MERCURY ACCUMULATION AND TISSUE DISTRIBUTION IN WATERBIRDS OVERWINTERING IN TEXAS

by

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LIST OF ABBREVIATIONS

Abbreviation	Description
dw	Dry weight
EIA	Energy Information Administration
EPA	Environmental Protection Agency
FDA	Federal Drug Administration
fw	Fresh weight
Hg	Mercury
MeHg	Methylmercury
TDSHS	Texas Department of State Health Services
THg	Total mercury
TPWD	Texas Parks and Wildlife Department
Se	Selenium
SIA	Stable Isotope Analysis
ww	Wet weight

ABSTRACT

Mercury (Hg) is global pollutant that is toxic to wildlife at low concentrations. In waterbirds, exposure to Hg has resulted in altered breeding behavior, reduced hatching success, and nest abandonment. There have only been a few studies which assessed Hg concentrations in waterbirds in Texas, despite Texas being one of the greatest Hg emitters in the U.S. and an overwintering location for migratory waterbirds. In this study, tissues (muscle, liver, breast feather, wing feather) from 16 species of waterbirds that overwinter in Texas [American coot (*Fulica americana*), American wigeon (*Mareca americana*), blue-winged teal (*Spatula discors*), canvasback (*Aythya valisineria*), gadwall (*Mareca strepera*), green-winged teal (*Anas carolinensis*), hooded merganser (*Lophodytes cucullatus*), lesser scaup (*Aythya affinis*), mottled duck (*Anas fulvigula*), northern pintail (*Anas acuta*), northern shoveler (*Anas clypeata*), redhead duck *(Aythya americana*), red-breasted merganser (*Mergus serrator*), ring-necked duck (*Aythya collaris*) sandhill crane (*Antigone canadensis*), and wood duck (*Aix sponsa*)] were collected by TPWD licensed hunters from nine locations throughout the state and analyzed for total mercury (THg) using a direct mercury analyzer. This study investigated THg concentrations among species, foraging guilds (granivore, herbivore, omnivore, herbivore), and feeding strategies (dabbler, diver, dabbler/diver, wader) to determine which tissues and species had the greatest THg concentrations, which species had methylmercury (MeHg) concentrations exceeding federal [EPA; 0.3µg/g wet weight (ww)] and state (TDSHS; 0.7

µg/g ww) advisory levels for human consumption, and which species had THg concentrations above known threshold levels for adverse biological effects in birds. This study also investigated the relationship between $\delta^{13}C$ and $\delta^{15}N$ and muscle THg concentrations, and lastly investigated if wing or breast feather THg concentrations could be used to predict muscle and liver THg concentrations. Overall, THg concentrations were greatest in piscivorous diving waterbirds such as hooded merganser and red-breasted merganser but were also elevated in northern shoveler (omnivorous dabbler) and lesser scaup (omnivorous diver). Seven of the 16 investigated species had THg concentrations in their muscle and/or liver tissue that put them at risk of experiencing adverse biological effects. These same species also exceeded EPA and TDSHS MeHg advisory levels for human consumption. Wing and breast feather THg concentrations did not successfully predict muscle or liver THg concentrations in gadwall or redhead duck. The results from this study indicate that Hg accumulates the most in species that primarily consume fish but can be above concentrations known to cause deleterious biological effects in non-fish-eating species. However, since all species are migratory, future studies need to investigate the extent to which Hg accumulates in tissues while overwintering in Texas.

I. INTRODUCTION

Waterbirds

Waterbirds consist of 32 families of aquatic birds including Anatidae (ducks, geese, and swans) and Charadriiformes (shorebirds and wading birds, terns, and gulls) which dominate the majority of the 871 species of waterbird found globally (Wetlands International, 2012). Waterbirds are important for healthy wetlands due to the many ecosystem services they provide. These ecosystem services fall into four categories - provisioning, supporting, regulating, and cultural - as designated by the Millennium Ecosystem Assessment (2005) and include supporting services such as acting as plant, animal, nutrient, and contaminant bioindicators (Green and Elmberg, 2014; Burger and Eichorst, 2007; Elmberg et al., 2010), animal and plant propagule transportation and dispersal (Frisch et al., 2007; Klein et al., 2008; Brochet et al., 2009, 2010), and pest control (Miles et al., 2002). Additionally, waterbirds have been shown to play a pivotal role in maintaining species diversity among plant species, whether by indirectly stimulating the coexistence of different plant species by regulating competition or by increasing species diversity by reducing a dominant plant species (Jasmin et al., 2008; Hidding et al., 2010). Cultural services provided by waterbirds, including recreational hunting, birdwatching, and ecotourism, generate billions of dollars annually. In the United States in 2016, 2.4 million hunters spent an average of 7 days hunting migratory birds spending an estimated $2.3 billion on hunting-related expenses, which included travel and equipment (USFWS 2016). While all investigated species in the current study are considered waterbirds, it should be noted that sandhill crane (*Antigone canadensis*) prefer wetland environments while breeding and nesting, they have been documented spending time upwards of 300 yards from bodies of water (Gerber et al., 2014) and therefore may not be spending as much time preying on fish.

Mercury as a Pollutant

Mercury (Hg) is a naturally occurring toxicant which is added to the environment through natural (e.g., volcanic eruptions and erosion of rocks) and anthropogenic (e.g., coal-fired power plants, combustion of fossil fuels, and small artisanal gold mining operations) sources (Pacyna et al., 2006, 2009, 2010; Selin et al., 2007; Swain et al., 2007; Pirrone et al., 2010; Driscoll et al., 2013). Once released into the environment, Hg can be transported far from its source through atmospheric currents and ocean circulation (Seigneur et al., 2004; Lindberg et al., 2007; Selin, 2009; Harris et al., 2012a,b). In aquatic environments, inorganic Hg (Hg^{2+}) is converted into organic methylmercury (CH_3Hg^+; MeHg), the most toxic form to wildlife and humans, by sulfate-reducing bacteria in the sediment and overlying water column (Han et al., 2004; Hall et al., 2009; Marvin-DiPasquale et al., 2009; Gilmour et al., 2013).

In freshwater, estuarine, and marine organisms, particularly fish, it is well known that Hg bioaccumulates over time so larger, older individuals within a species have higher Hg concentrations than smaller, younger individuals (Cai et al., 2007; Bank et al., 2007; Fry and Chumchal, 2012; Chetelat et al., 2013; Donadt et al., 2021). Mercury also biomagnifies in aquatic food webs, so species at the top of the food web such as tunas, swordfish, and sharks have the highest Hg concentrations (Hammerschmidt and Fitzgerald, 2006; Maz-Courrau et al., 2012; Pouilly et al., 2013; Harding et al., 2018).

