Jonathan Maycol Branco

Exposição ao mercúrio ambiental na Bacia Amazônica e seus efeitos em Andorinhas-Azuis (*Progne subis*)

Environmental mercury exposure in the Amazon Basin and its effects on Purple Martins (*Progne subis*)

São Paulo

2021

EXEMPLAR CORRIGIDO

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Epigraph

"It is not our part to master all the tides of the world, but to do what is in us for the succour of those years wherein we are set, uprooting the evil in the fields that we know, so that those who live after may have clean earth to till. What weather they shall have is not ours to rule."

J. R. R. Tolkien, The Return of the King

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Resumo

Na América do Norte, 29% da abundância de aves foi perdida nas últimas 5 décadas. Essa tendência, entretanto, não é homogênea em todos os grupos de aves e afeta alguns mais do que outros. Os declínios de aves insetívoras são maiores do que a média; e a mesma tendência acontece com as aves migratórias. Não é surpreendente, então, que *Progne subis* (andorinhas azuis), uma ave insetívora migratória, também tenha sofrido declínios. Embora não seja listada como ameaçada pela IUCN, a população geral de andorinhas azuis vem sofrendo um declínio de 0,8% ao ano nas últimas cinco décadas. Essa taxa, no entanto, varia na extensão da América do Norte e certas regiões têm taxas locais de declínio mais altas. É o caso de andorinhas azuis que ocupam as regiões sul, leste e central dos EUA e leste do Canadá. As andorinhas azuis dessas regiões têm sofrido as maiores taxas de declínio. Uma característica particular das andorinhas azuis que vivem nessas regiões é o fato de todas migrarem para a região amazônica da América do Sul, ao contrário das demais populações que migram para as regiões leste e sudeste da América do Sul. Dentre as muitas razões potenciais para esse declínio, a região amazônica possui altas concentrações de Hg, tanto de ocorrência natural quanto originado da mineração de ouro. Além disso, o rápido aumento de barragens hidrelétricas na região nas últimas cinco décadas promove a lentificação da região, um processo que tem demonstrado reter Hg particulado e permitir a metilação microbiana. O Hg, particularmente na forma metilada, é uma substância altamente tóxica que pode levar a uma série de efeitos potencialmente deletérios em animais. Na pesquisa apresentada no segundo capítulo, verifica-se que nas populações de andorinhas azuis de três estados dos EUA que migram para a Amazônia, a contaminação por mercúrio total (THg) se correlacionou com uma diminuição no índice de gordura e peso da ave, um resultado que pode ter um grande impacto em uma espécie que usa gordura como reserva de energia para a migração. Embora a concentração de THg encontrada nas aves amostradas esteja abaixo dos limites da literatura para impacto severo na saúde e na reprodução, esses resultados sugerem que o crescimento contínuo da lentificação da Amazônia pode levar a uma severa perda de biodiversidade em um futuro próximo.

Palavras-chave: Ecotoxicologia, Bacia Amazônica, Mercúrio, Migração, Ave Insetívora

Abstract

In North America, 29% of bird abundance have been lost in the last 5 decades. This tendency, however, is not homogenous across all bird groups and affects some groups more than others. Declines is insectivorous birds are higher than the average; and the same tendency happens to migratory birds. It is not surprising, then, that *Progne subis* (Purple Martins), a migratory insectivorous bird, has also been suffering declines. Although not being listed as threatened by IUCN, the overall population of Purple Martins has been suffering a decline of 0.8% per year for the past five decades. This rate, nevertheless, varies within North America and certain regions have higher local rates of decline. This is the case of Purple Martins that occupy the southern, eastern, and central regions of USA and eastern Canada. Purple Martins from these regions have been suffering the highest decline rates. A particular characteristic of Purple Martins living in these regions is the fact that they all migrate to the Amazon region of South America as opposed to the other populations that migrate to eastern and southeastern regions of South America. Among many potential reasons for this decline, the Amazon region has high concentrations of Hg, both naturally occurring and originating from gold mining. Furthermore, the rapid increase of hydroelectric dams in the region over the past five decades promotes the lentification of the region, a process that has been shown to retain particulate Hg and allow for microbial methylation. Hg, particularly in the methylated form, is a highly toxic substance that may lead to a range of potentially deleterious effects on animals. In the research presented in the second chapter, it is shown that for populations of Purple Martins from three US states that migrate to the Amazon, total mercury (THg) contamination correlated with a decrease in fat score and overall weight of the bird, a result that may have a huge impact in a species that uses fat as energy reserves for the migration event. Although the THg concentration found in the birds sampled fall under literature thresholds for severe health and reproduction impact, these results suggest that the continuing growth of the lentification of the Amazon may lead to severe loss of biodiversity in the near future.

Keywords: Ecotoxicology, Amazon Basin, Mercury, Migration, Insectivorous Bird

Chapter 1

General Introduction

Panorama of bird conservation

In 2019, the Cornell Lab of Ornithology announced a worrying number: nearly 3 billion North American birds have been extirpated since 1970 (Rosenberg et al., 2019), a loss of 29% of the abundance of birds from five decades ago. It is not only in North America that this scenario has been documented; birdwatchers and researchers have reported declines in bird numbers around the globe. For example, in 2020 India released a comprehensive report containing the distributional range, trends in abundance, and conservation status of the nation's birds ("State of India's Birds," 2020). They report that of the 867 species assessed, 52% were declining in numbers and 101 species were at risk. Worldwide, the IUCN red list contains 1486 bird species with vulnerable, endangered, or critically endangered status ("The IUCN Red List of Threatened Species," n.d.). In Brazil, Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) maintains a IUCN Red List-equivalent, the "Livro Vermelho da Fauna Brasileira Ameaçada de Extinção" (Red Book of Brazilian Fauna Threatened with Extinction). The latest edition of this book includes a total of 1,979 species of known Brazilian avifauna, 236 (11.9%) of which are threatened with extinction (ICMBio, 2018).

The reason for declines in bird abundance can seldom be attributed to a single factor. From the most common such as climate change, habitat loss, biodiversity decline, poaching and wildlife trafficking to lesser-known factors such as predation by pets, impact on glass windows or panes and consumption of plastic, bird species face numerous threats to their survival (Lebbin et al., 2010). The simultaneous exposure to myriad factors pose challenges to any conservation effort and require the initial identification of the specific threats to each bird species.

Insectivorous bird conservation status

Declines in insectivorous bird abundance are higher than the average for all birds. According to Rosenberg et al. (2019), 73.1% of North American aerial insectivorous species are in decline, with a 31.8% decline in the overall abundance. Among the often-posed hypotheses for this higher decline in insectivorous birds is the reduced food availability (Nebel et al., 2010), and pesticide contamination (Nebel et al., 2010; Hallmann et al., 2014).

The observed declines in insectivorous bird abundance coincides with the substantial decline of insect populations reported around the world: the so-called “insect apocalypse” that has been perceived by many metrics (Hallmann et al., 2017; Klink et al., 2020; Sánchez-Bayo & Wyckhuys, 2019). More worryingly, the factors behind the abrupt decline in insect abundance itself is not well understood.

The decline of insectivorous bird abundance, however, might not be completely related to the decline of insect populations. Nevertheless, pesticide usage is one of the most common hypotheses (Sánchez-Bayo & Wyckhuys, 2019). Contaminants, present in the insects they consume and in the environments in which they live, can be incorporated in their bodies (Ackerman et al., 2016; Custer et al., 1998; Custer et al., 2005). In this context, pesticides come to light as a potential factor driving the decline of birds. Given the high and ever increasing diversity of pesticides, these toxicants can potentially have deleterious effects at every level of biological organization in birds and other animals including genomic, reproductive, immunological, neurological and behavioral toxic effects among others (H Walker, 2003; Fry, 1995; Skolarczyk, Pekar, & Nieradko-Iwanicka, 2017; Van Scoy, Pennell, & Zhang, 2016). Besides pesticides, there are other environmental contaminants that can also result in deleterious effects in birds, such as mercury (Hg) and other heavy metals (Ackerman et al., 2016).

Mercury contamination in insectivorous birds

Heavy metals are environmental toxicants that can be acquired from the diet. One of those heavy metals, Hg, can bioaccumulate and biomagnify in birds (Ackerman et al., 2016). Bioaccumulation is the gradual accumulation of contaminants in an organism over time resulting in a higher contaminant concentration relative to the environment (Borgå, 2013). Biomagnification is the result of bioaccumulation happening in each stage of the food chain conferring higher concentrations of contaminants in higher trophic levels than in lower (Borgå, 2013).

With Hg contamination rates in insectivorous birds up to 125 times higher than in granivores and 3.6 times higher than in omnivores (Ackerman et al., 2018), these organisms are at a higher risk of suffering deleterious effects of Hg contamination. Being unsafe at any level, effects of Hg on health can encompass any organ or subcellular structure in animals (Bernhoft, 2012). In birds, Hg is known as a potent and persistent neurotoxicant and can elicit a variety of other pathologies including anemia, liver and kidney dysfunction, retarded gonadal development, and reduced growth and reproduction (Ackerman et al., 2016; Basu, Goodrich, & Head, 2014; Eisler, 2005; Fernandes Azevedo et al., 2012). Furthermore, Hg can broadly disrupt the endocrine system, affecting almost all endocrine glands including those associated with the reproductive, adrenal, and thyroidal axes (Zhu et al., 2000). Endocrine disruption can, therefore, elicit a variety of systemic impacts that compromise health, negatively alter behavior and development, and reduce fitness through diminished survival and reproductive output.

Source points of Hg can either result directly or indirectly from anthropogenic actions (e.g., industry, mining) or can be naturally occurring (Rytuba, 2003; Morel, Kraepiel & Amyot, 1998; Xu et al., 2015). The primary source of natural elemental Hg on earth is in mercury-bearing ores that can be weathered and release Hg in the soil, water bodies and volatize into the atmosphere (Rytuba, 2003; Morel et al. 1998). It can remain in the atmosphere for up to a year before distilling out of atmospheric circulation during precipitation events and returning to the surface and thus is broadly dispersed via atmospheric processes across the planet (Fitzgerald & Mason, 1997). This allows virtually unrestricted Hg pollution beyond its source point, resulting in dispersion of both naturally occurring and anthropogenic Hg across even the most remote regions of the Earth (Fitzgerald, Engstrom, Mason & Nater, 1998). However, Hg tends to precipitate on land more often than on water (Mason, Fitzgerald & Morel, 1994; Xu et al., 2015). This phenomenon predicts that Hg precipitation to be higher closer to its source (Morel et al. 1998). Furthermore, release of elemental Hg from natural ore deposits creates regions of higher Hg concentration closer to the source (Rytuba, 2003; Xu et al., 2015). The main occurrence of mercury-bearing ores on earth’s surface is in mineral belts that are a result of tectonic and volcanic events (Rytuba, 2003; Xu et al., 2015). Therefore, across the globe there are large variations in background levels of Hg depending on the existence of regional events releasing Hg to the environment.

