1988). It is possible that warblers have a similar latency. The highest blood [THg] reported among breeding songbirds in North America is around 15 ppm, ww (Jackson et al., 2015) and my warblers were in the high end range after two-weeks of dosing.

Extensive migrations involving continuous fasting flight for long periods of time are undertaken by many small songbird species. They consume large amounts of food to restore their energy and nutrients at stopover sites to fuel their migrations (McWilliams et al., 2004). Insectivorous birds are likely at a higher risk due to the high MeHg concentrations that have been found in insects and other arthropods in mercury contaminated environments (e.g., Cristol et al., 2008). My study showed that during the period of greatest food consumption, songbirds have the potential to rapidly accumulate MeHg at their stopovers. When fasting, Zebra Finches catabolized large amounts of lean tissue which was associated with elevated blood [THg] (Seewagen et al., 2016). During

flights, birds may also catabolize body protein (Gerson and Guglielmo, 2011) releasing MeHg bound to proteins and then causing neurotoxicity. When birds fly for many hours, the stored MeHg in other compartments such as organs may be released into blood stream as well. Maintenance of oxygen delivery is an essential pathway for avian endurance flight because flight requires efficient oxygen delivery to working muscles during intense exercise (Brackenbury, 1984). Once MeHg enters the circulatory system, it can bind to the thiol groups in hemoglobin (Brackenbury, 1984; Vallee and Ulmer, 1972), decreasing the oxygen carrying capacity of the blood. The inhibited flight capacity observed in the MeHg treated warblers may be suggestive of a reduced amount of oxygen being transported to the flight muscles. Additionally, MeHg is a known neurotoxin and readily transports across the blood-brain barrier. The neurological damage could disrupt motor nerve conduction or impair navigational abilities that migratory birds rely on during their journey. This study showed evidence of neurological impairment, with MeHg-treated birds flying more erratically with a higher number of strikes in the wind tunnel. This indicates that birds exposed to high concentrations of MeHg may be at greater risk during migration due to more erratic behaviors and inefficient flying with potentially higher energy costs, in addition to possible navigational deficiencies such as those described by Moye et al., (2016).

Although songbirds store large amounts of fuel, mainly as fat, in preparation for migratory departure, the long total distance of many journeys requires songbirds, especially Neotropical migrants, to rest and refuel at stopover sites several times before they reach their destination. With more erratic flight behaviors and inefficient flying, in addition to possible navigational deficiencies, migrants might take more time to arrive at

stopover sites. Thus, even though mercury dosing does not appear to alter body mass and body composition during refueling at stopover, delays due to poor flight ability could result in arrival at poor-quality habitats with limited food resources, which can lead to poor body composition. As a result, these may affect their successive journeys and breeding/overwintering performance. Moreover, birds must search vigorously for food in preparation for overnight or even multi-day flights. Kobiela et al., (2015) suggested that MeHg-exposed Zebra Finches experience hyper-sensitivity in the presence of predators, which might lead to longer waiting time to forage and result in loss of mass in the wild. Other laboratory studies also showed that MeHg can reduce the motivation to fly or decrease appetite to cause weight loss (Spalding et al., 2000). These patterns may indicate a longer refueling time or a lower refueling rate at stopover sites for individuals with a higher MeHg burden. Late arrival time and poor refueling performance may lead to a less competitive migration performance, a longer stopover duration and a poor body condition. It may be even more challenging for migrants that have to cross large ecological barriers, such as the Gulf of Mexico and the Sahara Desert. For heavily Hg-burdened birds, the ability to complete a non-stop flight covering thousands of kilometers and lasting many hours or days could be significantly compromised. In fact, recent field data indicate that high Hg exposure during the summer breeding season was associated with reduced survival during migration and overwintering periods in Blackpoll Warblers (*Setophaga striata*) and American Redstarts (*Setophaga ruticilla*) (Ma et. al, 2018; Chapter 4).

These laboratory studies have provided insights into potential adverse effects of MeHg on flight and migration in small migratory songbirds. Future research should focus on

longer flights to test for limits to endurance and differences in energy expenditure. My study suggested a higher energetic cost in the MeHg treated group, but it was not statistically different in a 2-h flight window with my limited sample size of suitable flights. Real migratory flights last for many hours with limited food/water availability and are substantially more challenging. Since migratory journeys range from short flights to non-stop flights lasting several days, it is important to understand how elevated MeHg burdens could impact the maximum endurance capacity for long distance migrants. To bring together the effects of MeHg burdens to migratory birds in the field, birds should be tracked following known exposure to MeHg.

To my knowledge, this is the first study to demonstrate the potential consequences of environmentally relevant short-term MeHg exposure on migratory songbird flight ability. The rapid bioaccumulation of Hg that I documented suggests that even songbirds originating from uncontaminated breeding or wintering areas (where exposure is chronic) could quickly become impaired if they refuel at a highly contaminated stopover site. Dietary MeHg exposure may not just have adverse effects on avian reproduction, but also migration.

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Chapter 4

4 Evidence of negative seasonal carry-over effects of breeding ground mercury exposure on survival of migratory songbirds

4.1 Introduction

Mercury (Hg) is a significant global pollutant that reaches remote areas such as polar regions and northern boreal forests through atmospheric deposition and other processes (Driscoll et al., 2013). The bioaccumulating form, methylmercury (MeHg), is a potent neurotoxin that causes adverse effects in humans and wildlife (Chan et al., 2003; Wolfe et al., 1998), particularly at higher trophic levels (Lavoie et al., 2013; Suedel et al., 1994). Mercury methylation occurs in anaerobic lake sediments and bottom waters (Gilmour et al., 1992), as well as in parts of watersheds such as wetlands with high moisture, low pH, and high organic matter content (Driscoll et al., 2007; Grigal, 2003; Rimmer et al., 2005). These conditions are common in the boreal forests of North America which are also the main breeding regions for migratory songbirds on that continent (Wells and Blancher, 2011).

Adverse effects of high doses of Hg on captive fish-eating birds have been well documented, including impaired growth and reproduction (Borg et al., 1970; Heinz, 1979; Spalding et al., 1994), however, population declines in wild piscivorous birds have not been directly attributed to Hg (Bustamante et al., 2016; Henny et al., 2017; Mitro et al., 2008; Pollet et al., 2017). Presumably, songbirds with relatively lower apparent trophic positions, should be less affected in terms of survival. However, trophic status, as well as the structure of the primary food webs supporting terrestrial songbirds have

seldom been determined. In fact, terrestrial passerines could be exposed to MeHg levels as high as obligate fish-eating species through the consumption of emergent aquatic insects, by foraging on higher trophic-level insect predators such as spiders (Cristol et al., 2008), and/or by living in environments with high MeHg availability (Abeyasinghe et al., 2017; Edmonds et al., 2010; Rimmer et al., 2005; Townsend et al., 2013). In addition, many songbirds make long-distance migrations during which they face substantial physiological and behavioral challenges associated with flight, refueling, navigation, and predator avoidance that can increase their risk of mortality (Newton, 2008). Thus, migration is an extremely challenging period for birds during which the highest rates of mortality may be observed (Paxton et al., 2017; Rushing et al., 2017; Sillett and Holmes, 2002).

Nestling birds grow feathers at the natal site, and in North America, most adult passerines undergo a complete feather molt prior to autumn migration (Rohwer et al., 2005). After this, songbirds generally undergo a partial (prealternate) body molt on the non-breeding grounds, but retain their summer-grown flight feathers (Pyle, 1997). Growing feathers are connected to blood vessels during formation, and MeHg that has accumulated in the body is transferred from other compartments (e.g., liver, kidney) into feathers (Braune and Gaskin, 1987). Once feathers mature, they become metabolically inactive and MeHg levels are stable (Appelquist et al., 1984; Bond et al., 2015). In fish-eating species, it is widely accepted that feather Hg concentrations represent the Hg body burden (Bearhop et al., 2000; Furness et al., 1986) and are highly correlated with Hg in other tissues (Braune 1987; Burger 1993). Therefore, with non-destructive sampling and easy acquisition at any time before the next complete molt, tail feathers, for example, can

be assessed as a proxy for Hg body burden at or near the breeding grounds prior to autumn migration.

Methylmercury has been demonstrated to impair bird immune function (Hawley et al., 2009; Lewis et al., 2013), foraging behavior (Kobiela et al., 2015), navigation (Moye et al., 2016), and flight ability (Carlson et al., 2014; Ma et al., 2018; Chapter 3), which could be particularly detrimental during long-distance migration. In this study, I used songbird feathers grown prior to autumn migration to examine the link between Hg exposure at breeding grounds and songbird survival until the subsequent spring return migration (a seasonal carry-over effect). I measured tail total feather concentrations (hereafter [THg]) in birds stopping to refuel at the same Canadian banding station in autumn and the following spring migration seasons. I hypothesized that high Hg exposure during summer reduces survival between departure from and return to the breeding grounds the following year. I therefore predicted a shift in the distribution of species-specific feather Hg values towards lower means in the spring (Figure 4.1) and expected this to be particularly apparent in species undergoing long migrations.