Contrary to what is observed in fish and cetaceans, past studies on Hg accumulation in waterbirds have found conflicting relationships with age, with species demonstrating either a positive, inverse, or no relationship between tissue Hg concentration and age (Furness and Hutton, 1979; Tavares et al., 2013; Goutte et al., 2014; Keller et al., 2014; Tartu et al., 2014). However, Hg biomagnification has been widely documented in many avian species which occupy high trophic levels such as gulls and cormorants (Beyer et al., 1997; Hosseini et al., 2013; Keller et al., 2014; Ahmadpour et al., 2016; Dolgova et al., 2018; Einoder et al., 2018), like what has been observed in fish and cetaceans (Dorea et al., 2006; Cai et al., 2007; Pouilly et al., 2013; Harding et al., 2018).

Impact of Mercury on the Health of Waterbirds

Waterbirds are unique in their ability to reduce the concentration of Hg, in particular MeHg, within their body because of their ability to transfer MeHg from the blood into developing feathers, thereby sequestering it (Furness et al., 1986; Monteiro and Furness, 2001; Condon and Cristol, 2009; Whitney and Cristol, 2017a). However, waterbirds exposed to MeHg when reproducing are at significant risk of deleterious effects because MeHg is quickly incorporated into the blood and redistributed throughout various organs and tissues before being maternally transferred to eggs (Bearhop et al., 2000; Heinz & Hoffman, 2004; Eagles-Smith et al., 2008).

At blood-equivalent total Hg (THg) concentrations below 1.0 μg/g wet weight (ww), exposure to THg can impact breeding behaviors such as pair bowing and head bobbing, decrease reproductive success and egg hatchability rates, and can impact general behaviors like nest protection, preening, and bill popping (Spann et al., 1972;

Burgess and Meyer, 2008; Heinz et al., 2009; Ackerman et al., 2016). With blood and feather THg concentrations ranging from 0.1-0.3 μg/g ww, white ibises (*Eudocimus albus*) were fed varying levels of dietary methylmercury chloride (CH_3HgCl) over a three-year period and experienced a decrease in overall productivity of eggs by 30%, males attempted to court females less, females approached courting males less, and overall showed a trend towards lesser parenting behavior (Frederick and Jayasena, 2010). Dietary CH_3HgCl fed to black ducks (*Anas rubripes*) over 28 weeks caused reductions in overall egg production, clutch size, and fecundity at only 3.0 μg/g ww resulting in only 16 ducklings surviving the study compared to 73 control ducklings (Finley and Stendell, 1978). At higher blood-equivalent THg concentrations (≥ 4.0 μg/g ww), bird eggs of the common mallard (*Anas platyrhynchos*) experienced reduced hatchability, a general decrease in overall productivity (Heinz et al., 2009; Herring et al., 2010; Kenow et al., 2011) and an increase in same sex pairings in white ibis (*Eudocimus albus*) (Frederick and Jayasena, 2010).

A recent review of 150 publications by Whitney and Cristol (2017a), which each assessed the impacts of Hg exposure on avian physiology determined that the threshold at which THg becomes lethal to 20% of avian populations is when concentrations in the diet exceed 5.0 μg/g ww. Markers used to assess avian health include, but are not limited to, reproductive behavior, neurologic function, endocrine system function, immunocompetence, general behavior, oxidative stress, and axonal degeneration, and nearly all birds assessed in this study were negatively impacted by exposure to Hg (Whitney and Cristol, 2017a). Chronic exposure to Hg can result in deleterious health effects like decreased fledgling production, adverse breeding behavior, nest abandonment, and impaired ability to handle stress (Spalding et al., 1994; Evers et al., 2005, Herring et al., 2012).

Impact of Mercury on Human Health

Humans are primarily exposed to Hg through seafood consumption (Bjorkman et al., 2007; Passos et al., 2008; Sunderland, 2007; Chan et al., 2010;), however, the consumption of waterbirds, e.g., ducks, could also be another lesser studied exposure pathway. Exposure to MeHg can result in deleterious neurological, cardiovascular, and immunological effects in humans (Diez 2008; Choi et al., 2009; Rice et al., 2014; Okpala et al., 2018). Methylmercury is a widely documented neurotoxin due to its ability to cross the blood-brain barrier and target the central nervous system (Kerper et al., 1992; Clarkson et al., 2007; Lohren et al., 2016) resulting in hearing, vision, and memory loss, muscle tremors, weight loss, and ataxia (Fujiki and Tajima, 1992; Mergler et al., 2007; Azevedo et al., 2012). In previous studies, MeHg has been linked to significant cardiovascular impairment such as increased blood pressure, lowered ability to pump blood (cardiomyopathy), chest pain (angina), myocardial infarction and ischemic strokes (Frustaci et al., 1999; Roman et al., 2011; Genchi et al., 2017). In addition, Hg is transferred *in utero* by crossing the placenta and is also passed to offspring postpartum through breast milk (Yang et al., 1997; Ask et al., 2002; Steuerwald et al., 2000; Grandjean et al., 2004; Rice et al., 2014) resulting in physical malformations, impaired critical thinking and problem-solving ability, impaired motor function, lower IQ, and decreased muscle growth in children (Harada, 1995; Grandjean et al., 1997; Castoldi et al., 2001; Oken et al., 2005)

Because of the many negative health impacts caused by MeHg exposure coupled with seafood being the dominant exposure pathway, federal and state Hg advisories regarding fish consumption have been issued. At the federal level, the Food and Drug Administration (FDA) issues a Hg advisory for commercial fisheries when the MeHg concentration in muscle tissue exceeds their 1 µg/g ww action limit, whereas the Environmental Protection Agency (EPA) issues Hg advisories when the MeHg concentration exceeds their 0.3 µg/g ww human health criterion. In Texas, the Texas Department of State Health Services (TDSHS) issues a Hg advisory for a particular fish species when the muscle MeHg concentration exceeds the 0.7 µg/g ww human health-based standard. While Hg advisory levels have not been issued for waterbirds, the same fish advisories can be applied.

Mercury in North American Birds

A literature review on Hg accumulation in North American waterbirds was conducted using Google Scholar™ and returned 1,290 unique publications which referenced Hg in papers published between January 2005 and March 2022 (search = allintext: mercury North America waterbird). When removing the "sort by range" function which allows setting specific years to search within, only 1,690 total results were present indicating that the majority (76%) of papers which focused on these subjects have occurred in the past 17 years.