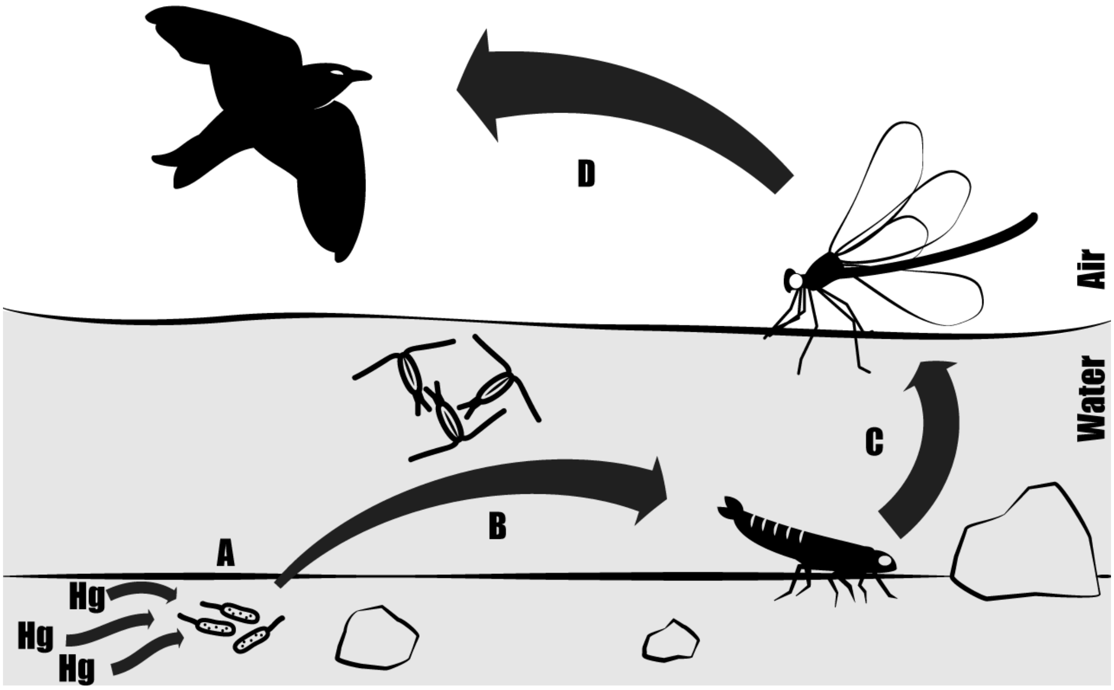

Figure 1.1. Mercury (Hg) incorporation pathway in insectivorous birds. Diluted Hg in water bodies is methylated and bioaccumulated by bacteria (A). The methylmercury (me-Hg) is then incorporated in any organism that feeds on the contaminated bacteria and biomagnifies as it advances through the food chain (B). Eventually, through the food chain, the me-Hg is taken up by aquatic insect larvae that incorporate and retain it through metamorphosis into the adult form (C). Contaminated aerial adult insects consumed by insectivorous birds allow for a further biomagnification in the latter, reaching the highest contamination level of me-Hg in this food chain if no predation of the insectivorous birds occur (D).

Hg from the environment is incorporated in birds and other animals via their diets (Morel et al. 1998). Elemental Hg does not biomagnify easily in organisms but in water can be converted by bacteria into methylmercury (me-Hg) or chloromethylmercury, highly toxic lipophilic compounds that magnify through food webs as it incorporates and is retained in fat tissues, with the highest trophic levels and longest-lived species exhibiting the highest body burdens of Hg (Hosseini, Nabavi, & Parsa, 2013; Morel et al. 1998). Once created, organic Hg is retained inside the microorganisms that produced them and is transferred to organisms that feed on them such as fish or aquatic insect larvae, bioaccumulating in tissues and biomagnifying in organisms that consume those animals, further increasing the concentration of me-Hg at each step of the food chain (Morel et al. 1998). Since Hg is an element, it does not degrade. Animals at higher trophic levels, such as insectivorous birds, will bioaccumulate Hg at the highest concentrations.

Migratory bird conservation status

North American migratory birds have declined at a higher rate over the past 5 decades than North American resident birds. 58.2% of migratory bird species are in decline in North America, resulting in an abundance decline of 28.3% for the entire group, compared to a positive change of abundance of 5.3% for resident birds (Rosenberg et al., 2019). Due to the highly stressful and energy consuming nature of migration (Hedenström, 2010; Gutiérrez et al., 2019), migratory birds are generally vulnerable after migration effort, when their energy reserves are depleted, and pre-migration, when they must build new endogenous reserves of energy and increase antioxidant capacity to prevent oxidative stress damage during long flights (Hedenström, 2010; Gutiérrez et al., 2019).

Furthermore, threats to migratory birds appear greater and to have a larger potential effect than threats for non-migratory species. For instance, given that migratory birds travel between largely different and distant habitats, they are more susceptible to acquiring and spreading viruses to and from different locations (Reed, Meece, Henkel, & Shukla, 2003). In the same way, it is to be expected that migratory birds are susceptible to exposure of a wider range of contaminants throughout their migratory range. Furthermore, climate change can have an impact on the bird's perceived timing of migration and delay egg laying due to changes in season duration (Laaksonen et al., 2006). Climate change can also result in phenological mismatches that have been shown to lead to fatal conflicts between migratory bird species and the resident birds when the nesting period changes to unusual dates (Samplonius & Both, 2019). In addition, habitat loss can be highly detrimental to migration success, as many migratory species require stopover sites mid-migration (Rogers et al., 2010).

Purple Martin (*Progne subis*) biology and conservation status

Purple Martins (*Progne subis*) are migratory insectivorous birds that spend their breeding season in North America and non-breeding season in South America. Although they have been subject to studies for decades, the biology of Purple Martins is not completely understood. Specifically, data are lacking with respect to migration patterns and distribution in South America. From recently collected geolocation data, there appears to be a general tendency of Purple Martin populations from the western USA and Canada to migrate to southeastern Brazil, whilst populations from southern, eastern and central regions of the USA and eastern Canada migrate to the Amazon (Fraser et al., 2012). As a result of this pattern, individuals from some regions of North America meet and share the same region in South America, while others shown little overlap (figure 1.2). On their non-breeding grounds, Purple Martins flock by the thousands and form nighttime roosts that may encompass hundreds of thousands of individuals that dissipate near sunrise as birds leave roost sites to forage.

The overall population of Purple Martins has declined in North America at a rate of about 0.8% per year for the past five decades (Sauer et al., 2017). Specifically, populations of Purple Martins from 21 out of 42 US states and four out of seven Canadian provinces where they occur have been decreasing since 1966 (Sauer et al., 2017). The US States which reported declines are located in the central, southern and eastern regions of the US and eastern Canada. The subspecies of Purple Martins that live in these regions (*P. subis subis*) winters in the Amazon (Fraser et al., 2012). On the other hand, states and provinces with populations from the northwestern subspecies (*P. subis arboricola*), known to migrate to coastal states of Brazil to the south and east (Fraser et al., 2012), are increasing in number (Sauer et al., 2017). Lastly, the subspecies from the southwest of US (*P. subis hesperia*) that has not been tracked using biologging technology (Fraser et al., 2012), has shown population decreases in both states where it occurs (Sauer et al., 2017).

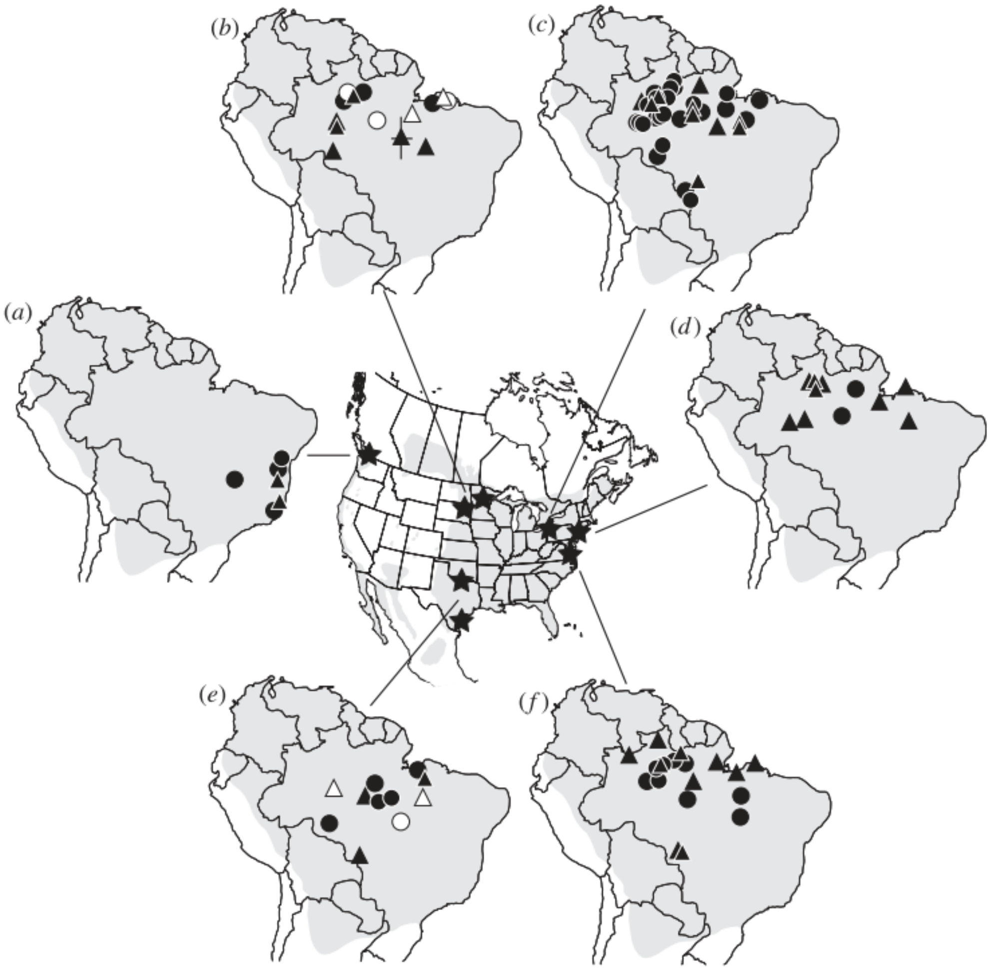

Figure 1.2. Purple Martins from the subspecies P. subis arboricola breed in the western USA and Canada and migrate to southeastern Brazil (a). Purple Martins belonging to the subspecies P. subis subis breed in the southern, eastern, and central regions of the USA and eastern Canada and migrate to the Amazon region (b to f). Triangles stand for males and circles for females, whilst the fill color separate states of origin in b and e (white is Minnesota in b and Oklahoma in e). Reproduced with permission of The Royal Society from Fraser, KC et al. (2012) Continent-wide tracking to determine migratory connectivity and tropical habitat associations of a declining aerial insectivore. Proc. R. Soc. B. 279 4901–4906

Even though they are declining, Purple Martins are not listed as threatened, and according to IUCN the population trend is deemed as stable (BirdLife International, 2016). This is true for populations of certain states and provinces of North America, particularly in the west coast (Sauer et al., 2017). However, there is evidence that the overall population of this species is in decline, as the breeding bird survey reveals a population trend of -0.8% of individuals a year across the entirety of the USA and Canada (Sauer et al., 2017).