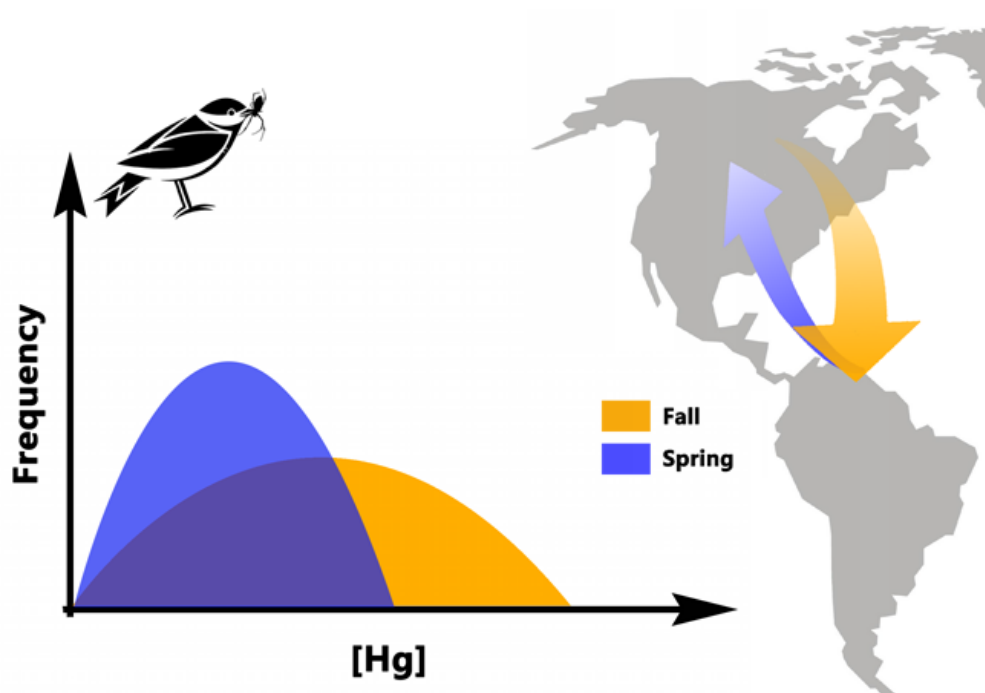


Figure 4.1 Proposed hypothesis given to explain mercury body burden reflects the annual population shift in migratory songbirds. It is hypothesized that mercury exposure on or near the breeding ground before autumn migration (reflected as total mercury concentration in feather [Hg]) negatively affects migration and overwinter survival. Thus, individuals with high Hg burden would be less likely to return, resulting a left shift in the population-level distribution of feather Hg values towards lower concentrations in the spring. Orange represents the autumn population while blue represents the spring population.

4.2 Materials and methods

4.2.1 Study species

Foraging guild is a commonly recognized predictor of Hg accumulation (Eagles-Smith et al., 2009), and I was specifically interested in the effects of [THg] on migration and overwintering success. I assumed the longer the migration distance, the more taxing this period would be for birds. I selected five songbird species based on their varied foraging guilds (De Graaf et al., 1985) and migration distances (Rodewald, 2015). Blackpoll Warbler *Setophaga striata*, breeds broadly across northern North America, and is known for its extraordinarily long transoceanic non-stop flights in the autumn from eastern North America to wintering areas in South America (DeLuca et al., 2015). As an insectivorous, upper-canopy gleaner, it mainly inhabits boreal forests in the summer. This species is thought to use a “loop migration” pattern (see Figure. 6 in Holberton et al., 2015, eBird Occurrence Maps, <http://ebird.org/content/ebird/occurrence/blackpoll-warbler/>). The American Redstart *Setophaga ruticilla*, is a common long-distance (primarily overland) migrant. As a lower canopy, insectivorous gleaner, it breeds in forests throughout North America, then migrates and stops over in a variety of habitats, and overwinters in Central to northern South America (Sherry et al., 2016). The Ruby-crowned Kinglet *Regulus calendula*, is a small lower-canopy, insectivorous gleaner that commonly breeds in spruce-fir forests in the northwestern United States and across Canada. Most kinglets migrate to the southern and southwestern United States and Mexico for the winter, but some simply move to lower elevations for winter. Thus, it is a short-distance migrant or resident in some cases (Swanson et al., 2008). The Swainson’s Thrush *Catharus ustulatus* is a long-distance migratory omnivore, that mainly forages on the ground in forested

habitats. This species breeds as far north as Alaska and northern Canada and winters primarily in northern South America (Mack and Wang, 2008). The White-throated Sparrow *Zonotrichia albicollis*, is a short- to medium-distance migrant that is primarily granivorous and forages mostly on the ground. It breeds widely across the boreal forests in North America and migrates mainly to the southern United States (Falls and Kopachena, 2010).

My study was conducted at Long Point Bird Observatory (LPBO, 42°34' 58" N, 80°23'53" W), Ontario, Canada. The location of this site allows collection of feather samples in both autumn and spring migration, particularly for the species in my study (see Appendix C for specific details of Blackpoll Warblers). Birds were captured by mist nets in autumn 2014 (September and October) and spring 2015 (April-June). For each bird, the fourth rectrix from each side of the tail was collected and stored in labeled paper envelopes until analysis. Sex and age class (juvenile: “hatching year” birds in autumn and the “second year” cohort in spring; adult: “after hatch-year” in autumn and “after second-year” cohort in spring) were determined through plumage, examination of skull pneumatization, and other features (Pyle, 1997).

4.2.2 Mercury determination

Based on the literature, the fraction of feather total Hg concentration as MeHg is greater than 95% (Rimmer et al., 2005; Edmonds et al., 2010). Thus, I used total [Hg] as a measure of MeHg body burden represented in feathers. Before analysis, feather samples were cleaned with 1% acetone and rinsed with 18.2 MOhm deionized water and dried at ambient temperature. Because of their light weight, feathers were covered by aluminum foil and measured intact using a direct mercury analyzer (DMA-80, Milestone Inc.,

USA), following US EPA Method 7473 (EPA, 1998) at the Biotron Centre for Experimental Climate Change Research, University of Western Ontario (UWO), London, Ontario, Canada, an ISO 17025 accredited facility. Instrument calibration utilized certified reference material (IAEA-86-Human Hair) as an accepted standard with similar matrix properties to the samples. Laboratory quality control samples included a method blank, calibration check standard (CCS) by using aqueous standard, and a duplicate every 20 samples at minimum. The quality assurance was presented as Mean \pm S.E. Mean percent recovery of IAEA-86 was $88.96 \pm 2.94\%$ ($n = 9$) and CCS $96.54 \pm 1.45\%$ ($n = 27$) during the running of the samples reported here. The relative percent difference between duplicates was $11.44 \pm 1.14\%$ ($n = 27$). The differences of duplicates were within acceptable range because of low sample amount for individual feathers (0.01 to 0.001 gram). All samples had concentrations greater than 10 times the minimum detection limit of the method.

4.2.3 Blackpoll Warbler feather deuterium analysis

According to the hypothesis, Blackpoll Warbler is most likely to be at risk from Hg toxicity based on their most challenging long-distance migration (DeLuca et al., 2015). Feather stable H isotope ratio ($\delta^2\text{H}_f$), a valuable geographic marker in North America, can be assigned probabilistically to the regions where feathers could have grown (Hobson and Wassenaar, 1996). For Blackpoll Warblers, I expected an approximate range from -190 to -90‰ for flight feathers grown on the breeding grounds (Hobson and Wassenaar, 1996). Thus, I measured $\delta^2\text{H}_f$ in Blackpoll Warbler feathers to ensure that the individuals sampled in both migration seasons originated from similar geographic locations, and if the feathers sampled in the spring had indeed been grown during the previous breeding

season prior to migration. If individuals were generally from the same breeding origins in spring and autumn (i.e. with similar $\delta^2\text{H}_f$), then my assay would provide stronger evidence of the potential effects of Hg burden. To further test origins of the two groups measured, I also include a probabilistic assignment of molt origins. A detailed description on this technique and the results can be found in Appendix C.