Geographically, there are over one hundred regions across North America that have been identified as hot spots of Hg exposure in avian populations including the western Aleutian Islands, Puget Sound, northern Montana, North and South Dakota, central Arizona, San Francisco Bay, and the Gulf Coast of Texas (reviewed in Ackerman et al., 2016). Numerous hotspots have been found in northeastern United States and Canada including New York, New Hampshire, Vermont, Massachusetts, Maine, and the Laurentian Great Lakes (Evers et al., 2007; Evers et al., 2011; Jackson et al., 2015). Of the 29,219 total tissue samples from western North America reviewed in Ackerman et al. (2016), blood THg concentrations below 0.2 μg/g ww were considered background levels as no adverse effects were observed below this level. 66% of samples had THg blood concentrations between 0.2-1.0 μg/g ww and were considered low risk with symptoms such as altered breeding behavior, reduced chance of successful breeding, and reduced egg hatchability. Twenty-eighty percent of blood THg concentrations were between 1.0 – 3.0 μg/g ww and were at moderate risk with more severe negative effects such as decreased chances of raising multiple chicks, reduced nest success, impaired reproduction, and reproduction failure. 8% exceeded 3.0 μg/g ww and 4% were considered at severe risk with blood THg concentrations exceeding 4.0 μg/g ww. Blood-equivalent THg concentrations between 3.0 – 4.0 μg/g ww are considered high risk and can be summarized by significant impacts on reproductive success due to decreases in productivity, decreases in egg hatchability, decreased productivity for first time breeding females, altered courtship behavior, and malpositioned embryo within the egg. Concentrations exceeding 4.0 μg/g ww can result in severe reproductive and physiological effects such as being up to 31% less likely to reproduce successfully, decreased egg hatchability and 50% likelihood of embryo malposition, decreased offspring survival rates, and a proposed liver concentration (8.5 μg/g ww) where mortality occurs.

Mercury in Texas Birds

According to the U.S. Energy Information Administration (EIA), Texas uses the most coal for energy production of any state in the country and is therefore the greatest Hg emitter (U.S. Energy Information Administration, 2020). Texas is also home to one of the largest avian migration pathways in North America, the Central Flyway which extends from Texas northwards to Canadian provinces of Alberta and Saskatchewan (Boere and Stroud, 2006). However, relatively few studies have been conducted on Hg accumulation and tissue distribution in waterbirds collected in Texas (King and Cromartie, 1986; Gamble and Woodin, 1993; Mora et al., 2005; Schulwitz et al., 2015) including the species investigated in this study, and none focused on the geographic regions included in this study.

Prior to 1970, most studies involving Hg concentrations in Texas birds focused on anthropogenically caused factors such as thinning eggshells because of exposure to pesticides that contained Hg (King et al.,1970) and Hg accumulation at dredge disposal sites (White and Cromartie, 1985). Livers from redhead duck (*Aythya americana*) collected from Baffin Bay in early and late winter of 1988 and 1989 were all observed to increase their THg concentrations as duration of stay increased in the bay (Gamble & Woodin, 1993). Furthermore, in Galveston Bay, a field study conducted in 1980-1981 found Hg present in all livers sampled (0.75 – 1.42 μg/g ww) of three species of waterfowl including neotropic cormorants (*Nannopterum brasilianum*), laughing gulls (*Leucophaeus atricilla*), and black skimmers (*Rynchops niger*) (King & Cromartie, 1986). A more recent 2015 study indicated that two north Texas lakes, Caddo Lake and Lewisville Lake, were found to be contaminated with Hg due to atmospheric deposition which affected passerine birds such as the eastern bluebird (*Sialia sialis*) and Carolina wren (*Thryothorus ludovicianus*) more heavily than their stationary nestlings and showed Hg was biomagnifying within the lakes because large piscivorous avian species had higher levels of Hg compared to insectivorous species (Schulwitz et al., 2015).

In addition to the lack of data on Hg accumulation in waterbirds overwintering in Texas, there is a demand for hunting in the state and thus a sizeable gap in public knowledge on what they may be consuming. In 2020, 1,120,620 million hunting licenses were sold in Texas, generating over $46,886,984 for the state (USFWS, 2020). With so few studies focusing on Hg accumulation in Texas waterbirds coupled with the fact that waterbirds are hunted for consumption, there is a need for data collection on commonly consumed species in the region so that agencies [e.g., Texas Parks and Wildlife Department (TPWD)] that write conservation, management, and recovery plans for waterbirds in Texas can better understand the risk to waterbird health at the individual and population-level as a result of Hg exposure.

Foraging Guild and Feeding Strategy Delineation for Mercury Analysis

Due to the vast number of waterbird species and accompanying inter- and intraspecific variability in Hg concentrations, assigning species into specific foraging guilds for ease of interpretation has been widely adopted (Wiens, 1989; Paszowski and Tonn, 2006; Gatto et al., 2008; Liordos, 2010; Chatterjee et al., 2020). The guild approach considers groups of species whose niche's overlap, regardless of taxonomic differences, and are generally distinguished by similarities in food preference, food substrate (e.g., where food is taken from), feeding strategy (dabbling, diving, or wading), and habitat use (DeGraaf et al., 1985; Eagles-Smith et al., 2009; Ackerman et al., 2016). Chatterjee et al. (2020) successfully assigned several waterbird species to five distinct foraging guilds using only foraging habitat and feeding technique, suggesting room for variability in factors which determine foraging guilds. Some common avian guilds include granivore (consume grains, seeds, and nuts), herbivore (plant matter like leaves, roots, and stems), omnivore (consume both plant and animal matter), and piscivore (consume mostly fish but are known to consume insects and crustaceans as well) (DeGraaf et al., 1985).

Most Hg found in birds comes from the diet for piscivorous species and exposure to air pollution for non-piscivorous species (Evers et al., 2005). Piscivore blood-equivalent THg values have been shown, on average, to be up to 16.5-times greater than granivore THg values (Ackerman et al., 2016). However, dietary preferences in birds can change throughout the year based on many factors such as availability, reproduction behavior, and feeding methods (Bethke, 1991; Afton et al., 1991; Olmos et al., 2001; Woodin and Michot, 2002) Additionally, changes in agricultural land use and loss of wetland environments can influence waterbird diet (Duncan et al., 1999; Rendon et al., 2008; Kloskowski et al., 2009).