Purple Martins suffer from the conflicts that occur with human populations in Brazil because of the preferred habitat choices of the species. Their habitat is often close to human populations, both in North America and South America (Brown & Tarof, 2013). The eastern subspecies (*P. subis subis*) are dependent on human structures to construct their nests and live in proximity to human populations (Brown & Tarof, 2013). The western subspecies, on the other hand, can construct nests of its own but still live close to human populations (Brown & Tarof, 2013). In Brazil during the 20th century, the species was previously considered symbolic of certain cities in the State of São Paulo, where it was recognized and appreciated. Nowadays, the flocks of hundreds or thousands of individuals that gather to roost at the end of the afternoon in parks or city squares in Brazilian cities, or on structures, such as towers, are unappreciated due to their noise and deposition of feces in these areas. Thus, humans have taken to persecuting Purple Martins in these areas by tree-cutting and harassment using fireworks to deter their roosting. Therefore, Purple Martins are the subject of significant human-wildlife conflict which could be contributing to the decline of specific populations that roost in urban areas and industrial plants (Hill, Kramer, & Levy, 2004). However, even though their presence in cities in the state decreased, the subspecies that occurs in Sao Paulo is not suffering from population decline as per surveys in North America (Fraser et al., 2012; Sauer et al., 2017). Thus, persecution and harassment alone are not enough to cause a decline in a perceivable degree in this species.

Amazon status

Specific reasons for the decline of Purple Martins have not yet been determined but might be related to the Amazon region in some form. Even though not all Purple Martin populations that migrate to the Amazon are in decline, most of the North American states and all Canada provinces from which Purple Martins migrate to the Amazon are reporting declines, as opposed to no declines being reported in US states and Canada provinces from which populations migrate to the southeastern coast of Brazil, which could suggest that a contributing factor occurs in the Amazon region (Fraser et al., 2012; Sauer et al., 2017). One important factor of the Amazon region is its higher than average Hg and me-Hg levels, both naturally occurring and as a result from the gold mining activities that happened in the region in past decades and still happens illegally in the region (Lino et al., 2019). Hg can also be further released from the soil into the environment by deforestation and erosion which are extensive in some regions of the Amazon (Lino et al., 2019). Furthermore, the use of inorganic Hg to facilitate the extraction of artisanal gold in past decades was common in the region (Pirrone & Mahaffey, 2005) and continues to this day. Even though the main source of elemental Hg content in the Amazon comes from the natural geological events that leads to a naturally enriched soil, the anthropogenic contribution of Hg is still evident in the Hg measured in the region and its continental shelf (Siqueira et al., 2018).

Furthermore, another potential contributor of me-Hg in the Amazon Basin are the hydroelectric dams located in the region. The Amazon Basin of Brazil is massive and home to 138 hydroelectric dams of varying sizes, most of them constructed in the past five decades, as well as a growing number of new dams being proposed or under construction (ANEEL, 2020; Castello et al., 2013). Damming alters the natural water flow, increasing the number of lentic habitats in the Amazon (Schiesari et al., 2020). This process has been shown to retain particulate Hg and allow for microbial methylation to increase up to 40-fold in the flooded areas (St. Louis et al., 2004; Zhao et al., 2017). Me-Hg levels were found to be consistently well above natural levels even nine years after the construction of experimental dams (St. Louis et al., 2004). In river-reservoirs, an increase of up to 92% of the natural me-Hg levels were found in the outflow rivers when compared to the levels in the inflow rivers (Zhao et al., 2017). Furthermore, since contaminants such as me-Hg are bioaccumulated and biomagnified through the food web, these changes in waterbody dynamics are going to reflect in increases in Hg content throughout the food web and extend the reach of the contamination impact (Schiesari et al., 2017). Consequently, the presence of a great number of hydroelectric dams throughout all the Amazon Basin is likely to exacerbate the naturally high levels of me-Hg in the region. It is expected, therefore, that birds migrating to the Amazon will be in contact with a higher Hg content than birds migrating anywhere else in the country.

Figure 1.3. Map of the largest hydroelectric dams (larger than 30MW in capacity) of the Amazon Basin in Brazil (source: ANEEL)

Mercury impacts on endocrine system of *Progne subis*

The effects of Hg on the endocrinology of Purple Martins (*Progne subis)* are still unknown. Hg contamination has been calculated for Purple Martins of the Canadian Province of Saskatchewan, which revealed a Hg concentration of 2.22 µg/g of feather, at which deleterious effects could potentially occur (Kardynal et al., 2020), but the contamination levels in other regions and the effects of this contamination in the endocrinology are yet to be verified. Nevertheless, given the existing literature on other birds and other vertebrates, it is likely that Hg contamination has a range of deleterious effects on Purple Martins including endocrine disrupting effects.

Being both migratory and insectivorous, the effects of Hg contamination may have an added complication on Purple Martins. Since insectivorous birds bioaccumulate Hg at higher levels (Ackerman et al., 2016) and Hg is an endocrine disruptor (Zhu et al., 2000), Purple Martins feeding and roosting in the Amazon may have altered endocrine function that could impair their physiological and metabolic capacity to migrate. For example, the adrenals are suppressed in function by Hg (Zhu et al., 2000). These glands produce, among other hormones, glucocorticoids (GC), which function to modulate organismal metabolism by regulating glucose homeostasis and adipocyte development, and are key to the adaptive stress response (Bauerle & Harris, 2016). Often called "stress hormones", GC function to influence behavioral and metabolic regulation to facilitate survival during and following exposure to stressful stimuli (Cockrem, 2007). Another important function of GC is immunomodulation; GC can promote either anti-inflammatory actions or pro-inflammatory actions, the latter normally in response to high GC levels in acute stress (Timmermans, Souffriau & Libert, 2019). Theoretically, compromised secretion of GC could alter the metabolism and lower fitness, especially under stressful situations. This is particularly relevant to Purple Martins that are exposed to Hg in their overwintering habitat and then must migrate many thousands of kilometers to their breeding grounds in North America.

Another endocrine axis that can be disrupted by Hg is the hypothalamo-pituitary-thyroidal axis. The thyroid produces hormones that are primary modulators of growth, development, thermogenesis, and metabolic rate, among others (Brent, 2012). Thyroid hormones are primarily composed of the prohormone thyroxine (T_4), the active form triiodothyronine (T_3) that is activated in tissues and regulates the actions of many genes, and the inactive reverse-T_3 (rT_3) as a means to regulate T_3 levels in tissues (Brent, 2012). Thyroid hormones are synthesized by iodination of tyrosine in the thyroid gland and their iodine component can be outcompeted by Hg (Soldin, O'Mara & Aschner, 2008). Due to growth and development roles, deficiency of thyroid hormones is particularly problematic for young individuals (Brent, 2012). Thyroid hormones are also responsible for the regulation of lipolysis and lipid synthesis (Shahid, Ashraf & Sharma, 2020), both processes crucially important in managing the required energy reserves for migration in birds (Gutiérrez et al., 2019). Thus, disrupted of thyroid hormone synthesis and secretion by Hg can impair the development of young birds and also affect the regulation of metabolism in adults, and by extension, their capacity to prepare for and complete the physical feat of trans hemispheric migration.

Feather analysis

Quantifying Hg content and endocrine function of birds requires biological sampling. Various bird tissues can be used to analyze for Hg content, each integrating Hg exposure at different timeframes (Eagles-Smith et al., 2008; Ackerman et al., 2016). Of those, feathers offer a good estimate of Hg contamination and concomitant hormone levels over the feather-growing period of a few weeks, since after feathers mature, they are no longer vascularized and thus they stop accumulating Hg (Burger, 1993).

Purple Martins complete a single molt per annum which is initiated in North America before migration, halts during migration, and is completed after arrival in South America (Niles, 1972). In South America, Purple Martins molt and regrow most of their tail feathers, with the occasional exception of their outer pair, and the majority of flight feathers (Niles, 1972). Thus, all but the outermost tail feathers would contain only accumulated Hg and hormones from a period of time after arriving in South America.

Progne subis as a bioindicator

The importance of bird conservation cannot be downplayed. These animals offer many ecosystem services such as pest control and pollination, among others (Şekercioğlu, Wenny & Whelan, 2016). Bird communities also offer economical value as many species are used as food sources or incentivize tourism for bird watching or simply by their perceived visual or auditory beauty thereby increasing appeal of the region (Şekercioğlu, Wenny & Whelan, 2016).

There is also a further reason why the study of bird conservation is important: birds are potentially good bioindicators. Bird communities are sensitive to habitat loss and fragmentation (Turner, 1996). Many have adapted to occupy highly specialized niches near or at the top of food chains and a common result of environmental disturbances is the extinction and substitution of specialized species for generalists (Krügel et al., 2000; Turner, 1996). Most bird species are also easy to spot and identify visually or audibly, and the taxonomy and systematics of the group is well-known (Furness & Greenwood, 1993). The combination of these characteristics in many birds make the group a plausible choice to use as bioindicators.

Purple Martins, being a transhemispheric migrant, have increased potential to as a bioindicator for both North America and South America. Furthermore, their ranges cover a great portion of both continents (Sauer et al., 2017; Fraser et al., 2012), including different biomes, latitudes, and other abiotic and biotic factors, thus providing researchers fine-control over the influence of each factor. By understanding the interrelationships among connectivity, population changes, and environmental factors that affect Purple Martins, it is possible to reveal conservation needs in specific regions across several countries.

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Chapter 2

Effects of Mercury Contamination on Weight, Fat and Endocrinology of Purple Martins (*Progne subis*)

Abstract

Purple Martins (*Progne subis*) are insectivorous birds that breed in North America before migrating to South America, where they complete their molt. Many individuals migrate to the Amazon Basin, a region of high mercury (Hg) contamination, which raises the possibility that observed declines in Purple Martins could be linked to Hg exposure. Hg is a toxic heavy metal; exposure to and bioaccumulation of Hg can result in numerous and systemic negative health outcomes, including disruption of hormones like corticosterone (CORT) and triiodothyronine (T_3). Both play important roles in metabolic regulation in migratory birds. Feathers accumulate Hg and hormones during growth and thus levels quantified in feathers can be used as a proxy to assess a bird's Hg exposure and endocrine profile. The objective of this study was to evaluate interrelationships among total Hg (THg), CORT and T_3 concentrations in feathers grown in the Amazon and reproductive output and body condition (mass, fat score) endpoints. We assayed tail feathers from 80 Purple Martins collected from birds at their breeding grounds in Florida, Virginia, and Wisconsin. The concentration of THg in Purple Martin feathers ranged from 1.103 μg/g dw to 8.740 μg/g dw, which is above the level at which physiological impacts have been observed in studies of other avian species. Neither hormone quantified correlated with Hg concentration at the time of feather growth. However, we found evidence that THg concentration may negatively impact the ability of Purple Martins to accumulate fat, which impair a migratory bird's survivorship due to the high energy requirement of migration. This result also suggests carryover effects of Hg contamination at the wintering grounds to the summer breeding grounds, where body condition was assessed.

Introduction

Purple Martins (*Progne subis*) are migratory insectivorous birds that spend their breeding season in North America and non-breeding season in South America. Although they have been subject to studies for decades, the biology of Purple Martins is not completely understood. Purple Martins have declined in North America at a rate of about 0.8% per year for the past five decades (Sauer et al., 2017). Regions reporting declines are located in the central, southern and eastern portions of the U.S. and the provinces eastern Canada. From geolocation data, the subspecies of Purple Martins that reproduce in these regions (*P. subis subis*) winter in the Amazon (Fraser et al., 2012).

Reasons for population decline are not yet known but likely relate to conditions or exposures at the wintering grounds in Brazil given that populations of Purple Martins from most US states and all Canada provinces that are known to migrate to the Amazon are in decline (Fraser et al., 2012; Sauer et al., 2017). One important abiotic attribute of the Amazon region is high mercury (Hg) and methylmercury (me-Hg) levels, both naturally occurring and from gold mining activities in the region over the past decades and continuing, albeit illegally, to this day (Pirrone & Mahaffey, 2005, Lino et al., 2019). Hg can also be further released from the soil into the environment by deforestation and erosion, which are increasingly extensive in some regions of the Amazon (Lino et al., 2019).