Prior to isotopic analysis, feathers were rinsed with 2:1 chloroform: methanol to remove surface oils and dried at ambient temperature overnight in a fume hood. I employed the comparative equilibration approach described in Wassenaar and Hobson, (2003) to determine the $\delta^2\text{H}_f$ value of the non-exchangeable hydrogen of feathers by using two calibrated keratin hydrogen-isotope reference materials from the USGS (EC-01: -197‰ , EC-02: -54.1‰) at the LSIS-AFAR facility, UWO. I performed $\delta^2\text{H}$ measurements on H_2 derived from high-temperature (1350°C) flash pyrolysis of 350 ± 20 μg subsamples using a Eurovector Elemental Analyser (Milan, Italy) interfaced with an Isoprime (Manchester, UK) continuous-flow isotope-ratio mass spectrometer. All results are reported for non-exchangeable H expressed in the typical delta notation, in units per mil (‰), and normalized on the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale. Measurements of two within-run ($n = 5$ each) keratin laboratory reference materials indicated a measurement precision of $\pm 2\text{‰}$.

4.2.4 Data analysis

The [THg] data in each species were generally right skewed and deviated from a normal distribution (Shapiro-Wilk test) and so were \log_{10} transformed. Two spring-captured Blackpoll Warblers were omitted from the analysis because their $\delta^2\text{H}_f$ (-67.7

and -84.1‰) suggested that these feathers may have been replaced south of the breeding grounds and were not coherent with the rest of the individuals sampled. Sex was not included in the models as the sex of some individuals could not be identified. I used analysis of covariance (ANCOVA) to test for effects of season, age class, and migratory origin ($\delta^2\text{H}_f$) on feather [THg] in Blackpoll Warbler. For the remaining species, two-way ANOVA was used to test for effects of season and age class on feather [THg]. Non-significant factors and interactions were removed sequentially from the statistical models. Log_{10} transformed data were used in statistical tests, but non-transformed values are presented throughout. All results are presented as arithmetic means (unit: $\mu\text{g/g}$) \pm S.D. (sample size), and levels of significance set at $p < 0.05$ for effect of age and $p < 0.1$ for effects of season given my directional prediction of lower feather [THg] in spring. Statistics analyses were performed using SAS version 9.1 and figures were made in R version 3.3.1.

4.3 Results and discussion

4.3.1 Seasonal effects of mercury on long-distance migratory insectivorous species

Consistent with my prediction, two long-distance migratory insectivorous species (Blackpoll Warbler and American Redstart) had higher feather [THg] in autumn than spring (Table 4.1, Figures 4.2, 4.3). Significantly, spring migrating Blackpoll Warblers had approximately 50 percent lower mean feather [THg] than autumn migrants. The $\delta^2\text{H}_f$ of the individual Blackpoll Warblers sampled during both autumn and spring migrations were from populations that shared geographically similar breeding grounds (Autumn: $-152 \pm 16.0 \text{‰}$ ($n = 38$); Spring: $-147 \pm 6.3\text{‰}$ ($n = 24$), $F_{1, 60} = 2.07$, $p = 0.16$; further

details and assignment maps in Appendix C). There were no significant interactions among age classes, seasons and $\delta^2\text{H}_f$ (all p values > 0.23) as well as no main effects of $\delta^2\text{H}_f$ or age class on Blackpoll Warbler feather [THg] (Table 4.1).

Table 4.1 General linear model results comparing feather [THg] of migratory songbirds between migration seasons and between age classes. Birds were captured by mist nets in autumn 2014 (September and October) and spring 2015 (April–June), at Long Point Bird Observatory (LPBO, 42°34'58"N, 80°23'53"W), Ontario, Canada.

| Species | Season | Age class | Season * Age class |
|------------------------|---|--|------------------------------------|
| Blackpoll Warbler | F_{1,60} = 4.11, p = 0.047 | F _{1,59} = 0.04, p = 0.84 | F _{1,58} = 0.01, p = 0.91 |
| American Redstart | F_{1,65} = 3.95, p = 0.051 | F _{1,64} = 0.04, p = 0.85 | F _{1,63} = 0.05, p = 0.83 |
| Ruby-Crowned Kinglet | F _{1,62} = 0.34, p = 0.56 | F_{1,63} = 14.43, p = 0.0003 | F _{1,61} = 0.17, p = 0.68 |
| Swainson's Thrush | F _{1,74} = 2.04, p = 0.15 | F_{1,75} = 3.25, p = 0.08 | F _{1,73} = 0.56, p = 0.46 |
| White-Throated Sparrow | F _{1,74} = 0.91, p = 0.34 | F_{1,75} = 23.85, p <.0001 | F _{1,73} = 3.38, p = 0.07 |

Error degrees of freedom differ between tests because non-significant interactions and main effects were removed from the final models. The bold font indicates the statistics of the final models.

For Blackpoll Warbler and American Redstart, I compared the upper tails of the spring and autumn distributions of feather [THg] values to determine the proportion of the population that was presumably lost between autumn and spring. For example, I found that 95% of the spring Blackpoll Warblers had a feather [THg] below 1.43 $\mu\text{g/g}$ whereas only 59% of the autumn samples were below this concentration. My results thus indicate that up to 36% of autumn migrant Blackpoll Warblers may have been negatively affected by Hg exposure due to the apparently non-random loss of only individuals with the highest [THg]. Ideally, feather [THg] would only be compared between similar age classes for the two time periods as I expect differential performance and survival of young (Sullivan 1989; Menu et al., 2005; McKim-Louder et al., 2013; Mitchell et al., 2015). That is, only hatch-year birds in autumn and second-year birds in spring would be compared as well as after hatch-year birds in autumn and after second year birds in spring. However, I did not have large enough sample sizes across all age classes to conduct these analyses and so I pooled age classes. Nonetheless, individuals with elevated [THg] in the autumn (i.e. $> 1.43 \mu\text{g/g}$) included 10 adults and 6 hatch-year birds (juveniles) while the comparable returning spring samples contained no adults and 2 second-year birds (juveniles). Thus, my sample was reasonably well balanced and indicates that regardless of age, higher [THg] birds were underrepresented in the returning cohort. The Blackpoll Warbler is an exceptional migrant that undertakes non-stop, multi-day flights over the Atlantic Ocean to reach wintering areas in South America (DeLuca et al., 2015). As a neurotoxin, Hg may impair their navigation (Moye et al., 2016), flight performance (Ma et al., 2018; Chapter 3), and foraging ability (Kobiela et al., 2015). Thus, it is reasonable to posit that Hg burden may contribute to reduced

survival during migration and winter for this species. The significant difference between autumn and spring feather Hg is of particular concern for the Blackpoll Warbler, which has declined in North America over the past 45 years (Sauer et al., 2013). My finding indicates these declines of Neotropical migrant songbirds such as Blackpoll Warbler may, at least in part, be related to increases in Hg exposure.

The American Redstart also migrates long distances, sometimes crossing the Gulf of Mexico to winter as far south as northern South America (Sherry et al., 2016). For the LPBO sample, previous studies of migratory connectivity suggest these birds were more likely to have wintered in the Caribbean compared to longer-distance migrants from Central America (Norris et al., 2006). Regardless of wintering population, Redstarts migrated considerably shorter distances compared to Blackpoll Warblers, and Redstarts are not known to make the multi-day transoceanic flights. Consistent with my hypothesis, the apparent percentage loss of individuals due to Hg was smaller; 95% of spring, and 79% of autumn populations were lower than 1.95 $\mu\text{g/g}$, indicating that an estimated 16% of population decline could be related to Hg exposure.