While diet accounts for most Hg found in birds, feeding techniques such as dabbling, diving, and wading, can also impact Hg concentrations in waterbirds (Pearce et al., 1976; Heinz, 1979; Driver and Derksen, 1980; Hoffman et al., 1998; Braune and Malone, 2006; Heinz et al., 2009). However, studies investigating the relationship between feeding strategy and Hg accumulation in waterbirds are less prevalent compared to foraging guild analyses. Dabblers such as gadwall (*Mareca strepera*) and the northern pintail (*Anas acuta*) generally feed by either skimming the surface for plants, insects, grains, and larva or by submerging their heads under the water of shallow marshes and flooded fields in a process called "tipping up" (Mitchell et al., 1992). Diving species like the red-breasted merganser (*Mergus serrator*) and ring-necked duck (*Aythya collaris)* take a more aggressive approach use their powerful legs to break the surface of the water to hunt small fish, insects, crustacean, and other subsurface food (Croll et al., 1992). Waders such as the sandhill crane (*Antigone canadensis)* and American avocet (*Recurvirostra americana*) typically rely on modified bills to glean plant material, invertebrates, and small vertebrates from the surface or by subsurface probing of lake bottoms and mud (Kelly et al., 2003).

Use of Stable Isotopes to Understand Mercury Concentrations in Birds

Stable isotope ecology uses elemental isotopic ratios [e.g., carbon (C) and nitrogen (N)] to better understand foraging habits, trophic positions, and overall food web ecology (Hobson et al., 1992, 1993, 1996; Jarman et al., 1996; Jennings et al., 2008). Stable isotope analysis (SIA) has been successfully used to help understand Hg concentrations in birds (Yoshinaga et al., 1992; Thompson et al., 1998). Stable isotope assessments offer the ability to investigate aspects of animal diets by observing differences in $\delta^{13}C$ and $\delta^{15}N$ values typical of their relevant food webs rather than physical examination of stomach contents or scat (Hobson & Wassenaar 1999). These same isotopes additionally offer the ability to determine variation of dietary patterns within a species (Hobson & Wassenaar 1999). $\delta^{15}N$ is used to determine the relative trophic level position, with higher $\delta^{15}N$ values indicating a higher trophic position relative to lower δ15N values (Minagawa and Wada, 1984; Peterson and Fry, 1987; Chumchal and Hambright, 2009; Chumchal et al., 2011). Given that fractionation of $\delta^{15}N$ occurs as trophic position changes, the relationship between $\delta^{15}N$ and THg can be used to determine whether birds with higher δ15N values have a higher tissue burden of THg.

$\delta^{13}C$ isotope ratios, unlike $\delta^{15}N$, remain relatively unchanged at each trophic level within a food web and can therefore be used to look at dietary changes or preferences within and among species (France and Peters, 1997; Cherel and Hobson, 2007). $\delta^{13}C$ can be used to look at dietary preferences due to the discovery that the C3 plants utilizing Rubisco catalysts are more deplete (-35‰ to -20‰) vs the more enriched PEP catalyst involved in C4 metabolism (-18‰ to -7‰) (Farquhar et al., 1989; West et al., 2004; Newton 2016). In the absence of physical stomach contents for dietary analysis, $\delta^{13}C$ values can be coupled with THg concentrations to infer the Hg burden of terrestrial vs. oceanic carbon and which source contributes more of overall $\delta^{13}C$ values (Post 2002). Comparing seasonal (summer and winter) differences of $\delta^{13}C$ and THg observed in double-crested cormorants (*Phalacrocorax auratus*) and Caspian terns (*Hydroprogne caspia*) breeding in Lake Ontario suggests that Hg loaded in the winter can carry over to the summer, indicating that Hg values observed in one season may not be a direct result of exposure in that region (Lavoie et al., 2014).

Species to be Investigated

Sixteen species of waterbird that overwinter in Texas were investigated in this study. Most (14 of the 16) species fall into one family of waterbird, Anatidae, which include ducks, geese, and swans. These species included American wigeon (*Mareca* *americana*), blue-winged teal (*Spatula discors*), canvasback (*Aythya valisineria*), gadwall, green-winged teal (*Anas carolinensis*), hooded merganser *(Lophodytes cucullatus*), lesser scaup (*Aythya affinis*), mottled duck (*Anas fulvigula*), northern pintail, northern shoveler (*Anas clypeata*), red-breasted merganser, redhead (*Aythya americana*), ring-necked duck, and wood duck (*Aix sponsa*). The remaining two species were from the Gruidae and Rallidae families [sandhill crane and American coot (*Fulica americana*), respectively].

Tissues to be Investigated

Once Hg enters the body, it is redistributed to different tissues where it can accumulate at various rates dependent upon several physiological and environmental factors including habitat use, foraging guild, molt strategy and timing, duration of wetland stays, and breeding timing (Burger et al, 1993; Bond and Diamond, 2008; Eagles-Smith et al., 2009; Eagles-Smith and Ackerman, 2014; Pedro et al., 2015; Sullivan and Kopec, 2018; Bottini et al., 2021; Thorne et al., 2021). It is widely documented that feathers can act as a Hg sequestration site in birds (Goede and Bruin, 1986; Burger et al., 2011; Whitney and Cristol, 2017b) and therefore feathers are a good tissue to use to understand Hg exposure. Of the tissues commonly included in Hg studies (muscle, blood, feathers, liver, and kidney), when speciated, 90-95% of THg in muscle, blood, and feathers has been determined to be MeHg (Fournier et al., 2002; Ackerman et al., 2016), whereas 88% of THg in bird liver being MeHg (Eagles-smith et al., 2009). Lastly, MeHg is more variable in bird kidneys with between 69 and 82% of THg being MeHg (Rutkowska et al., 2019), therefore, THg should not be interchanged with MeHg in liver and kidney unless using a conversion factor.

The tissues examined in this study (muscle, liver, breast feather, and wing feather) have been chosen because duck muscle and occasionally liver are consumed throughout the United States, potentially providing a source of Hg to humans. Breast and wing feathers were included because they allow for minimally invasive sampling and can be used to investigate the Hg body burden when harvesting the entire bird is not feasible (Braune, 1987; Hahn et al., 1993; Blevin et al., 2013; Schulwitz et al., 2015; Karimi et al., 2016; Beckler et al., 2016; Cherel et al., 2018).

Objectives of the Study

This study determined muscle, liver, wing feather, and breast feather THg concentrations in 16 species of waterbird that overwinter in Texas and aimed to answer the following: can a less invasive tissue like feathers predict THg within internal tissues, what species and tissues are accumulating the most Hg, are muscle and liver (which are the most commonly consumed duck tissues by humans) THg concentrations high enough to pose a threat to waterbird and human health, can stable isotope ecology coupled with THg data be used to infer dietary information and trophic status. The goals of this study can be organized into six objectives:

1. Assess interspecies variability in THg concentration among each tissue, with the prediction that tissues of piscivores and omnivores will have higher THg concentrations than tissues from granivores and herbivores and that divers will have higher THg concentrations than dabblers, dabbler/divers, and waders.
2. For each species, determine the intraspecies variability in muscle, liver, and feather

THg concentrations, with the prediction that THg concentrations will be highest in wing feather, followed by liver and muscle, and lowest in breast feather.