Hydroelectric dams are other potential contributors of me-Hg in the Amazon Basin. This region of Brazil is home to 123 hydroelectric dams of varying sizes in operation as well as a growing number of new dams being proposed or under construction (ANEEL, 2020). Damming a river alters the natural water flow, allowing the retention of particulate Hg in the flooded areas, and, by extension, facilitates increased microbial methylation of Hg up to 40-fold in the flooded areas (St. Louis et al., 2004; Zhao et al., 2017). Me-Hg levels were found to be consistently well above natural levels even 9 years after the construction of experimental dams (St. Louis et al., 2004). In riverine reservoirs, an increase of up to 92% of the natural me-Hg levels were found in the outflow rivers when compared to the levels in the inflow rivers (Zhao et al., 2017). Consequently, the presence of a great number of hydroelectric dams throughout the Amazon Basin likely exacerbates the naturally high levels of me-Hg in the region.

The effects of Hg on the health of Purple Martins (*Progne subis)* are not fully understood. Hg contamination of Purple Martins of the Canadian Province of Saskatchewan revealed a mean Hg concentration of 2.22 µg/g (SD: ± 0.93) of feather (Kardynal et al., 2020), levels sufficiently high to cause negative health outcomes in other birds (Ackerman et al., 2016). However, the Hg levels of Purple Martins from other regions and the effects of this contamination in the physiology of this species are yet to be verified. Nevertheless, given the existing literature on other vertebrates, it is likely that Hg contamination has a range of deleterious effects on Purple Martins. Hg is a potent and persistent neurotoxicant that can elicit a variety of pathologies including anemia, liver and kidney dysfunction, retarded gonad development, and reduced growth and reproduction, potentially leading to the mortality of the bird in high enough concentrations (Ackerman et al., 2016; Basu, Goodrich, & Head, 2014; Eisler, 2005; Fernandes Azevedo et al., 2012). Furthermore, Hg can broadly disrupt the endocrine system, including those associated with the reproductive, adrenal, and thyroidal axes (Zhu, Kusaka, Sato, & Zhang, 2000).

Hg bioaccumulates and biomagnifies in birds (Ackerman et al., 2016). Insectivorous birds are reported to biomagnify Hg from their diet at a higher rate than herbivorous and carnivorous birds (Ackerman et al., 2018). Being both migratory and insectivorous, the effects of Hg contamination can have an added complication on Purple Martins. Since insectivorous birds bioaccumulate Hg at higher levels (Ackerman et al., 2016) and Hg is an endocrine disruptor (Zhu et al., 2000), Purple Martins feeding and roosting in the Amazon may have altered levels of hormones important for migration, among other annual cycle stages. For example, the adrenal glands synthesize and secrete glucocorticoids (GC), such as corticosterone (CORT), which modulate a variety of metabolic (e.g., glucose homeostasis, adipocyte development) and immune processes (Bauerle & Harris, 2016). Often referred to "stress hormones", GC influence many aspects of behavior and metabolism that improve survival during and following exposure to stressful stimuli (Cockrem, 2007). Further, GC function in immunomodulation, either promoting anti-inflammatory actions or pro-inflammatory actions, the last normally in response to high GC levels in acute-stress (Timmermans, Souffriau & Libert, 2019). Compromised secretion of GC might reduce survivorship under stressful situations given the importance of this hormone in regulating responses to both physiological and perceived stress (Timmermans, Souffriau & Libert, 2019).

This is particularly relevant to Purple Martins that are exposed to Hg in their overwintering habitat and then must migrate many thousands of kilometers to their breeding grounds in North America.

Similarly, the thyroidal axis can be disrupted by Hg. This endocrine axis produces hormones that are primary modulators of growth, development, thermogenesis, and metabolic rate, among others (Brent, 2012). Thyroid hormones are primarily composed of the prohormone thyroxine (T_4) and the more biologically active form, triiodothyronine (T_3), which is activated in tissues and regulates the actions of many genes (Brent, 2012). Thyroid hormones are synthesized and secreted by the thyroid gland. Synthesis involves iodination of tyrosine, a biochemical process that can be outcompeted by Hg (Soldin, O'Mara & Aschner, 2008). Due to the growth and development roles of THs, deficiency of T_3 is particularly problematic for young individuals (Brent, 2012). Thyroid hormones are also important modulators of lipolysis and lipid synthesis (Shahid, Ashraf & Sharma, 2020), both processes are crucially important to managing energy reserves for migration and reproduction in birds (Gutiérrez et al., 2019). Thus, the disruption of thyroid hormones synthesis and secretion by Hg can impair reproduction, development, and migration.

Various tissues (e.g., blood, muscle, fat, feather etc.) of birds can be quantified for Hg and hormone content, each of which record different time-frames of incorporation (Eagles-Smith et al., 2008). For example, correlation in mercury content between internal tissues tend to be high since they accumulate mercury throughout the lifetime of the birds (Eagles-Smith et al., 2008). This correlation, however, is weaker between internal tissues and feathers because feathers only accumulate Hg during growth (Eagles-Smith et al., 2008). Because of this, feathers offer a good estimate of Hg contamination and concomitant hormonal levels over the feather-growth period of a few weeks, since after feathers mature, blood vessels atrophy and thus cannot contribute circulating hormones and metals (Burger, 1993). Feather molt in Purple Martins starts in North America before migration, when they mostly change their body feathers and, not uncommonly, some of their primary and secondary flight feathers, before the molt halts during migration, and finishes after arrival in South America (Niles, 1972). In South America, Purple Martins molt and regrow most of their tail feathers, with the rare exception of their innermost pair, and the majority of flight feathers (Niles, 1972). Thus, these feathers grown in South America only contain accumulated Hg and hormonal levels from a period of time after arriving in South America.

We set out to test the hypothesis that exposure of Purple Martins to Hg contamination in the Amazon leads to negative physiological outcomes by quantifying and evaluating the interrelationships among total Hg (THg), CORT and T_3 content of feathers grown in the Amazon and body condition (mass, fat score) endpoints. We predicted that there would be no significant differences in feather Hg content between breeding groups of Purple Martins because all groups molt in the Amazon Basin of Brazil and are therefore exposed to the same level of environmental Hg when the feathers are growing. Furthermore, since Hg is known to disrupt CORT and T_3, we predicted that reproductive and body condition endpoints would correlate with Hg concentration.

Methods

Field sampling

Because tail feathers from Purple Martins are only grown in South America (Niles, 1972), feathers can be sampled either in South America or North America and capture the same endocrine and toxicological signature. We sampled in North America when the birds were preparing to nest, allowing for measurement of mass and fat score after presumptive contamination on the wintering grounds in Brazil.

In North America, Purple Martins nest in in man-made nest-boxes. Citizen science volunteers (landlords) construct and maintain the nest-boxes and monitor the Purple Martin occupants through a program called Project Martin Watch that is administered by the Purple Martin Conservation Association (PMCA). Data on the arrival and departure dates of individuals are recorded by the landlords. Some landlords also take notes on the number of eggs, nestlings, and fledglings among other data. At a subset of these locations, the animals are banded and measured, and samples of tail feathers collected by ornithologists during the breeding season. All this information is curated by the PMCA. For this study, the third innermost pair of tail feathers (T_3 rectrices) was plucked by hand from birds sampled in three US states: Wisconsin, Virginia, and Florida. In total, two feathers each from 80 individuals were used for the analysis. All birds sampled were adults that returned from their migration to South America.

Wisconsin and Virginia:

In Wisconsin (44.3° N, 88.4° W) 84 individuals were screened while 32 were screened in Virginia (38.6° N, 77.2° W). Two tail feathers from 25 arbitrarily selected adult individuals from each location were collected by the landlords. Birds were captured at their nests using door-drop traps. The birds were also assessed for sex for both locations. Biometric data were not available for these locations.

Florida:

In Florida (28.4° N, 81.5° W), birds were sampled at Walt Disney World Resorts, where around 20 Purple Martin houses exist, with numbers varying slightly from year to year. Each house contained 18-24 nests. Birds there were trapped using door-drop traps in the nests during the night and were measured and sampled during the following morning. Since these birds have ID bands and are accompanied by researchers over the years, their age is known by the team. Notes were taken about each bird's age, sex, mass, and fat score concomitant with the collection of two tail feathers. Fat score was assessed by scoring amount of subcutaneous fat visible according to the methods from Bairlein (1995). The researcher visually grades fat content of the two main fat deposits in birds (furcular and abdominal) in a standardize class scale that ranges from 0 to 8 as the amount of visible fat increases. The resulting class is the fat score attributed to the individual bird (Bairlein, 1995). Over 600 individuals from Florida were screened and, from these, feathers from 30 arbitrarily selected individuals were used in this analysis. The individuals selected included 15 males and 15 females and were selected over a broad range of age (adults in their second to seventh year) and fat score (1 to 4) as to best diversify the analyzed group.

Laboratory analysis

Mercury analysis:

Fifteen to 20 mg of chopped feather was placed in 15 mL Polytetrafluoroethylene (PTFE) bottles and underwent an open acid digestion adapted from Mohammed et al. (2017). For the digestion, 1.5 mL hydrochloric acid (HCl; Fisher

Chemical, MA, USA), 1 mL of 5% potassium permanganate ($KMnO_4$; Fisher Chemical) in 0.1% HCl, 100-200 uL hydrogen peroxide (H_2O_2), and 7 mL deionized (DI) water was added to each sample. These samples were left to digest overnight at room temperature and then placed in a water bath at 85°C for 2 h for further digestion. After digestion, the samples were vacuum filtered. Each sample was diluted to 15 mL with DI water prior to analysis. QAQC samples, extraction blanks, and a 10 ppb certified tuna fish flesh homogenate reference standard (IAEA-436) were prepared along with samples to ensure quality control and determine extraction efficiencies.

Hydroxylamine hydrochloride (100 uL $NH_2OH \cdot HCl$; Medivators) was added to each sample just before analysis to reduce the Hg to ground state atoms. Total Hg (THg) was quantified using the FIMS-100 cold-vapor atomic absorption analyzer with an attached auto-sampling unit (Perkin Elmer) and argon gas. Tin (II) chloride dihydrate ($SnCl_2$; Fischer Chemical) was used as the carrier and HCl as the reductant. Analysis blanks and standards were prepared within 2 h of each analysis. Standards were made from 1000 µg/mL Hg standard stock (PerkinElmer) and serially diluted to 10 ppb, 5 ppb, and 0.5 ppb concentrations. Analysis QAQC (10 ppm Hg; Inorganic Ventures, VA, USA) was diluted to 1 ppb and run as an unknown sample.

Corticosterone and T_3 analysis:

Feather CORT and T_3 levels were analyzed separately with kits from MP Biomedicals (Santa Ana, California, USA). Assay buffer and wash buffer were prepared for both analyses. Assay buffer was made using a 1:5 dilution of buffer concentrate (buffer X065, Arbor Assays, Ann Arbor, MI, USA) with water and the wash buffer was made using a 1:20 dilution of buffer concentrate with water.

Chopped feather (≤1 cm pieces) samples were digested using a native *Bacillus licheniformis* keratinase (FEED-0001; Creative Enzymes, Shirley, NY, USA) and an alkaline phosphate buffered saline (PBS; pH 9.0). One day prior to assay, all samples were resuspended in 500 µL assay buffer, shaken for 1 h and then stored at 4°C overnight. A duplicate was run for all samples and standards with an internal control being run on all plates. Digestion produces a pellet with equal mass to the feather and a liquid supernatant with hormones being present in both components. Tests of parallelism and accuracy were conducted. Acceptable accuracy was defined as an $R^2 > 0.95$ with a slope between 0.7 and 1.3 (ideal slope = 1) (Dillon et al. 2021). The resulting slope for the parallelism was 1.041 for pellets and 0.909 for supernatant, with a R^2 of 0.998 for both pellets and supernatants. For a more detailed methodology see Dillon et al. (2021).