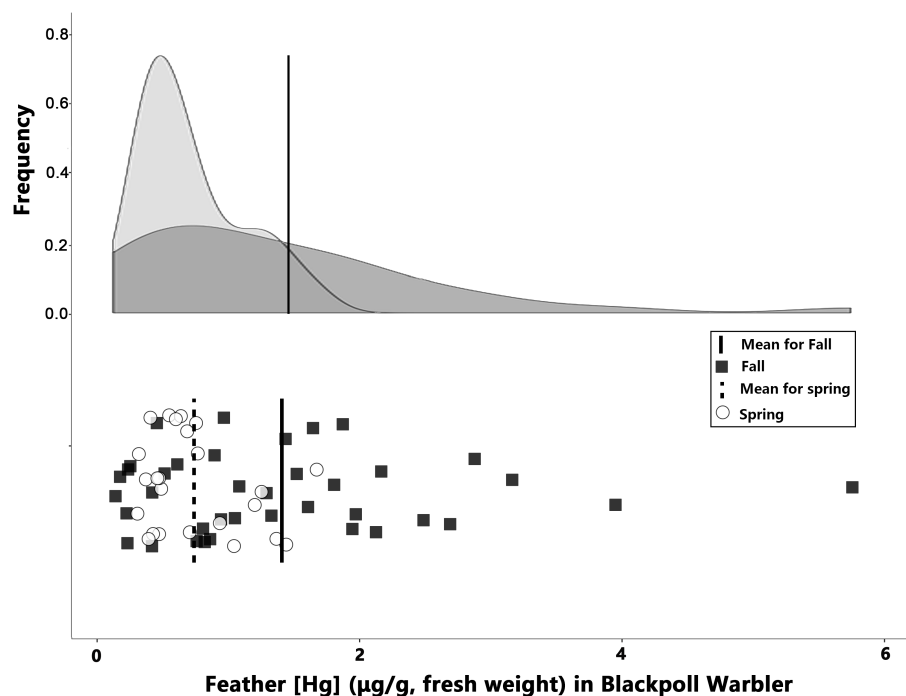


Figure 4.2 The feather [THg] ($\mu\text{g/g}$) distribution in Blackpoll Warbler for autumn and spring seasons. Birds were captured by mist nets in autumn 2014 (September and October) and spring 2015 (April-June), at Long Point Bird Observatory (LPBO, $42^{\circ}34'58''$ N, $80^{\circ}23'53''$ W), Ontario, Canada. Upper panel: a density plot where the dark grey area represents the autumn population, the light grey area represents the spring population, and the vertical solid line indicates the value below which 95% of spring values fall. Lower panel: black squares represent individual birds from autumn migration and white circles represent individual birds from spring migration. The solid line indicates mean [THg] in autumn migration ($1.41 \pm 1.17 \mu\text{g/g}$) and the dashed line indicates mean [THg] in spring migration ($0.74 \pm 0.39 \mu\text{g/g}$). 95% of the spring samples were below $1.43 \mu\text{g/g}$ [THg] whereas 59% of the autumn samples were below this concentration, suggesting approximately 36% of population decline could be related to Hg exposure.

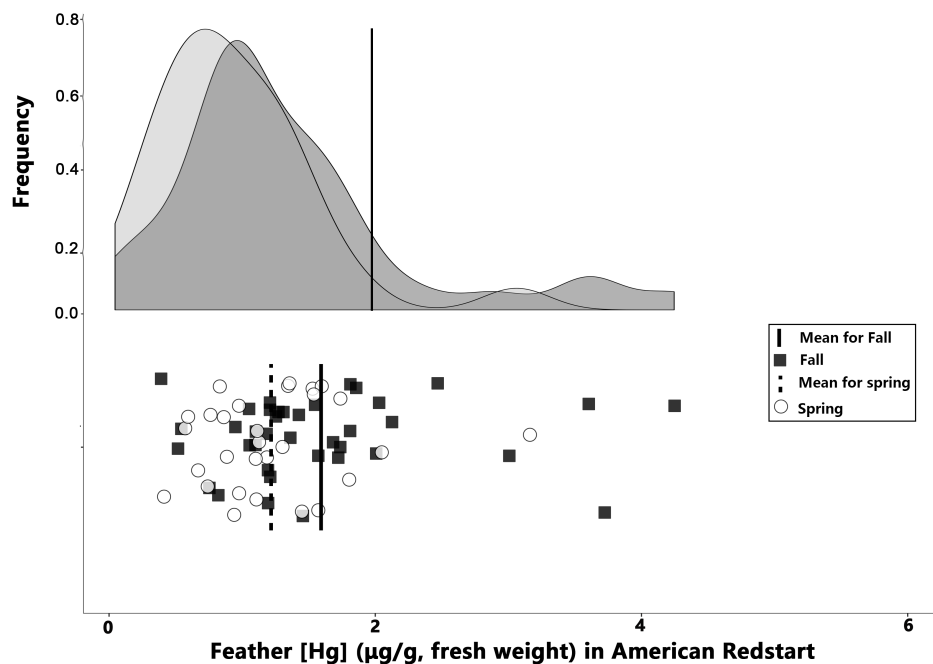


Figure 4.3 The feather [THg] ($\mu\text{g/g}$) distribution in American Redstart for autumn and spring seasons. Birds were captured by mist nets in autumn 2014 (September and October) and spring 2015 (April-June), at Long Point Bird Observatory (LPBO, $42^{\circ}34'58''$ N, $80^{\circ}23'53''$ W), Ontario, Canada. Upper panel: a density plot where the dark grey area represents the autumn population, the light grey area represents the spring population, and the vertical solid line indicates the value below which 95% of spring values fall. Lower panel: black squares represent individual birds from autumn migration and white circles represent individual birds from spring migration. The solid line indicates mean [THg] in autumn migration ($1.59 \pm 0.86 \mu\text{g/g}$) and the dashed line indicates mean [THg] in spring migration ($1.22 \pm 0.55 \mu\text{g/g}$). 95% of spring samples were below $1.95 \mu\text{g/g}$ whereas about 79% of autumn samples were below this concentration, suggesting approximately 16% of population decline could be related to Hg exposure.

4.3.2 Age effects in other species

There were no differences between spring and autumn [THg] for either the short-distance insectivorous migrant (Ruby-crowned Kinglet), or the omnivorous/granivorous species (White-throated Sparrow and Swainson's Thrush), independent of migration distance (Table 4.1 and Appendix C Figure C.2). However, I detected significant effects of age on feather [THg] in Ruby-crowned Kinglet and White-throated Sparrow, and a trend in Swainson's Thrush, but the pattern varied among species. These findings indicate that other factors like diet and trophic shifts during different life stages, and the extent and demands of the migratory route influence which migratory songbird species are affected by Hg exposure. Further discussions of age effects can be found in Appendix C.

4.4 Conclusion and future priorities

My study only considered five bird species migrating through one geographic region during one migration year. My findings for two long-distance migratory insectivores were consistent with my prediction that there may be important connections between Hg exposure, the process of migration, and songbird survival. While it has generally been held that feather [THg] much greater than I have measured is associated with toxicity in birds (e.g. 5-40 ppm for impaired reproduction, Eisler, 1987; Evers et al., 2008), my results suggest that much lower feather [THg] can be indicative of negative fitness effects in long-distance migratory passerines.

Future research should test the impacts of Hg exposure on migration and survival of songbirds by (1) examining a broader array of species in North America and on other continents like Asia where Hg exposure may be greater due to higher Hg use and emission; (2) using samples representing longer time periods, possibly through

retrospective studies of museum specimens; (3) investigating which life stage (migration or overwintering) is more likely to reduce survival due to Hg exposure; (4) establishing the relationship between feather [Hg] and Hg body burden during molt, and reference or threshold values of adverse effects of Hg in songbirds; and (5) conducting experimental field dosing studies to measure directly how dietary Hg affects individual migration performance and survival. Bridging these knowledge gaps will help us gain a broader and more mechanistic understanding of the potential role of Hg in songbird population declines and contribute to research-informed policy decisions about the reduction of Hg-related environmental impacts.

4.5 References

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Chapter 5

5 Conclusions

5.1 Overall findings and significance

Previous studies of the effects of MeHg on birds have largely been on reproductive success, and focused on upper-trophic-level piscivorous species (e.g., Evers et al., 2008), with less attention paid to songbirds (Jackson et al., 2011). The direct influence of MeHg on songbird migration, which is arguably the most taxing phase of a songbirds lifecycle, has rarely been investigated before the work reported in this thesis. Further, a limited number of studies (Ackerman et al., 2017; Jackson et al., 2015) have reported on Hg in some passerine tissues (e.g., blood and eggs) but present an incomplete picture of Hg burden. Here, feathers have been demonstrated to be a powerful indicator of both Hg exposure and breeding ground origin using deuterium isotope ratios.

By measuring feather Hg in 1946 individuals from 15 species at Canadian breeding grounds (in Chapter 2), I confirmed the relationships among diet structure, geographical region and Hg levels in feathers. I characterized (1) overall breeding origins (Eastern, Central and Western of Canada) by combining information from stable isotopes of hydrogen ($\delta^2\text{H}_f$), sampling banding station and band recoveries; (2) diet structure (exclusively insectivorous, partially insectivorous, non-insectivorous) by the proportion of invertebrates in diets through the whole life cycle. Overall, my findings reinforced the importance of trophic position (the food they eat) in MeHg exposure in a common monitoring tissue (feather). This study reveals that the proportion of invertebrates that are consumed in a whole life cycle may be reflected in the concentrations of Hg in

feather. In addition, a strong geographical trend in feather [THg] (logitudinally, West < Central < East and latitudinally, North < South in the Western region) was observed across Canadian breeding grounds. Specifically, lower [THg] were found in Western birds with low $\delta^2\text{H}_f$, indicating MeHg is less bioavailable in Northwestern breeding areas. In contrast, individuals that came from other breeding grounds likely had the same risk of Hg availability regardless of whether they came from northern or southern areas. Taken together, songbird feather MeHg is predominantly reflective of specific geographical regions across Canada, while trophic position (diet composition) could explain the variations in Hg values of songbird species occupying a particular region. Based on the data shown in my study, the long-distance insectivorous species originating from the Eastern region appear to be at the greatest risk of Hg exposure and subsequent effects.