3. Determine if any of the 16 species have blood-equivalent THg concentrations which exceed toxicological risk levels, with the prediction that piscivorous species will be at risk of adverse biological effects due to elevated THg blood-equivalent concentrations.
4. Determine if any of the 16 species have THg ww concentration that exceeds the EPA human health criterion (0.3 μg/g) and TDSHS human health-based standard (0.7 μg/g) advisory levels for fish, with the prediction that muscle and liver from piscivorous and omnivorous species will have a higher percentage of individuals that exceed advisory levels than granivores and herbivores.
5. Measure the muscle $\delta^{13}C$ and $\delta^{15}N$ values and investigate the relationship between $\delta^{13}C$ and $\delta^{15}N$ and muscle THg concentration, with the prediction that $\delta^{13}C$ values will be similar across foraging guild or feeding strategy and species with higher $\delta^{15}N$ values will have greater THg concentrations.
6. Determine if THg concentrations in wing and breast feathers can be used to predict THg concentrations in internal tissues (muscle, liver) with the prediction that feather THg concentrations can be used to predict the THg concentration in internal tissues.

II. METHODS

Sample Collection

Sixteen species of bird (total n = 149; Table 1) were sampled by TPWD licensed hunters between November 2018 and January 2019. Sample collection occurred in West Texas (Brownsfield), inland Texas (Choke Canyon, Eagle Lake, and Martindale), and five locations along the Gulf coast (Aransas Pass, Bayside, Corpus Christi Bay, Port Mansfield, and the Guadalupe Delta Wildlife Management Area near Tivoli) (Figure 1). Except for Brownfield, all other waterbirds were collected in South Texas. The sample size of each species collected at each location can be found in Appendix A.

In addition to recording location and species information, all birds were weighed before muscle, liver, wing feathers, and breast feathers samples were collected and placed in individually labeled 50 ml trace metal clean tubes (muscle, liver) or labeled 1 gallon Ziploc bags (feathers). Samples were then transported to Texas State University and stored at -20°C until further processing.

Sample Preparation

Muscle and liver samples were thawed, trimmed to remove surface tissue, and the ww recorded, after which they were freeze dried for 48 hours at -54°C (Labconco FreeZone$^{2.5}$; Labconco, Kansas City, MO), the dry weight (dw) recorded, and then homogenized into a powder. To allow for conversion between dw and ww THg concentrations, the muscle and liver moisture content for each species is reported in Table 1.

Feathers were cleaned to remove exogenous contamination and dried following the method described in Ackerman et al. (2007) and Bond et al. (2015). In summary, feathers were rinsed to remove surface contamination using DI water, manually agitated in a 1% Liquinox (Alconox Inc., White Plains, NY) detergent solution for 60 seconds every 10 minutes for one hour (6 one minute shake sessions) before being rinsed 4-5 times in DI to remove the detergent, and lastly dried at 60°C for 24 hours. The entire breast feathers were processed, however, due to their large size, only the vane of the wing feathers was used. This region of the feather was chosen to be analyzed because Hg in the vane is evenly distributed, resulting in no significant difference between inner and outer vane sections (Goede and Bruin, 1984; Hahn et al., 1993).

Mercury Analysis

The concentration of THg in all samples was measured using a Direct Mercury Analyzer (DMA-80; Milestone Inc., Shelton, CT) which uses thermal decomposition, gold amalgamation, and atomic absorption spectrometry as described in U.S. EPA Method 7473 (U.S. EPA, 2007). On average (minimum and maximum mass in parentheses), 25.1 mg (22.6 to 26.9 mg) of muscle tissue, 24.5 mg (15.6 to 27.4 mg) of liver tissue, 22.0 mg (12.9 to 25.9 mg)) of wing feathers, and 20.4 mg (19.0 to 23.3 mg) of breast feathers was analyzed in quartz boats.

To ensure accuracy of results, quality control included blanks (empty quartz boats), certified reference materials [CRMs; DORM-4 fish protein (0.412 µg/g THg), National Research Council Canada (NRCC); DOLT-5 dogfish liver (0.444 µg/g THg), NRCC; and ERM-CE464 tuna (5.24 µg/g THg), European Reference Materials), and duplicate samples. Blanks (n = 67) had a mean THg concentration of 0.0001 μg/g and the mean percent recovery of the CRMs was 97.0% for DORM-4 (n = 44), 93.8% for DOLT-5 (n = 13), and 96.4% for ERM-CE464 (n = 18). The mean relative percent difference between duplicate samples was 1.6% for muscle (n = 20), 1.6% for liver (n = 21), 1.7% for wing feather (n = 20), and 2.2% (n = 22) for breast feather.

Stable Isotope Analysis

For each investigated species, between 0.64 to 1.4 mg of non-lipid extracted muscle tissue (total n = 90; up to n = 7 per species) was packaged into tin capsules and shipped to the UC Davis Stable Isotope Facility (Davis, CA) for $\delta^{15}N$ determination using an elemental analyzer (PDZ Europa ANCA-GSL) interfaced to a continuous flow isotope ratio mass spectrometer (IRMS; PDZ Europa 20-20; Sercon Ltd., Cheshire, UK). Results were given in δ-notation using:

$$\delta_{Sample}\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R is a ratio of the heavy to light isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$). The standards used were Vienna Pee Dee Belemnite and atmospheric nitrogen for $\delta^{13}C$ and $\delta^{15}N$, respectively. Duplicate samples (n = 5) had a mean relative % difference of 0.45% and 0.41% for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Although only $\delta\ ^{15}N$ was requested, $\delta^{13}C$ values were also provided for each sample, however, a lipid correction factor is required because the samples were not lipid extracted prior to analysis. The following equation was used because of its ability to perform well given a wide range of C:N ratios:

$$\delta^{13}C_{predicted} = \delta^{13}C_{bulk} + D\ I + D\ f(C:N)$$

where f(C:N) = 3.9 / (1+(287 / 93) (1+(1 / 0.246 (C:N) – 0.775))), D and I are fixed values of 6‰ and -0.180, respectively, as determined in McConnaughey and McRoy (1979) and Kojadinovic et al. (2008).

Calculating Blood-Equivalent THg Concentrations

In order to compare muscle and liver THg concentrations in this study with known Hg threshold levels for adverse biological effects in avian species, muscle and liver THg concentrations were converted to blood-equivalent concentrations using the following equations taken from Eagles-Smith et al. (2008).