Statistics

Samples from the three bleeding locations were used when analyzing correlation to Hg, CORT and T_3 concentration as response variables. When biometric variables were analyzed, only Florida samples were used due to data availability. Both simple and multiple linear regression were used to analyze hormones as response variables, with THg, breeding location, sex and age as predictor variables. For THg concentrations as response variable, linear models were used with breeding location, sex, and age as predictor variables.For bird's mass as a response variable, linear models were used with hormones, THg, sex and age as predictors. The same predictors were used for fat score, but an ordinal logistical regression (OLR) from the MASS package in R was used instead of a linear model to accommodate for the possibly variable distance between fat scores (Venables & Ripley, 2002). In each case, a stepAIC function from the MASS package was used to reduce the number of multiple variable models to only the most likely (Venables & Ripley, 2002). Given the small sample size, Akaike Information Criterion with a correction for small sample sizes (AICc) was used to select the best model between the model selected by stepAIC and the simplest models (Burnham & Anderson, 2004). For AICc selection, the canonical ΔAICc (delta AICc) of 2 was used to determine equal likelihood of models, and from these, the model with the lowest number of parameters (K) was selected when available. As exceptions, given that the glm function in R does not provide AIC values for quasi-poisson models (R core team, 2020). For comparison with toxicity thresholds of blood THg the following formula from Eagles-Smith et al. (2008) was used:

$$\ln\left(Blood\ THg\ \frac{\mu g}{g}\ ww\right) = 0.673 \times \ln\left(Feather\ THg\ \frac{\mu g}{g} dw\right) - 1.673$$

This formula provides an estimate of the blood THg but has a low explanation of the variance (R^2: 0.32). Therefore, we decided to do all statistical analyses using the values for feather THg.

Results

The minimum THg concentration found in Purple Martin Feathers was 1.103 µg/g dry weight (dw) and the maximum was 8.740 µg/g dw, with a mean of 2.807 ± 0.153 µg/g dw (fig. 2.1). The minimum inferred blood THg concentration was 0.201 µg/g wet weight (ww), and the maximum was 0.807 µg/g ww, with a mean of 0.367 ± 0.013 µg/g ww (fig. 2.1). The minimum concentration of CORT in feathers was 3.530 pg/mg dw, and the maximum was 134.090 pg/mg dw, with a mean of 11.890 ± 1.794 pg/mg dw (fig. 2.1). The minimum concentration of T_3 concentration was 24.210 pg/mg dw and the maximum was 109.160 pg/mg dw, with a mean of 55.305 ± 2.142 pg/mg dw (fig. 2.1).

The lowest AICc for models with variation of THg concentration as a response variable had age as a predictor (table 2.1). However, the null model was selected as the most likely due to its ΔAICc being lower than 2. Furthermore, breeding location had a much higher AICc (ΔAICc: 159.186), indicating that there was no difference in Hg contamination in Purple Martins between breeding locations (fig. 2.2).

The model selected by AICc that best explains CORT variation uses both breeding location and THg as predictors (table 2.2). However, the second-best model containing only the breeding location had a ΔAICc of 0.3 and a lower number of parameters and was therefore selected as the most likely model. The selected model estimates a higher CORT concentration in Purple Martins from Florida (mean: 20.6 pg/mg) than in Virginia (mean: 6.2 pg/mg) and Wisconsin (mean: 6.9 pg/mg) (fig. 2.2).

The model selected by AICc as the most likely model in explaining T_3 variation uses breeding location as the predictor (table 2.3). The selected model estimates a higher T_3 concentration in Purple Martins from Florida (mean: 70.6 pg/mg) than in Virginia (mean: 45.5 pg/mg) and Wisconsin (mean: 46.4 pg/mg) (fig. 2.2).

The variable selected by AICc to best explain mass variation was THg. Addition of sex as a variable provided a slightly lower AICc but with a ΔAICc below 2 (ΔAICc: 1.0) the model with only THg concentration as predictor was deemed the most likely (table 2.4). This model predicts a decrease in bird's mass with increasing feather THg concentration (fig. 2.3).

For fat score as a response variable the AICc selection showed that the best model uses THg, sex and age as predictor variables. No other tested model had a ΔAICc below 2 (table 2.5). The selected model predicts a change in probabilities toward lower fat scores for higher THg contamination (fig. 2.4). It also predicts lower fat scores for older birds and females tend to have higher fat scores than males. (fig. 2.4).

Discussion

Purple Martins from Florida, Wisconsin and Virginia did not significantly differ in feather THg concentration. This result was expected given that Purple Martins from these three states migrate to the Amazon Basin and, therefore, grew their tail feathers in the same region (Fraser et al. 2012; Niles, 1972). The concentration of THg quantified in the feathers of these individuals were similar to concentrations found in Saskatchewan, Canada (Kardynal et al. 2020), a breeding population also known to migrate to the Amazon basin.

Notably, THg measured in the feather is expected to correlate with the blood THg levels during the period of feather-growth and not the lifetime accumulated Hg in other body tissues (Eagles-Smith et al. 2008). As such, we cannot extrapolate total feather Hg to body burden of Hg. Therefore, the correlation between age and Hg being rejected by AICc was expected as each individual bird was subjected to similar Hg exposure at the time of feather-growth regardless of the bird's age. Nevertheless, it is still expected that accumulated Hg will result in stronger toxicity as the bird gets older and accumulates more Hg. Thus, lifespan bioaccumulation of total body Hg could be one reason for the negative and independent effect of age in the fat score. However, total body Hg measurement would be required to test this hypothesis.

The feather CORT of most birds fell between 4.35 and 21.7 pg/mg (90% of values). This might suggest a basal level for feather CORT in this species. The other values were outside one standard deviation for two individuals and outside four standard deviations in one individual. Since these values reflect circulating CORT over the feather-growing timeframe, it is possible that these higher values indicate that the birds in question have faced a high-stress situation while completing their molt in South America. However, correlation of feather CORT to feather THg was rejected by AICc selection, indicating that the Hg contamination that the individuals encountered were not high enough to cause a perceivable effect in this hormone. In loons (*Gavia immer*), there is evidence that an increase in blood THg of 1 µg/g ww results in an increase of 14.6% in CORT (Evers, De Sorbo & Savoy, 2005). Variation in concentrations of blood THg of 0.6 µg/g ww would result in a maximum variation in CORT levels caused by Hg of around 8.7%, assuming the same correlation occurs in this species. The inter-assay variation in this analysis was only 2.2% and should be able to detect this difference if nothing else interfered (Dillon et al. 2021). That variation, however, would likely not be detectable due to the higher random variation found even in the lowest concentrations of CORT in this species.

Feather T_3 concentration was more evenly distributed (min: 35.35, max: 109.16, mean: 70.553, SD: 19.2119). This hormone concentration did not show clear outliers or tendencies at any extreme. Feather T_3 correlation with feather THg was rejected by AICc implying that, as with CORT, in this species this range of variation in Hg contamination was not sufficient to cause any perceivable effect on T_3.

Since Hg is accumulated throughout the lifespan of a bird, it is likely that older birds would have higher total body Hg concentrations and that would show correlation with the hormones. However, AICc selection rejected a correlation between age and CORT levels or age and T_3 levels, which suggest that if these hormones respond to Hg, they are more responsive to momentary events than to the lifetime Hg accumulation this species is going through.

On the other hand, both CORT and T_3 were higher in individuals from Florida than from Wisconsin and Virginia. Since all these individuals are known to migrate to the same region in South America but not necessarily at the same time (Fraser et al., 2012), the more likely explanation for this difference in CORT might be related to the timing of migration and events unrelated to Hg that happened during or right after migration. Over the years, it has been documented by landlords that Purple Martins migrate earlier to and from breeding grounds in lower latitudes than in higher latitudes (PMCA, n.d.). The difference in T_3, might also be linked to differences in timing of migration or even the lower latitude of Florida, since metabolism of T_3 in birds fluctuates seasonally with photoperiodism and is stimulated during longer-days (Nakao, Ono & Yoshimura, 2008). Furthermore, since the feather growth period occurs right after arrival in South America (Niles, 1972) hormone concentrations could be reflective of circulating levels from before migration.

Nevertheless, feather THg correlated with the body mass measured later at the breeding site, suggesting that Hg affected the ability of the bird to accumulate fat for migration or that it might have delayed the migration and the subsequent recovery of fat reserves, resulting in a bird at lower body condition the time of breeding. It can also be verified by analyzing the correlation of fat score with Hg, which showed that birds with higher Hg were likely to have lower fat scores at the breeding season. The impacts on survivability and overall fitness that this physiological response has on the affected birds are hard to tell just by looking at the results. However, fat accumulation is crucial prior to long migrations to guarantee enough energy reserves to succeed in this costly endeavor (Hedenström, 2010; Gutiérrez et al., 2019). It is possible that higher concentrations of Hg contamination occur in Purple Martins migrating to the Amazon Basin. But Purple Martins affected by higher levels of Hg may not survive the migration if fat and total mass of the birds decreases to unsustainable levels consequently. Therefore, it is possible that the results found present a survivorship bias if higher exposure to Hg leads to death of the birds prior to feather collection in the breeding grounds. Nonetheless, the results found suggest that Hg contamination from South America affects the health of Purple Martins negatively.

Due to the lack of correlation found between THg and CORT and T_3, the mechanism behind the decrease in mass and fat score cannot be safely linked to these hormones. However, given the possible differences in the hormonal concentration found in feathers may be due to differences in timing of migration and that correlations may be too small against the higher variability of hormones found, this link cannot be safely refuted as well (Evers, De Sorbo & Savoy, 2005). Another possible mechanism would be a delay in the migration period, causing the birds to arrive later in their breeding site and not allowing enough time for them to recover the loss of fat reserves during migration. Furthermore, since Hg can affect any structure, from molecular levels and DNA expression to tissues (Bernhoft, 2012), there are multiple possible mechanisms for this effect. Independent of the mechanism, weigh loss is a long-time known effect of exposure to organic and inorganic Hg even in low concentrations of the heavy metal and that could explain the results (Friberg & Vostal, 1971).

Few reviews establish toxicity thresholds for feather THg; but one review of several experimental and observational studies in different bird species showed that concentrations as low as 5 ug/g correlates with impaired reproduction (Eisler, 1987). Furthermore, it is possible to compare the THg found in this study to toxicity thresholds from other reviews on several species by using the formula provided by Eagles-Smith et al. (2008) to convert total feather Hg concentration to total blood Hg concentration. In their work, THg content in 6 tissues (blood, eggshell, feather, liver, muscle, and kidney) of 4 waterbird species was measured and compared in birds collected in the field in both the prebreeding and breeding season (Eagles-Smith et al. 2008). The authors point that the correlation between feather THg and other tissues had too high a variation (R^2: 0.32). However, the methodology used by the authors samples both blood and feather at the same time which, expectedly, will not correlate well. A better correlation would be expected if blood samples were taken at the moment of feather-growth. Nonetheless, the calculated blood THg concentrations (min: 0.201 µg/g ww, mean: 0.368 µg/g ww, max: 0.8074 µg/g ww) crosses several thresholds for impairment including oxidative stress (0.2 µg/g ww), altered gene expression (0.3 µg/g ww), and several reproduction-related impairments (0.3-0.8 µg/g ww) (as reviewed by Ackerman et al., 2016 and Scheuhammer et al., 2007). These thresholds, however, were taken in different species and while they may or may not hold true for Purple Martins, they still show the toxicity potential of the concentrations found in this study.