How refueling at MeHg-contaminated stopover sites would contribute to MeHg bioaccumulation, and how such exposure could affect subsequent flight performance during migration was previously not known. The MeHg dosing and wind tunnel flight studies (in Chapter 3) provide a small but important glimpse into how MeHg may affect migration in the field. The dosing study showed that migratory Yellow-rumped Warblers rapidly accumulate dietary MeHg in blood, brain and muscle, liver and kidney in just 1-2 weeks. Exposure to a 0.5 ppm MeHg diet did not affect vertical takeoff performance, but in 2-h wind tunnel flights, MeHg-treated warblers had impaired flight performance. In the field, the findings in the wind tunnel test might have been more obvious when migrants need to search for food, avoid predators, experience unpredictable weather, and fly multiple hours to reach next available stopover. Overall, my research suggests that migratory songbirds are potentially at risk to quickly increase Hg body burden through

contaminated diets at breeding grounds and stopovers, which could impair their flight ability in the following migration.

If Hg, as an environmental stressor, influences survival during migration periods and over winter, I would expect to observe an overall population-level reduction in survival during migration related to reduced feather [THg]. This would result in high [THg] birds being lost between fall departure and spring return cohorts as elevated Hg body burden reduces migration performance and causes a high probability of mortality. In five migratory passerine species, I compared tail Hg concentrations that were grown prior to autumn migration and retained until the following spring (in Chapter 4). This prediction was met for two long distance migratory insectivores. Most alarmingly, the spring returning cohort of Blackpoll Warbler, a species that undergoes long non-stop autumn migration to South America, had nearly 50% lower Hg concentrations than those that departed in the autumn, suggesting significant losses of individuals with the highest body Hg. This novel discovery is a timely contribution to the greater understanding of the impacts of Hg exposure on survivorship in these precipitously declining migratory species and provides a testable hypothesis and methodology that can now be pursued.

Together, the findings in this thesis suggest that Hg burden in songbirds may not just be affected by trophic position as previously thought, but also by four overarching factors that appear to govern both overall levels of Hg exposure as well as vulnerability to its effects on breeding, migration and survival: 1) the proportion of invertebrates in diet over the whole life history; 2) the geographical location of summer breeding grounds; 3) premigratory accumulation at stopover, and; 4) migration distance. These results will

redirect avian Hg ecotoxicological research from large fish-eating species to passerines, specifically those long distance migrants with steeply declining populations.

5.2 Songbirds of conservation concern and mercury exposure risk

My research provides strong evidence that selection pressures associated with environmental MeHg exposure prior to migration may reduce migration success and annual survival. Specifically, MeHg exposure on the breeding areas could have a carry-over effect to influence survival of insectivorous songbirds that undergo extensive and demanding migratory journeys. Also, impaired endurance flight performance could be linked with MeHg exposure and rapid accumulation at heavily contaminated stopover sites. These findings bridge the knowledge gaps of MeHg effects among breeding, stopover and migration stages and highlight the importance of incorporating information from other phases of the annual cycle. Without considering the annual cycle, the effects of MeHg exposure on small long-distance migrants may be significantly underestimated. In Figure 5.1, I provide a conceptual model for the effects of environmental Hg in the context of migratory songbird annual cycle, based on integrating seasonal dynamics and the potentials for Hg to limit and regulate migratory songbird fitness. The areas of recent progress are labeled as “known” and future research directions are labeled as “unknown”. The bold font indicates the findings are based on a songbird study and the bold orange font highlights the contributions from my project. The details of these findings and the references can be found in Chapter 1. Given that migrants can spend most of the year away from the breeding grounds and face seasonally-specific threats and limitations, I hope this model can provide insights on the effects of Hg at each stage (breeding, overwintering or migration) of life cycle.

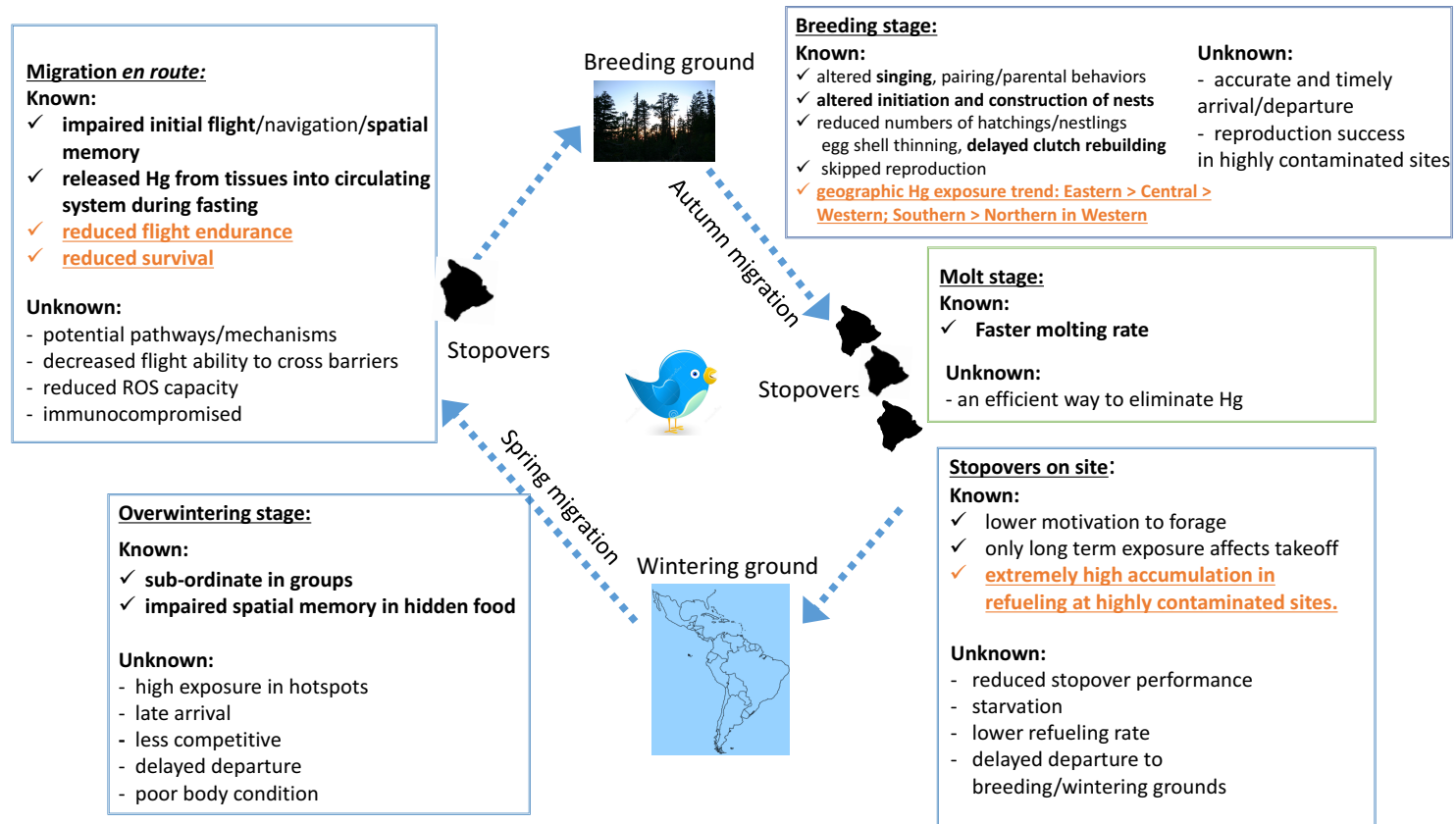


Figure 5.1 A model of environmental mercury on migratory songbirds in the context of annual cycle. The areas of recent progress are labeled as “known” and future research directions are labeled as “unknown”. The bold font indicates the findings are based on a songbird study and the bold orange font highlights the contributions from my project. The references cited were given in Chapter 1.

Overall, long distance migratory insectivores may be at high risk of Hg exposure, especially those from Eastern Canadian breeding grounds. Notably, for Blackpoll Warblers that undertake extensive and demanding migratory journeys, feather [THg] of even 1.5 ppm could potentially reduce migration success and survival. Thus, based on the findings in Chapter 4, the reference value of migration success risk induced by Hg is more conservatively estimated at 2 ppm in tail feathers for long-distance insectivores, which migrate up to Central/South America. In considering all of the data presented in this thesis, about 25% of long distance migrant birds exceeded this reference value in the autumn. If the individuals sampled in Eastern region (Chapter 2) are considered on their own, close to 70% of long distance insectivores may be at risk of reduced survival due to negative carry-over effects of exposure to elevated Hg.

5.3 Future directions

Although the data strongly suggest that Hg is contributing to survival in some long-distance insectivorous species, especially for the populations that origin from Eastern Canada, more evidence is needed to determine whether the culling is occurring during the migration phase or at the southerly overwintering grounds. A further investigation that samples tail feathers of a variety of Neotropical long-distance insectivores from their breeding, migration stopover and overwintering sites can answer this question.