Muscle (R^2 = 0.90): $\ln(\text{Blood THg}_{\mu g/g\ ww}) = 1.080 \times \ln(\text{Bird Muscle THg}_{\mu g/g\ dw}) - 1.024$

And

Liver (R^2 = 0.88): $\ln(\text{Blood THg}_{\mu g/g\ ww}) = 0.970 \times \ln(\text{Bird Liver THg}_{\mu g/g\ dw}) - 1.929$

Once converted, samples were determined to have levels of THg which can results in waterbirds experiencing observable effects of Hg exposure, impairment, substantial impairment, and severe impairment. The lowest concentration which resulted in observable impacts on avian health occur at 0.2 µg/g ww and include oxidative stress responses, altered gene expression, and decreased egg hatchability. Concentrations above 1.0 µg/g ww (impairment) effect behavior and physiology such as altered breeding behavior, reduced breeding success the following breeding season, reduced productivity, further reduced egg hatchability, and impaired behavior. Substantial impairment occurs at approximately 2.0 µg/g ww and is summarized by impairments to reproduction including reduced egg hatchability and reduced breeding success, and reduced productivity. Severe impairment occurs at 3.0 µg/g ww and can result in decreased immune responses, impaired productivity, and complete reproductive failure (Ackerman et al., 2016).

Statistical Analysis

All statistical analysis was completed using SigmaPlot v14.0 with the confidence level set at $\alpha = 0.05$. All THg data was natural log transformed prior to statistical analysis to better meet assumptions of normality and equal variance.

A one-way analysis of variance (ANOVA) and Tukey post hoc test was used to determine whether there was a significant difference in THg concentration within a tissue among species (species with $n \geq 10$), among feeding guilds and feeding strategies, and between tissues within a species (species with $n \geq 5$). A one-way ANOVA was also used to determine if there was a difference in muscle $\delta^{13}C$ and $\delta^{15}N$ values among species, foraging guilds, and feeding strategies among species (species with $n \geq 3$). If the data failed the assumptions of normality or equal variance, a Kruskal-Wallis one-way ANOVA on Ranks and Dunn's pairwise comparison was used.

Finally, linear regressions were used to examine the relationship between muscle $\delta^{13}C$ and THg concentration and $\delta^{15}N$ and THg concentration based on feeding guild and feeding strategy using log-transformed Hg data to better meet statistical assumptions. Linear regressions were also used to predict whether the THg concentration in wing and breast feathers could be used to predict the THg concentration in muscle and liver for two species with the largest sample sizes (gadwall and redhead).

III. RESULTS

Interspecies Variability in THg Concentrations

Median, mean, standard deviation (SD), and minimum and maximum THg concentrations organized by species and tissue are provided in Table 2. Among the species included in the study, the greatest mean muscle, liver, breast, and wing feather THg concentrations were reported in hooded merganser, red-breasted merganser, and northern shoveler, whereas the lowest mean muscle and liver THg concentrations were reported in sandhill crane, American wigeon, and redhead duck. The three species with the lowest mean wing feather THg concentrations were canvasback, redhead, and American wigeon and the lowest breast feather THg concentrations were reported in canvasback, American wigeon, and northern pintail. Among the 16 species investigated in this study, five species (both merganser species, canvasback, lesser scaup, and northern shoveler) reported the greatest mean THg concentration in the liver, whereas the remaining 11 species reported the greatest concentration in the wing feathers.

The interspecies variability in THg concentrations for each examined tissue is shown in Figure 2. For each tissue, there was an overall significant difference in THg concentrations among species with a sample size ≥ 10 (one-way ANOVA or Kruskal-Wallis one-way ANOVA on Ranks; muscle: $H = 72.6$, $df = 5$, $p < 0.001$; liver: $H = 68.9$, $df = 5$, $p < 0.001$; breast feather: $F_{(5,98)} = 24.07$, $p < 0.001$; wing feather: $F_{(5,98)} = 25.6$, $p < 0.001$).

The foraging guild with the greatest mean (± SD) THg concentrations (µg/g dw) across muscle, liver, breast feather, and wing feather were piscivores (1.63 ± 1.11, 9.49 ± 5.21, 6.02 ± 3.79, and 7.66 ± 5.16, respectively), followed by omnivores (0.188 ± 0.224, 0.999 ± 1.33, 0.767 ± 0.815, and 1.23 ± 1.06, respectively), granivores (0.118 ± 0.0801, 0.400 ± 0.218, 0.266 ± 0.198, and 0.626 ± 0.537, respectively) and lowest in herbivores (0.0460 ± 0.0599, 0.247 ± 0.241, 0.290 ± 0.223, and 0.365 ± 0.282, respectively). For each tissue, significant differences in THg concentration were reported among foraging guilds (Figure 3; one-way ANOVA or Kruskal-Wallis one-way ANOVA on Ranks; muscle: $H = 47.7$, $df = 3$, $p < 0.001$; liver: $H = 43.4$, $df = 3$, $p < 0.001$; breast feather: $H = 43.9$, $df = 3$, $p < 0.001$; wing feather: $F_{(3,144)} = 51.2$, $p < 0.001$). Piscivores had significantly greater muscle, liver, and feather THg concentrations than the other three guilds ($p < 0.001$) and the only non-piscivorous relationship among foraging guilds was between omnivore and herbivore mean THg wing feather concentrations ($p < 0.001$).

The feeding strategy with the greatest mean (± SD) muscle, liver, breast feather, and wing feather THg concentration (µg/g dw) was divers (0.874 ± 1.09, 1.47 ± 1.68, 3.19 ± 3.90, and 4.12 ± 5.09, respectively), followed by dabblers (0.154 ± 0.191, 0.798 ± 1.13, 0.576 ± 0.731, and 0.909 ± 0.982 respectively), waders (0.0157 ± 0.0147, 0.0800 ± 0.0949, 0.465 ± 0.424, and 0.903 ± 0.735, respectively) and were lowest in dabbler/divers (0.0480 ± 0.0842, 0.232 ± 0.324, 0.295 ± 0.234, and 0.319 ± 0.252, respectively). For each tissue, a significant difference in THg concentrations was also reported among feeding strategies (Figure 4; Kruskal-Wallis one-way ANOVA on Ranks; muscle: $H = 67.3$, $df = 3$, $p < 0.001$; liver: $H = 66.3$, $df = 3$, $p < 0.001$; breast feather: $H = 19.2$, $df = 3$, $p < 0.001$); wing feather: $H = 31.8$, $df = 3$, $p < 0.001$). For muscle and liver, significant differences were reported among all pairwise relationships except between diver and dabbler ($p = 0.139$ and $p = 0.107$, respectively) and between dabbler/diver and wader ($p = 0.537$ and $p = 0.382$, respectively). For breast feathers, differences were found between diver and dabbler/diver ($p < 0.001$) and between diver and dabbler ($p < 0.001$).