It is not likely that a feather THg concentration range between 1.103 µg/g dry weight (dw) and 8.740 µg/g dw is high enough to case direct mortality in Purple Martins because in most species of birds analyzed the proposed mortality threshold is at least 10.5 times the maximum THg concentration found in this study (Ackerman et al., 2016; Scheuhammer et al., 2007). According to literature thresholds, at the higher extreme of Hg found in this study (~0.8 µg/g ww of equivalent blood THg), reproductive success might be reduced by half, but only one individual sampled had a concentration this high and most individuals had less than half this concentration, where the reduction in reproductive success should be less than 10% (Ackerman et al., 2016; Scheuhammer et al., 2007). Values at this range might not be enough to cause the population declines found in the subspecies that migrate to the Amazon (Sauer et al., 2017). However, that assumes that the THg concentration range found is representative of all Purple Martins going to the Amazon, which might not be true if Purple Martins do not survive the migration when contaminated with higher concentrations and, therefore, cannot be sampled by the methods used in this research. Furthermore, since all three breeding locations sampled were in decline and migrate to the Amazon a correlation between Hg contamination from the Amazon and population decline cannot be tested. Further studies should aim to analyze Hg contamination in breeding locations from other states, with different population trends, as well as birds in the Amazon.

In this research, feather has proven to be effective for measuring Hg contamination in a migratory bird. Feather Hg concentration represent an average Hg concentration over a predictable timeframe. It is likely a better measurement than blood Hg when comparing birds from different locations, populations, years, or species. Feather Hg, however, has the caveat of showing Hg accumulation of specific periods and may not satisfy the needs of a research if there is no feather growth in the desired investigation period. In that regard, blood Hg may still be the most reliable measurement for recent Hg exposure. However, blood Hg is more likely to change due to brief exposures to Hg and may not represent a good measurement of continuous exposure to toxic concentrations of Hg. Thus, as with other tissues, feathers can be a great source of contamination and hormonal information when used in the appropriate circumstances.

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Appendix: tables and figures

Table 2.1. AICc values of models of THg concentration as response variable. Both the model with age as a predictor and the null model were deemed likely due to ΔAICc being lower than 2. However, the null model was deemed more likely given the lower K. (ΔAICc = delta AICc, ModelLik = model likelihood, AICcWt = AICc weight, LL = log-likelihood and Cum.Wt = cumulative weight).

Table 2.2. AICc values of models for corticosterone concentration as response variable. Two models were deemed likely due to ΔAICc being lower than 2. Both include the breeding location (State) as a predictor, but one also includes THg as a predictor. However, the model with breeding location (State) as the sole predictor of corticosterone concentration was selected as being more likely than the other model due to lower K. Table is not representative of all possible combinations of variables tested and instead shows only the models with a single model and the model selected by stepAIC.

Table 2.3. AICc values of models for T_3 concentration as response variable. Two models were deemed likely due to ΔAICc being lower than 2. Both include the breeding location (State) as a predictor, but one also includes THg as a predictor. However, the model with breeding location (State) as the sole predictor of T_3 concentration was selected as being more likely than the other model due to lower K.

Table 2.4. AICc values of models for bird's mass as response variable. Two models were deemed likely due to ΔAICc being lower than 2. One of the models uses only ln(THg) as a predictor variable while the other also includes bird's sex as another predictor variable. The model using only ln(THg) as a predictor was selected as being more likely than the other model due to lower K.

Table 2.5. AICc values of models for bird's fat score as response variable. Only one model was deemed likely due to ΔAICc being lower than 2. The selected model uses THg, bird's sex and bird's age as predictors.

Table 2.1 – AICc values of models of THg concentration as response variable							
	K	AICc	ΔAICc	ModelLik	AICcWt	LL	Cum.Wt
Age	3	122.563	0	1	0.585	-57.820	0.585
Null	2	123.770	1.207	0.547	0.320	-59.662	0.905
Sex	3	126.207	3.644	0.162	0.094	-59.642	1
State	4	281.749	159.186	2.71E-35	1.59E-35	-136.604	1

Table 2.2 – AICc values of models for corticosterone concentration as response variable							
	K	AICc	ΔAICc	ModelLik	AICcWt	LL	Cum.Wt
State+THg	5	652.653	0	1	0.536	-320.916	0.535
State	4	652.957	0.304	0.859	0.461	-322.208	0.994
Age	8	663.726	11.072	0.004	0.002	-322.834	0.997
THg	3	663.958	11.305	0.004	0.002	-328.819	0.998
Null	2	664.897	12.244	0.002	0.001	-330.369	1
Sex	3	667.042	14.389	0.001	0	-330.361	1

Table 2.3 – AICc values of models for T_3 concentration as response variable							
	K	AICc	ΔAICc	ModelLik	AICcWt	LL	Cum.Wt
State	4	657.223	0	1	0.724	-324.341	0.724
State+THg	5	659.150	1.927	0.382	0.276	-324.164	1
Age	8	687.758	30.535	2.34E-07	1.69E-07	-334.850	1
Null	2	692.921	35.698	1.77E-08	1.28E-08	-344.382	1
Sex	3	693.343	36.120	1.43E-08	1.04E-08	-343.518	1
THg	3	695.083	37.860	6.01E-09	4.35E-09	-344.382	1

Table 2.4 - AICc values of models for bird's mass as response variable							
	K	AICc	ΔAICc	ModelLik	AICcWt	LL	Cum.Wt
ln(THg)+Sex	4	153.185	0	1	0.502	-71.792	0.502
ln(THg)	3	153.291	0.106	0.948	0.476	-73.184	0.978
Age	3	160.985	7.800	0.020	0.010	-77.031	0.988
Null	2	161.866	8.680	0.013	0.007	-78.710	0.995
Sex	3	162.459	9.274	0.009	0.005	-77.768	1

Table 2.5 – AICc values of models for bird's fat score as response variable							
	K	AICc	ΔAICc	ModelLik	AICcWt	LL	Cum.Wt
THg+Sex+Age	7	66.860	0	1	0.998	-26.035	0.998
THg	5	79.067	12.207	0.002	0.002	-34.325	1
Sex	5	84.963	18.103	0.001	0.001	-37.272	1
Age	5	85.521	18.661	8.87E-05	8.84E-05	-37.552	1
Null	4	89.015	22.156	1.54E-05	1.54E-05	-40.370	1

Figure 2.1. Concentration of THg, CORT and T_3 in feathers across all samples. THg minimum was 1.103 µg/g dry weight (dw) and the maximum was 8.740 µg/g dw, with a mean of 2.807 µg/g dw and a standard error (SE) of 0.153. Minimum concentration of CORT in feathers was 3.530 pg/mg dw, and the maximum value was 134.090 pg/mg dw, with a mean of 11.890 pg/mg dw and a SE of 1.794. T_3 concentration varied between 24.210 pg/mg dw and 109.160 pg/mg dw, with a mean of 55.305 pg/mg dw and a SE of 2.142.

Figure 2.2. Top - Concentration of THg shows no correlation with breeding location. The mean THg in Florida was 2.956 µg/g dry weight (dw) with a SE of 0.329. The mean THg in Virginia was 2.841 µg/g dw with a SE of 0.211. The mean THg in Wisconsin was 2.588 µg/g dw with a SE of 0.219. **Middle -** Differences in ln of corticosterone (CORT) concentration between breeding locations. The mean CORT in Florida was 20.631 pg/g dry weight (dw) with a SE of 4.293. The mean CORT in Virginia was 6.212 pg/g dry weight (dw) with a SE of 0.268. The mean CORT in Wisconsin was 6.870 pg/g dry weight (dw) with a SE of 0.332. **Bottom -** Differences in T_3 concentration between breeding locations. The mean T_3 in Florida was 70.553 pg/g dry weight (dw) with a SE of 3.508. The mean T_3 in Virginia was 45.535 pg/g dry weight (dw) with a SE of 2.574. The mean T_3 in Wisconsin was 46.422 pg/g dry weight (dw) with a SE of 2.063.

Figure 2.3. Correlation between THg and Mass. THg is shown in ln to facilitate visualization. Intercept value of correlation is 56.418 with a slope estimate of -3.534.

Figure 2.4. Probability of each fat score given a certain THg concentration. Each graph shows different probability trends for combinations of age and sex. THg effect on fat score is negative, decreasing the probability of higher fat scores and increasing the probability of lower fat scores. Both age and sex had an independent negative effect on Fat Score with older birds having overall lower fat scores and females having higher fat scores than males.

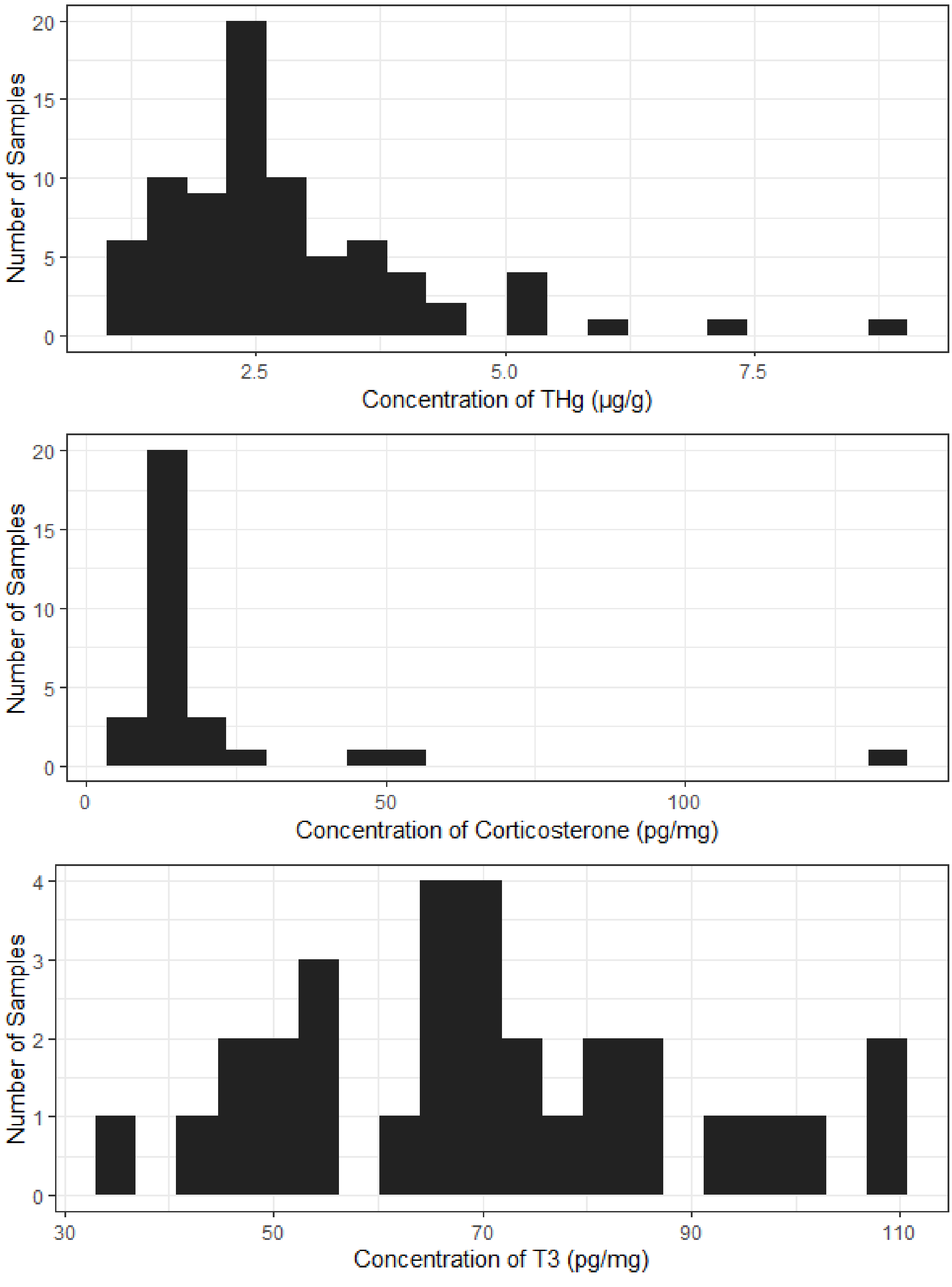

Number of Samples
Concentration of THg (µg/g)
Number of Samples
Concentration of Corticosterone (pg/mg)
Number of Samples
Concentration of T3 (pg/mg)