The laboratory studies have provided insights into potential effects of MeHg on endurance flight and migration in small migratory songbirds. Future research should focus on the possible pathways that cause flight impairment to better understand the toxicity of MeHg in small migratory passerines. There are many indications that

environmental Hg affects neurological functioning, bioenergetics, and behaviors differently in a wide array of avian taxa (Chan et al., 2003; Whitney and Cristol, 2017). These differences may be due to genetic variation and it has been proposed that different species exhibit variable Hg demethylating capacities (Scheuhammer et al., 2007). The roles of some proposed detoxification mechanisms, like Hg-Selenium relationships (Koeman et al., 1975) need to be further investigated.

The distribution and impacts of songbird MeHg exposure in the Neotropics has not been fully assessed, although it has been reported that there are increasing Hg emissions in this region (Pirrone et al., 2010). Neotropical migrant songbirds spend about half of the year at overwintering sites (Rodewald, 2015). Overwinter performance has been linked to arrival timing and physical conditions at breeding grounds, which are important determinants of reproductive success (Marra et al., 1998). However, this is the least studied stage for Hg exposure in songbirds (See Figure 5.1). All of the effects of Hg on bird neurology and physiology could affect their performance at their wintering grounds. Since overwintering locations have been shown to impact [THg] in two fish-eating bird species (Lavoie et al., 2015), it is essential to evaluate if Hg exposure (either on the breeding grounds, during migration, and/or on the wintering grounds) impairs overwintering performance.

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Appendices

Appendix A: The permit from the Canadian Wildlife Service (SA-0208) under Animal Ethics Protocol 2010-020 University of Western Ontario Animal Care Committee.

Chris Guglielmo

From:
Sent: May-12-14 3:29 PM
To:
Cc:
Subject: eSirius Notification - New Animal Use Protocol is APPROVED2010-216::5



AUP Number: 2010-216
PI Name: Guglielmo, Christopher
AUP Title: Energetics, Fuel Use, Water Balance And Immunocompetence During Exercise In Migrating Birds
Approval Date: 05/12/2014

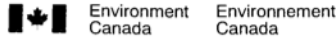
Official Notice of Animal Use Subcommittee (AUS) Approval: Your new Animal Use Protocol (AUP) entitled "Energetics, Fuel Use, Water Balance And Immunocompetence During Exercise In Migrating Birds" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal.2010-216::5

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura
on behalf of the Animal Use Subcommittee
University Council on Animal Care





**CANADIAN WILDLIFE SERVICE - PERMIT
PERMIS - SERVICE CANADIEN DE LA FAUNE**

| | | | |
|--|--|---|---|
| Issued to / Délivré en vertu de University of Western Ontario | | Permit holder / Titulaire du permis Scientific | Permit no. / No. du permis SA 0208 |
| Issued under section / Délivré en vertu de l'article 6(b) | | of / de MIGRATORY BIRD REGULATIONS | |
| Name / Prénom Guglielmo | Name / Prénom Christopher | Department / Département Dept. of Biology | |

1151 Richmond Street North
London On
N6A 5B7

| | |
|--|---|
| Date of issue / Date de délivrance April 10, 2014 | Date of expiry / Date d'expiration December 31, 2016 |
| (Empty space for notes or conditions) | |

Special Conditions - Conditions spéciales

- The permit holder may accept accidentally killed migratory bird specimens, donations by authorized collectors, and forfeited/seized birds.
- Specimens shall only be used for research purposes.
- Carcasses may be taken to a licensed taxidermist for mounting.
- Permit and birds shall be retained at the address on this permit at all times.
- Permit holder shall submit a written report, by January 31, each year, to the Canadian Wildlife Service, 867 Lakeshore Road, Burlington, ON., L7R 4A6, indicating the number of birds of each species salvaged and mounted during the previous calendar year and all birds destroyed.
- Specimens may be donated to the Royal Ontario Museum, Toronto, Ontario.
- All specimens not retained for research purposes are to be destroyed by disposal in the laboratory waste management system of the University of Western Ontario.
- All birds mounted or otherwise are the property of the Canadian Wildlife Service held in trust and are to be surrendered to the Service upon notice.
- No specimens of migratory birds may be purchased, sold, offered for sale, or otherwise be made subject of a commercial transaction.
- Nominees to this permit are: Department of Biology faculty/staff acting under the direction of the permittee.

ALL SPECIMENS TO BE RETAINED AT: Department of Biology, University of Western Ontario, London.



Canada

Appendix B: Supplementary material for Chapter 3

Materials and methods

Wind tunnel flight performance scoring protocol (see Table B.1 below)

Table B.1 Wind tunnel flight performance scoring table

| Score | Description |
|--------------|--|
| 1 | Little to no flight propensity: Bird always tries to land, no sustained flight for >20 seconds; Bird crashes immediately, tries to fly backwards, etc. |
| 2 | Small amount of flight propensity: Bird is mainly trying to land or escape, no sustained flight for >3 minutes; Needs a lot of encouragements to stay in the air. No α -flight*, may hover. |
| 3 | Some sustained flight: Bird does not just try to land, will have sustained flights between 3 to 8 minutes; May need encouragement, may have α -flight. |
| 4 | Good flyer: Bird flies well, sustains flight for >8 minutes; May need encouragement but stays airborne in close to α -flight. |
| 5 | Great flyer: Regularly sustains flights for >10 minutes with little to no encouragement; Mostly α -flight or near α -flight. |

α -flight is defined as the bird in full flight position, with the legs stretched behind the body.

Food preparation process

To prepare 10 ppm stock solution, I transferred 1.00 g of 1000 ppm standard MeHg solution (Methylmercury Chloride, MeHgCl, Alfa Aesar, Stock no. 33553) into 99.00 g Milli Q water to make the 10-ppm stock solution. To mix with diet, I poured 2040 mL tap water into a pot then placed on the heater. When water was boiled, agar and components 1 to 7 (see Table B.2) were added and mixed well. When the solution was cooled down at 50 °C, 10 ppm stock solution was added and mixed immediately with a hand mixer to homogenize in the fume hood. I transferred 1223.5 ml of mixture into each of two separately marked MeHg containers (0.5 and 1.0 ppm) and then add additional water to MeHg solution as the table described below. Following this, the solution was transferred to a container with lid closed and stored in the fridge. Each batch was tested to ensure it was within 10 % of the expected THg concentration. Control diet was made in the same way without adding MeHg solution and additional water.

Table B.2 Composition of Methylmercury (MeHg) diets

| | Components | MeHg Diets | | |
|----|-----------------------|------------|--------|------|
| | | 0ppm | 0.5ppm | 1ppm |
| 1 | Casein (g) | 120 | | 120 |
| 2 | Dextrose (g) | 540 | | 540 |
| 3 | Briggs salt mix (g) | 53 | | 53 |
| 4 | Celufil (g) | 29 | | 29 |
| 5 | Vitamins (g) | 17 | | 17 |
| 6 | Canola oil (g) | 96 | | 96 |
| 7 | Agar (g) | 42 | | 42 |
| 8 | Water (mL) | 2040 | | 2040 |
| 9 | Additional water (mL) | 0 | 75 | 0 |
| 10 | MeHg solution (mL) | 0 | 75 | 150 |

Experimental designs for Study1 and Study2.

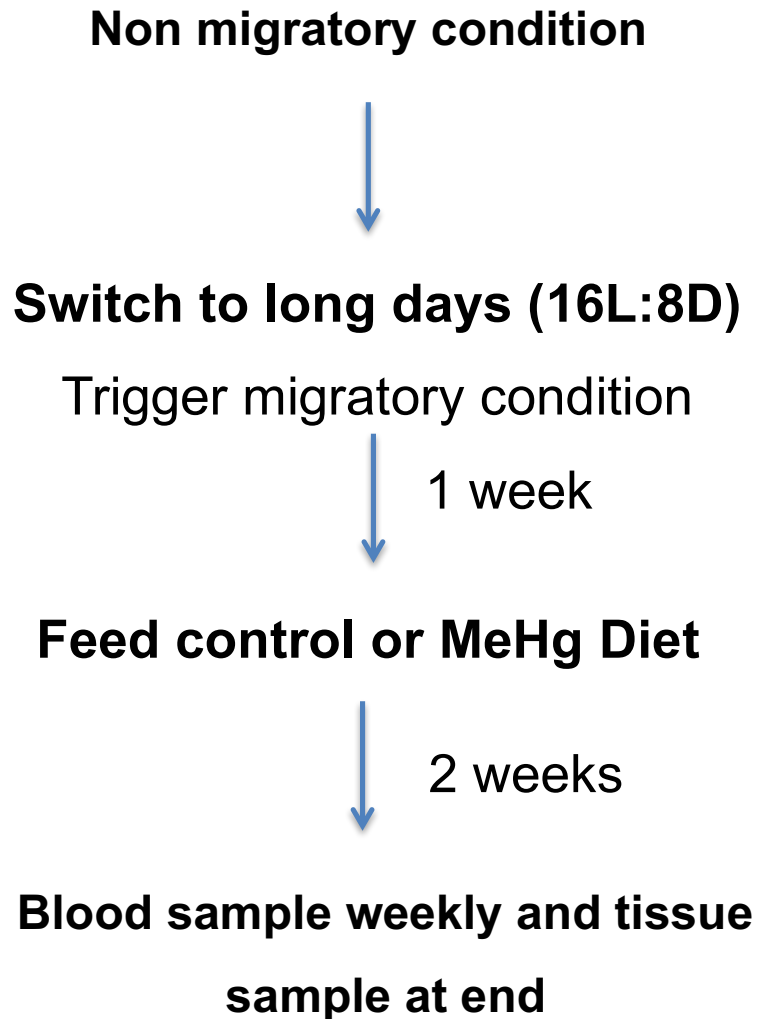


Figure B.1 Experimental design of Study 1 in Chapter 3.