Differences in wing feather THg concentrations among feeding strategies were significant for all pairwise relationships except between diver and wader ($p = 0.743$) and between dabbler and wader ($p = 1.00$).

Intraspecies Variability in THg Concentrations

Total Hg concentrations were examined among the four investigated tissues in all species with a sample size ≥ 5 (n = 10; Figure 5). Median THg concentrations were greatest in the wing feathers of six of the species (American coot, blue-winged teal, gadwall, northern pintail, ring-necked duck, and sandhill crane), liver for two species (green-winged teal and red-breasted merganser), and breast feathers for two species (American wigeon and redhead duck). Muscle median THg concentrations were the lowest among the four tissues investigated in all species (Figure 5, Table 2).

Overall significant differences (one-way ANOVA or Kruskal-Wallis one-way ANOVA on Ranks; df = 3 for all species) in mean tissue THg concentration were found in all investigated species: American coot ($H = 15.49$, $p < 0.001$), American wigeon ($F_{(3,20)} = 12.7$, $p < 0.001$), blue-winged teal ($F_{(3,16)} = 18.5$, $p < 0.001$), gadwall ($H = 71.6$, $p < 0.001$), green-winged teal ($F_{(3,40)} = 10.0$, $p < 0.001$), northern pintail ($F_{(3,36)} = 10.9$, $p < 0.001$), redhead ($H = 54.9$, $p < 0.001$), red-breasted merganser ($F_{(3,36)} = 11.7$, $p < 0.001$), ring-necked duck ($F_{(3,24)} = 15.2$, $p < 0.001$), and sandhill crane ($F_{(3.56)} = 59.3$, $p < 0.001$). All pairwise comparisons ($p < 0.05$) are shown in Figure 5.

Species with THg Concentrations above Adverse Biological Effects Threshold Levels

Species which had muscle and/or liver blood-equivalent THg concentrations that exceeded adverse biological effects threshold levels can be found in Table 3. Of the 16 species included in the study, seven species (American coot, blue-winged teal, hooded merganser, lesser scaup, northern pintail, northern shoveler, and red-breasted merganser) were found to have muscle and/or liver-blood equivalent concentrations ≥ 0.2 µg/g putting them at risk for observable effects. Muscle and liver blood-equivalent THg concentrations which may result in impairment (≥ 1.0 µg/g ww) were observed in hooded merganser and red-breasted merganser, while THg concentrations known to cause substantial impairment (≥ 2.0 µg/g ww) occurred in 20% of red-breasted merganser blood equivalent liver samples.

Species with MeHg Concentrations above Hg Advisory Levels

Species which had wet weight muscle and/or liver MeHg concentrations (ww) that exceeded EPA and/or TDSHS advisory levels are shown in Table 4. Of the 16 investigated species, only the two merganser species had individuals with a muscle MeHg concentration above the 0.3 µg/g ww EPA human health criterion, whereas eight of the investigated species had individuals with a liver MeHg concentration above the EPA human health criterion (21.4% of all liver samples). Only muscle from one hooded merganser had a MeHg concentration that exceeded the 0.7 µg/g ww TDSHS human health-based standard, whereas three species (hooded merganser, red-breasted merganser, and northern shoveler) had liver MeHg concentrations that exceeded the TDSHS standard (100%, 100%, and 55%, respectively).

Stable Isotopes and Relationship with THg Concentrations

The summary statistics for the muscle $\delta^{13}C$ and $\delta^{15}N$ values broken down by species, foraging guild, and feeding strategy, are shown in Table 5. $\delta^{13}C$ values for all species ranged between -27.4 to -13.7 ‰ with redhead duck being the most C enriched (mean $\delta^{13}C$ = -15.9 ‰) and hooded merganser and wood ducks having the most deplete C values (mean $\delta^{13}C$ = -23.6 and -23.6 ‰, respectively) (Table 5). Significant differences in $\delta^{13}C$ were observed between species (Kruskal-Wallis; $p < 0.001$, $H = 36.69$, $df = 13$). Redhead duck had significantly different $\delta^{13}C$ compared to lesser scaup ($p = 0.001$). No significant difference in mean $\delta^{13}C$ was detected among foraging guild [one-way ANOVA; $F_{(3,80)} = 1.4$, $df = 3$, $p = 0.249$; Figure 6], however, a significant difference in $\delta^{13}C$ values among feeding strategies was determined [one-way ANOVA; $F_{(3,80)} = 6.9$, $df = 3$, $p < 0.001$; Figure 6].

Mean $\delta^{15}N$ values ranged from 6.3 to 15.4‰ across all species (Table 5). The most N enriched species was lesser scaup (mean $\delta^{15}N$ = 12.9‰) and the most deplete was sandhill crane (mean $\delta^{15}N$ = 6.9‰) (Table 5). Significant differences in $\delta^{15}N$ values existed among species (one-way ANOVA; $F_{(13,68)} = 6.6$, $p < 0.001$). The red-breasted merganser had significantly different $\delta^{15}N$ values to American wigeon, American coot, blue-winged teal, redhead, and sandhill crane (all $p \leq 0.05$) and the lesser scaup had significantly greater $\delta^{15}N$ values than American wigeon, blue-winged teal, redhead, and sandhill crane (all $p \leq 0.05$). Post hoc results also indicated that $\delta^{15}N$ values for hooded merganser ($p < 0.05$), green-winged teal ($p < 0.001$), northern pintail ($p < 0.001$), and northern shoveler ($p < 0.05$) were all significantly greater than sandhill crane $\delta^{15}N$ values. There was an overall significant difference in $\delta^{15}N$ values among foraging guilds

(Kruskal-Wallis one-way ANOVA on Ranks; $H = 20.28$, $df = 3$, $p < 0.001$) and feeding strategies (Kruskal-Wallis one-way ANOVA on Ranks; $H = 28.64$, $df = 3$, $p < 0.001$) (Figure 6).

Relationships between THg versus $\delta^{15}N$ and between THg versus $\delta^{13}C$ were determined for all species combined, among foraging guilds, and among feeding strategies using linear regressions (Figure 7). Two significant relationships were found within foraging guilds between THg and $\delta^{13}C$ among herbivore ($p = 0.009$) and among piscivore ($p = 0.032$), however, the model for herbivore failed to account for the variation in the dataset ($R^2 = 0.24$) and barely met the R^2 criterion for piscivore ($R^2 = 0.50$). A significant relationship was also observed between THg and $\delta^{13}C$ within the feeding strategy dabbler/divers ($R^2 = 0.68$, $p < 0.001$). Significant relationships existed between THg and $\delta^{15}N$ for all foraging guilds and feeding strategies combined ($p < 0.001$) but failed to account for variation within the dataset ($R^2 = 0.19$). Among foraging strategies, significant relationships in THg versus $\delta^{15}N$ were found among herbivore and among omnivore. However, such low R^2 values suggest that each model was considerably weak at accounting for variation within the dataset. Lastly, a significant relationship was identified among the dabbler/diver feeding strategy ($R^2 = 0.65$, $p < 0.001$).