Figure 2.1

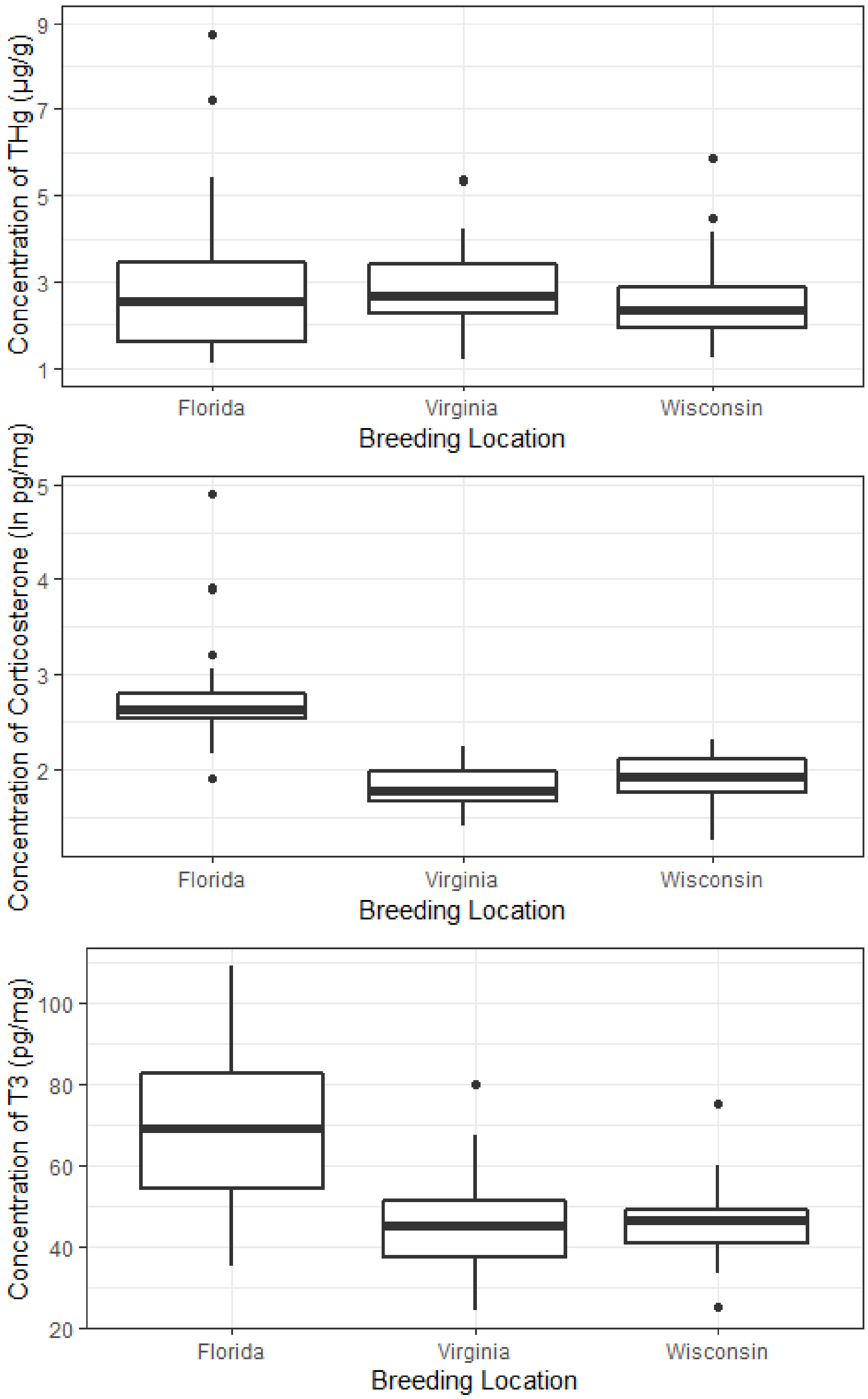

Concentration of THg (µg/g)
9
7
5
3
1
Florida
Virginia
Wisconsin
Breeding Location
Concentration of Corticosterone (ln pg/mg)
5
4
3
2
Florida
Virginia
Wisconsin
Breeding Location
Concentration of T3 (pg/mg)
100
80
60
40
20
Florida
Virginia
Wisconsin
Breeding Location

Figure 2.2

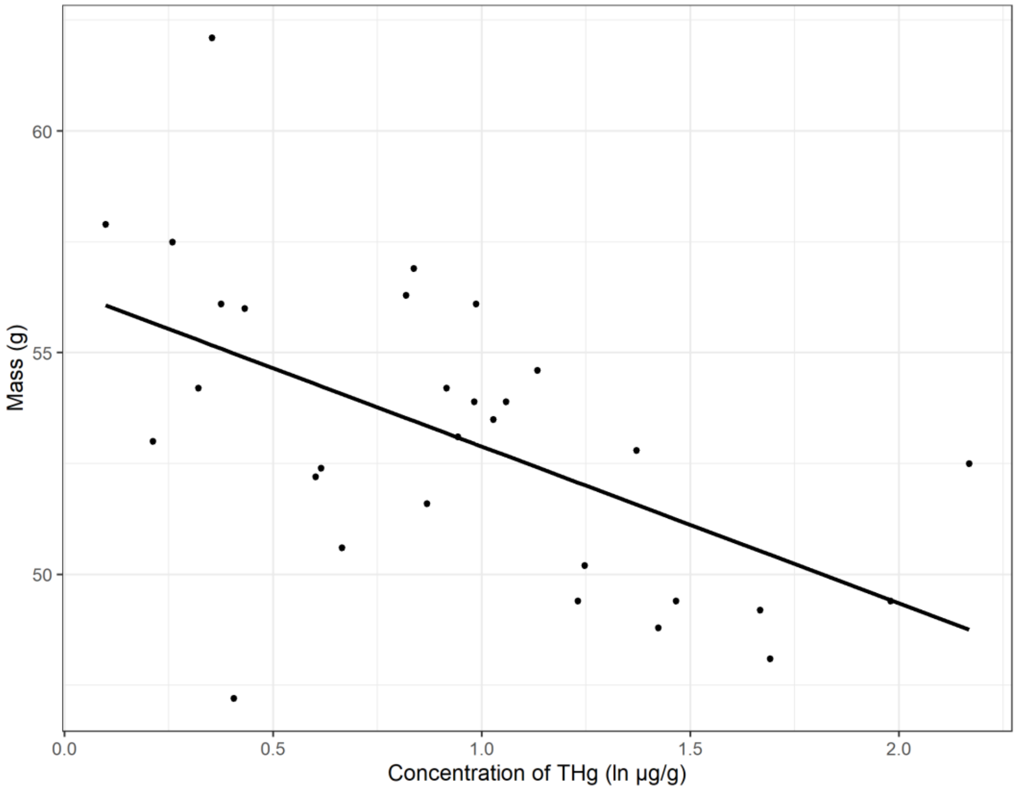
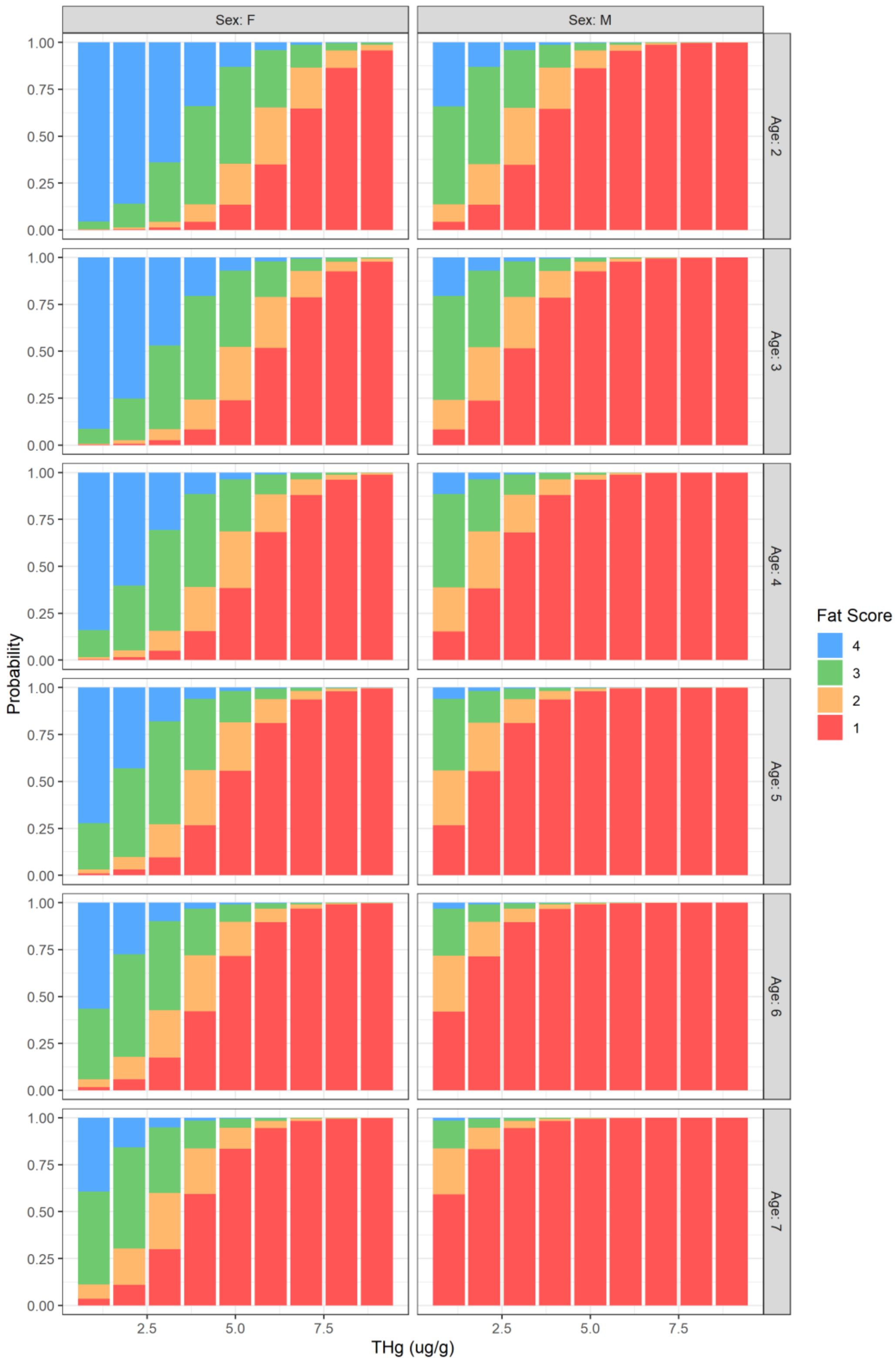