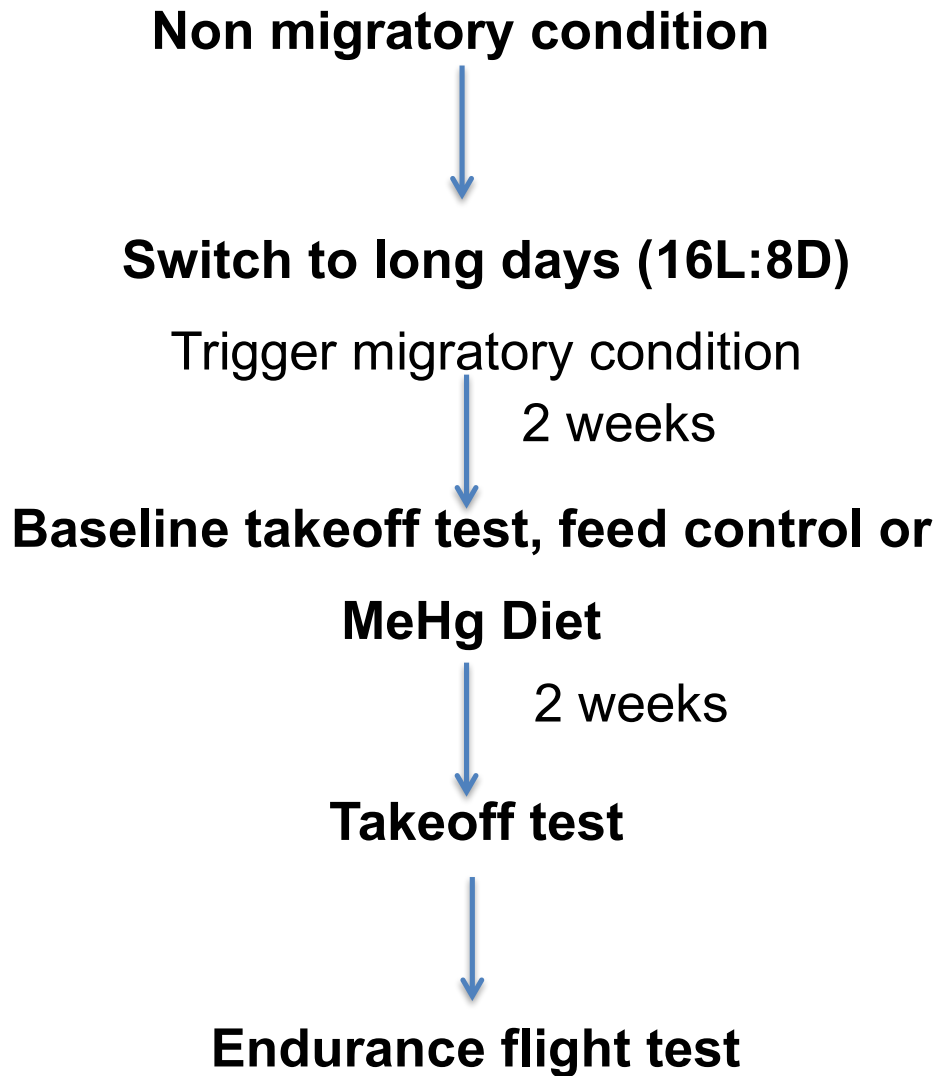


Figure B.2 Experimental design of Study 2 in Chapter 3.

Appendix C: Supplementary materials for Chapter 4

Materials and Methods

Probable breeding sites for Blackpoll Warbler

A likelihood-based assignment technique (Hobson et al., 2009; Wunder, 2010, Van Wilgenburg and Hobson, 2011) was applied to assign molt origins. I created a map (isoscape) of predicted $\delta^2\text{H}$ in feathers ($\delta^2\text{H}_f$) by applying algorithms presented in Hobson et al., (2012) to rescale the precipitation amount-weighted growing season $\delta^2\text{H}$ in precipitation ($\delta^2\text{H}_p$) map of Bowen et al., (2005) into equivalent feather values. Specifically, I applied the regression equation $\delta^2\text{H}_f = -17.57 + 0.95 \delta^2\text{H}_p$, based on data collected from multiple species of Neotropical migratory birds, to rescale the feather isoscape, and used a spatial “mask” operation to extract only those areas of the continent falling exclusively within the species’ breeding range based on a digital breeding-range map (BirdLife International and NatureServe, 2011). A variance estimates of 14.4 ‰ was assumed from the residuals of the precipitation-to-feather calibration (Hobson et al., 2012).

Result and Discussion

Probable breeding sites for Blackpoll Warbler

Figure C.1 indicates that both the autumn and spring cohorts of Blackpoll Warbler originated from the same general area of northwestern Canada. This strengthened my hypothesis that Hg exposure was linked to reduced survival in the same general group of individuals sampled. I also note, based on *ebird* animations

(<http://ebird.org/content/ebird/occurrence/blackpoll-warbler/>), that my sampling site (Long Point Bird Observatory, a labeled star in Figure C.1) most likely samples birds moving in an east to west direction in autumn and a northwestern movement in spring consistent with birds originating in the west as predicted by the isotope data. In short, I have provided good evidence that the spring and autumn cohorts were effectively sampling the same source population and that there is no reason to expect differential exposure to Hg on the breeding grounds driving the patterns I see.

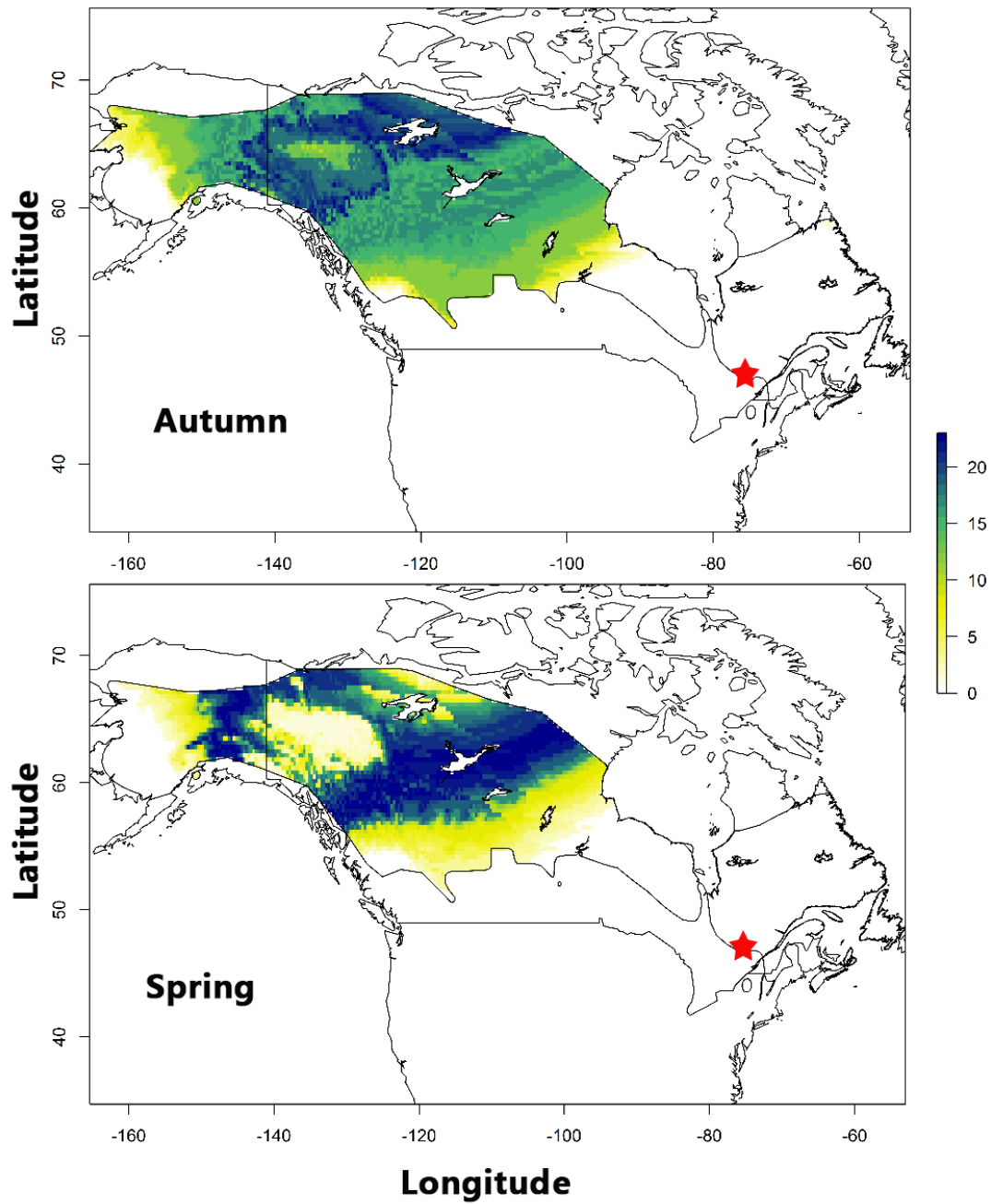


Figure C.1 Probable breeding origins of Blackpoll Warbler sampled during migration in autumn and the following spring at the Long Point Bird Observatory (LPBO, indicated by the star). Assignment based on tail feather deuterium values (see text). Color legend refers to number of individuals assigned to each pixel.

Age effects

Age class explained variation in feather [THg] of Ruby-crowned Kinglet and White-throated Sparrow, and there was a trend towards statistical significance in the Swainson's Thrush (Table 4.1, main text of Chapter 4). However, age affected Hg in different ways (Figure C.2). Adult Ruby-crowned Kinglets had 1.7 times higher feather [THg] than juveniles. This can be explained if Ruby-crowned Kinglet adults feed on higher trophic level, and hence higher Hg prey (e.g. spiders, pseudo-scorpions) than younger birds (e.g. caterpillars, larvae) (Swanson, 2008) or if there is a strong Hg accumulation in their body pools in adults. In contrast to this exclusively insectivorous species, juveniles had higher feather [THg] than adults in the two omnivorous species. Specifically, juvenile White-throated Sparrow feather [THg] was 2.3 times greater than in adults, and tended to be 1.4 times greater in juvenile Swainson's Thrush than adults. This could be explained by a more plant-based diet (e.g. fruit and seeds) of adults (Mack and Wang, 2008, Falls and Kopachena, 2010). Warner et al.,(2012) suggested that compared to adults, the young of Tidal Marsh Sparrows (Coastal Plain Swamp Sparrow, *Melospiza georgiana nigrescens*, and Seaside Sparrows *Ammodramous maritimus*) have a higher protein (insectivorous) diet which may at least partially explain the higher [THg] in juveniles. In terms of migration distance, Ruby-crowned Kinglets migrate short distances in most cases, experiencing a less taxing migration period, while White-throated Sparrows migrate short to medium distances, and Swainson's Thrushes are long-distance migrants. Thus, feather [THg] did not change seasonally in these three species possibly due to diet structure or migration distance.

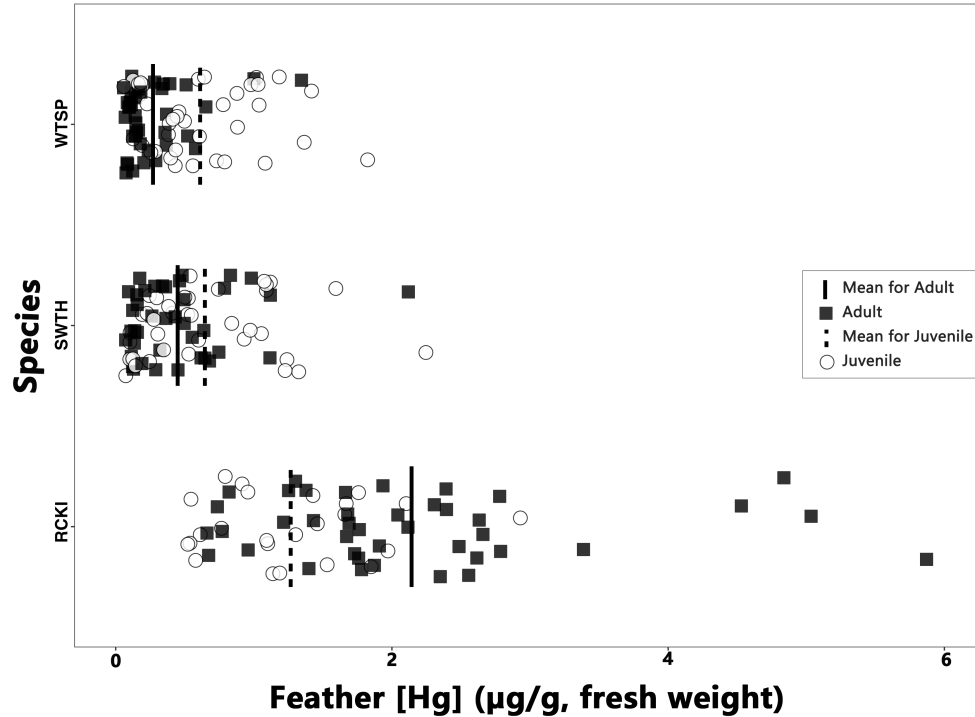


Figure C.2 The distribution of feather [THg] ($\mu\text{g/g}$, fresh weight) in Ruby-crowned Kinglet (RCKI), White-throated Sparrow (WTSP), and Swainson's Thrush (SWTH). Birds were captured by mist nets in autumn 2014 (September and October) and spring 2015 (April-June), at Long Point Bird Observatory (LPBO, $42^{\circ}34' 58''$ N, $80^{\circ}23'53''$ W), Ontario, Canada. Black squares represent adults while white circles represent juveniles. The solid line indicates mean [THg] of adults and the dashed line indicates mean [THg] of juveniles. Mean [THg] of adult Ruby-crowned Kinglet was $2.14 \pm 1.18 \mu\text{g/g}$ and of juveniles was $1.27 \pm 0.60 \mu\text{g/g}$. Mean [THg] of adult White-throated Sparrow was $0.27 \pm 0.26 \mu\text{g/g}$ and of juveniles was $0.61 \pm 0.42 \mu\text{g/g}$. Mean [THg] of adult Swainson's Thrush was $0.49 \pm 0.40 \mu\text{g/g}$ and of juveniles was $0.65 \pm 0.50 \mu\text{g/g}$.

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Curriculum Vitae

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Education

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Work and Research Experience

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Publications

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Yanju Ma, Brian A. Branfireun, Keith Hobson and Christopher G. Guglielmo, Evidence of negative seasonal carry-over effects of breeding ground mercury exposure on survival of migratory songbirds, *Journal of Avian Biology*. 2018, 49, 1-8.

Yanju Ma, Bo Su, Zhen-jin Meng. autumn and winter survey of waterbirds in national natural reserve of Beilun estuary, *Guangxi Sciences*. 2011, 18, 73-78(in Chinese).

Yanju Ma, Ang Yu. The toxicity research progress of nano-TiO₂, *Environmental Science and Management*. 2009, 34, 33-37(in Chinese).

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2017, Travel award, 13th International Conference on Mercury as a Global Pollutant, Providence, Rhode Island, USA.

2013-2017, Western Graduate Research Scholarship (WGRS, \$10,450 per year) & Graduate Teaching Assistantship (\$12,699.24 per year), University of Western Ontario, Canada.

2015, Fall Graduate Travel Award of Biology Department, University of Western Ontario, Canada.

2015, Travel award, Annual Meeting of Canadian Society of Zoologists, Calgary, Canada.

2013, Spoon-billed Sandpiper Survey in Southern China (HK 12,272.6), Hong Kong Bird Watching Society, Hong Kong, China.

2009-2012, Waterbird survey in Guangxi Beilun Estuary National Nature Reserve (RMB 20,000 per year), Fangchenggang, China.

2006-2009, National Scholarship (full tuition and allowance covered, RMB14,400 per year) for Graduate Students, Xiamen University, China.

2004, Departmental Scholarship of Nanjing University of Information Science & Technology, University of Environmental Science and Engineering, Nanjing, China.