Figure 2.3



Sex: F
Sex: M
Age: 2
Age: 3
Age: 4
Age: 5
Age: 6
Age: 7
Probability
1.00
0.75
0.50
0.25
0.00
2.5
5.0
7.5
THg (ug/g)
Fat Score
4
3
2
1

Figure 2.4

Chapter 3

General Conclusion

Future of Amazon Basin mercury contamination

The presence of naturally high Hg is characteristic of the Amazon Basin; the soil is rich in the elemental Hg and the numerous rivers that constitute the Amazon Basin spreads it through a wide region (Lino et al., 2019; Siqueira et al., 2018). The anthropogenic contribution of elemental Hg to the region over past decades is evident but is not sufficient to drastically alter the concentration of this element in the region (Pirrone & Mahaffey, 2005; Siqueira et al., 2018). Instead, examples of human actions that are more likely to impact the distribution of this element in the region include deforestation and soil erosion, events that expose mercury from the soil and plant matter to other organisms and water bodies (Lino et al., 2019). But the major effect that humans might have on Hg cycle in the Amazon are probably a result of the constructions and operation of over a hundred hydroelectric dams in the region, a number predicted to increase to over two hundred with dams that are currently in construction or planned (ANEEL, 2020; Castello et al., 2013). The resulting increase in the number of lentic habitats caused by damming a river and subsequent increase of microbial methylation of Hg may lead to a higher concentration of methylmercury (me-Hg) throughout entire food webs in the region (Schiesari et al., 2017; Schiesari et al., 2020; St. Louis et al., 2004; Zhao et al., 2017).

Increased me-Hg in the region, a form of Hg that more easily bioaccumulates in tissues (Hosseini, Nabavi, & Parsa, 2013; Morel et al. 1998), could lead to its increased concentrations of total Hg (THg) in Purple Martins feathers over the following decades. Although the concentration of me-Hg found in the research presented at chapter 2 do not cross lethal thresholds of the literature (Ackerman et al., 2016; Scheuhammer et al., 2007), this scenario might change in the future as more and more hydroelectric dams are constructed. What the concentrations of THg in Purple Martins are as of 2019, and the effects that it had on the physiology of Purple Martins may serve as a benchmark parameter for research done with this species in the future.

In addition, keeping track of THg concentrations in this species may contribute to our understanding of how changes in Amazonian me-Hg content in the environment translate to changes of THg in a high trophic level species, as well as the effects that different concentrations of THg may have in an insectivorous migratory bird.

Mercury in other species and regions

High Hg concentrations have been documented in other species living in the Amazon, including humans. Human populations living in the Amazon have some of the highest concentrations of Hg ever recorded in the world today and an intake of Hg that can surpass 4 µg/kg/day, much higher than the 0.23 µg/kg/day exposure limit recommended by the World Health Organization (as reviewed by Passos & Mergler, 2008). High intake of Hg by humans comes from a traditional fish-rich diet of the people in the area (Passos & Mergler, 2008). Since me-Hg is produced by bacteria living in sediments of water bodies, fish and other aquatic life bioaccumulate and biomagnify Hg at higher levels than terrestrial organisms that do not share the same food web (Hosseini, Nabavi, & Parsa, 2013; Morel et al. 1998). This can also be noted in birds, as granivorous birds often have much lower Hg levels than birds that feed on insects that have an aquatic stage in life (Ackerman et al., 2018). These values can be even higher in piscivorous birds living in contaminated areas, such as Scarlet Ibises (*Eudocimus ruber*), a bird that feeds mostly on crustaceans and insects captured in tidal areas, and in one study had a mean feather Hg concentration of 41 µg/g, a value 14.6 times higher than the mean values we found for Purple Martin feathers (Klekowski et al, 1999).

However, Hg contamination as an environmental problem is not limited to the Amazon Basin and is highly impacted by ecological connections, food webs and organismal dispersal (Schiesari et al, 2017). Narwhal (*Monodon monoceros*) tusks reveal that climate change and sea-ice loss may result in habitat and food web changes that expose top-predators to high concentrations of Hg contamination through biomagnification (Dietz et al., 2021). Furthermore, it has been shown that a migratory aquatic bird species with high Hg contamination coming to a region with lower Hg locally elevate the Hg of that region over decades by depositing contaminated feathers, causing an increase in Hg contamination and genetic mutations in a co-occurring resident species (Klekowski et al, 1999). Thus, showing that animals that migrate from regions of high Hg contamination to regions of lower contamination can spread Hg contamination even to non-predators.

Relevance of mercury to the conservation of Purple Martins

Despite not considered threatened with extinction by IUCN at the time of writing (BirdLife International, 2016), the general population of Purple Martins (*Progne subis*) has suffered annual declines of 0.8% per year for the past five decades (Sauer et al., 2017). It is likely that this trend will continue and, because of this, the chances of this species becoming undeniably threatened in the next decades are high. Particularly, this is even more likely to occur in regions where the population trends are even grimmer, such as in many eastern US states and Canadian provinces with some populational rates of decline exceeding 1.5% per year (Sauer et al., 2017). Investing in the conservation of this species before it gets to a point where recovery is more difficult could be more efficient and thus spare resources on the long-term.

Investigating the relevance of mercury (Hg) contamination to the conservation of Purple Martins is unlikely to solve the population decline of this species. Several contributing factors probably intertwine to result in the observed population trend, including climate change, habitat destruction, and exposure to environmental contaminants, of which Hg is just one contributor (Lebbin et al., 2010). Nonetheless, given the prevalence of this contaminant in the Amazon Basin, one important habitat for the subspecies that is suffering from the highest declines, it is justifiable to investigate its impact on Purple Martins (Fraser et al., 2012; Morel et al. 1998; Sauer et al., 2017).

The research we carried out provides evidence that the contamination acquired in the Amazon Basin alone in a single season is enough to correlate negatively with body mass and fat score of Purple Martins (see chapter 2 and figures 2.3 and 2.4). It is hard to estimate how big of a concern this is for the health of the affected Purple Martins because the mechanism(s) decreased mass and fat score is not yet known. However, considering that the model predicts a decrease in mass of 13% between the lowest mercury concentration in the samples and the highest (see chapter 2 and figure 2.3) it is reasonable to expect that this could be detrimental to the survival of Purple Martins, particularly for individuals that might already be dealing with other natural and anthropogenic stressors. I cannot conclusively state that levels of Hg contamination found directly impact Purple Martin survivorship. As such, the results from the research presented at chapter 2 cannot prove that Hg contamination is causing the population declines in Purple Martins. However, given the broad suite of pathologies associated with Hg contamination (Ackerman et al., 2016; Basu, Goodrich, & Head, 2014; Eisler, 2005; Fernandes Azevedo et al., 2012; Zhu et al., 2000), and the results from our research, it is likely that Hg exposures may at the very least make conservation attempts more difficult. My findings provide evidence that the influence of Hg contamination cannot be overlooked when discussing the conservation of Purple Martins.

Next steps for this research

A natural progression of this research would be to analyze Hg content in the other subspecies of Purple Martins: *Progne subis arboricola*, from states and provinces in the northwestern region of North America, known to migrate to coastal states of Brazil to the south and east (Fraser et al., 2012; Fraser et al., 2017); as well as *Progne subis hesperia*, the subspecies from the southwest of US that has not yet been tracked using biologging technology (Fraser et al., 2012; Fraser et al., 2017). Since breeding populations of *Progne subis arboricola* are generally not suffering declines seen in the other two subspecies (Sauer et al., 2017), analysis of their Hg contamination could provide crucial information in determining whether Hg content correlates with population trends. Furthermore, sampling in roosts at South America, whether feathers or blood, could further elucidate regional differences in Hg contamination.

Other possible succession includes analysis of Hg contamination of Purple Martins at their breeding grounds in North America. This could be done by sampling mercury content in the blood of adults during the breeding season or by sampling tissues in chicks. Because chicks complete their first year's growth in North America, their Hg content will reflect their exposures in North America only. However, it should be noted that bioaccumulation rates of Hg in tissues of chicks may differ from adults even when they acquire it in the same overall region (Eagles-Smith et al., 2008; Wood et al., 1996). Therefore, caution should be taken by researchers when comparing Hg contamination in adults and chicks.

Lastly, another possible route to continue this line of research would be to quantify total body Hg. In the research presented in this paper, only feathers were used, which allows for measurement of Hg of a known timeframe: the duration of feather-growth (Burger, 1993). However, Hg bioaccumulates in other tissues throughout the lifespan of the specimen, which can lead to more severe health effects later in life because of chronic effects of mercury toxicity (Sofia, Husodo & Sugiharto, 2016; Taber & Hurley, 2008). It is possible that the independent effect of age in fat score (see Chapter 2 and figure 2.4) could be related to Hg accumulation throughout the bird's life. Therefore, quantifying Hg in other tissues, such as liver and muscle, or even an entire carcass would help understand the impacts of Hg contamination in physiological endpoints of Purple Martins. This, allied with sampling of Purple Martins of different ages in all three subspecies would give a fuller picture of Hg content that could impact survivorship, reproduction and other aspects of their biology that could compromise individual survival and fitness and ultimately, population trajectories.

Conclusion

The results of the research conducted here adds to the growing body of literature that document the effects of Hg contamination in birds, as well as studies of the effects of Hg contamination in other species that occur in the Amazon Basin. Due to the generalized decrease bird populations of North America (Rosenberg et al., 2019), it is necessary to approach every factor that could be contributing to the decline or failure to recover so that conservation efforts can be better focused on the most concerning events. Due to the nature of the research presented in this dissertation requiring the survival of the contaminated Purple Martins for an entire season and the migration event, the results cannot provide sufficient evidence that Hg contamination of this species in the Amazon is a major contributor to the decrease that species has faced in the past five decades (see chapter 2 and Sauer et al., 2017). However, the higher extreme of THg concentration found in the birds sampled could present major problems for the species, due to the much lower mass and fat score (see chapter 2 and figures 2.3 and 2.4) and crossing thresholds for a much lower reproductive success (Ackerman et al., 2016; Scheuhammer et al., 2007). As of the samples collected in 2019, concentrations this high are not frequent in the species, but are occurring in some individuals (see chapter 2). Therefore, if the contamination increases to a point where more individuals reach these thresholds of THg concentration, Purple Martins could face even higher population declines.

It is likely that environmental me-Hg will increase in the Amazon Basin in the future due to the dramatic increase in hydroelectric dams planned or in construction (Castello et al., 2013). If the construction of hydroelectric dams results in a much higher me-Hg in the whole ecosystem of the region, species that inhabit the region could face severe toxic effects of the element. That is true not only to Purple Martins but also for migratory species that make use of the Amazon Basin and resident species of the region. Similarly, this is true for human populations that live in the region and even for species from other regions that are connected to species that inhabit the Amazon Basin. The reach of contamination from Amazonian me-Hg is, therefore, hard to fully grasp but has the potential to be much broader than the extent of the Amazon Basin.

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