Predicting Internal Tissue Concentrations using Wing and Breast Feathers

Wing feather THg concentrations were not a good predictor of muscle or liver THg concentrations in redhead and gadwall ($p > 0.05$). Breast feather THg concentrations were not a good predictor of gadwall muscle and liver THg concentrations and redhead liver concentrations ($p > 0.05$). Breast feather THg concentrations were only successful at predicting redhead muscle THg concentrations (Muscle = -3.023 + (0.393*Redhead duck breast feather), $p = 0.043$), however, the R^2 was low (0.149).

IV. DISCUSSION

Mercury has been shown to impact avian health and Texas is one of the greatest emitters of atmospheric Hg. However, to date, this is the most detailed analysis of THg accumulation and tissue distribution in waterbirds overwintering in Texas. Previous studies on the subject are limited in number and scope with the majority focusing on THg concentrations in between one and four species and across one to two tissues. Seventy five percent of the 731 publications which mention “mercury”, “waterbird”, and “Texas” (Google Scholar™: allintext: mercury waterbird Texas) occurred before 2015 suggesting that there is a lack of recent data on the topic.

This study affirmed the presence of inter- and intraspecies variation in THg concentrations in muscle, liver, breast feather, and wing feather in 16 species of waterbird which overwinter in Texas, determined 2 species which may be risk of deleterious health effects due to THg exposure, and determined 3 species with muscle and or liver MeHg concentrations exceeding advisory levels for human consumption. The study also used the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ to determine if dietary differences among species and trophic level distinctions between species, respectively, could be inferred from the data and also determined that for the investigated species, there is no relationship between $\delta^{13}C$ and $\delta^{15}N$ isotopes and THg concentrations in waterbird muscle. Lastly, the study examined whether wing and breast feather THg concentrations are a predictor of muscle and liver THg concentrations and found that for gadwall and redhead duck, there was no relationship.

Interspecific Variation in THg Concentrations

Significant interspecific differences in tissue THg concentrations were observed among the investigated species. In general, THg concentrations in the muscle, liver, breast feather and wing feather of guilds which feed at higher trophic levels were consistently greater than guilds which feed at lower levels with fish-eating mergansers having the greatest THg concentrations regardless of tissue indicating that Hg is biomagnifying within the food web. However, since all species are migratory to some extent, THg values observed in the present study may represent THg accumulated prior to arrival in Texas. For example, mottled duck are considered "resident" to North America despite having been documented traveling up to 430 km due to changes in habitat such as moving to ponds and fields when they are flooded but preferring more permanent bodies of water during normal conditions (Fogarty and LaHart, 1971) whereas blue-winged teal are long distance migrants and have been recorded traveling 6,114 km away from their banding location at an estimated 201 km per day (Bellrose 1980).

Piscivorous species such as the hooded and red-breasted merganser included in this study are among the top trophic level for waterbirds (Burger and Gochfield, 2000), and have been documented having muscle THg concentrations over 8-times higher than herbivores such as the Canadian goose (*Branta canadensis*) and omnivorous species such as the green-winged teal and ring-necked duck (Evers et al., 2005). Braune & Malone (2006) analyzed THg in breast muscle of various species of migratory ducks collected throughout Canada and found that the merganser species included in their study (common (*Mergus merganser*), hooded, and red-breasted merganser) had the highest THg concentrations compared to sea ducks, bay ducks, and dabblers. The highest THg concentration was observed in a common merganser (1.5 µg/g ww) which is comparable to the highest muscle THg value seen by hooded merganser (1.28 µg/g ww), but greater than the maximum value seen in red-breasted merganser in this study (0.683 µg/g ww). Median muscle THg (ww) in gadwall (0.025 µg/g), green winged teal (0.071 µg/g), northern pintail (0.056 µg/g), and redhead duck (< 0.07 µg/g) collected in Canada (Braune and Malone, 2006) were greater compared to those species in the present study (0.012 µg/g, 0.038 µg/g, 0.036 µg/g, and 0.006 µg/g, respectively) with exception of redhead duck, which was below their detection limit (< 0.07 µg/g). Green-winged teal (1.3 ± 1.4 µg/g fresh weight) and ring-necked duck (1.5 ± 0.8 µg/g fw) mean feather THg (feather location not specified) collected in New England, New York, and eastern Canada were two and three times greater than mean feather THg of green-winged teal (0.65 ± 0.56 µg/g dw) and ring-necked duck (0.49 ± 0.36 µg/g dw), respectively.

Among foraging guilds, piscivores had the highest THg levels in any tissue because of biomagnification from fish consumption, followed by omnivores which consume a range of different trophic levels within a food web, followed by herbivores and granivore feed at the lowest trophic level (Figure 3). There were wide ranges of THg concentrations found between two species within the same foraging guild which could be a result of differences in selected prey species (Green et al., 2002; Kear 2005; Burger and Gochfield, 2005). For example, mean blue-winged teal wing feather THg (1.53 µg/g dw) was much greater than that of sandhill crane wing feather (0.901 µg/g dw) despite both being grouped as omnivore, likely due to the diet of blue-winged teal consisting of a higher percentage of animal tissues (39.4% of aggregate stomach mass, 33% being *gastropod* pieces; Collins et al., 2017) compared to the 5-10% animal matter (which include *gastropoda*, *oligocheata*, and *insecta*) observed in sandhill crane diet (Reinecke and Krapu, 1986).

Another factor which may influence the difference in THg concentrations observed within guilds is the length of exposure to Hg. For example, hooded mergansers are resident to North America with year-round populations in Central and Eastern U.S. and Canada (Dugger et al., 2020), while red-breasted mergansers have year-round populations outside the U.S. in Europe and Greenland and only arrive along the Texas coast in November to overwinter (Craik et al., 2020) and therefore may be spending less time in Hg contaminated areas.

Species which implement diving as a feeding strategy had higher THg concentrations in their tissues compared to non-diving strategies or those which used multiple feeding strategies because these species are either piscivorous or omnivorous, consuming fish which biomagnify THg or other prey species which are higher in trophic level than prey consumed by dabblers and waders which are mostly primary producers (Figure 4). Diving ducks which use their wings or feet to physically break the water surface typically consume small to large fish and crustaceans (Mccaw III et al., 1996; Arzel and Elmberg, 2004) that are higher on the food web than potential food sources preferred by dabblers or waders, which include knotweed (*polygonum sp.*), submerged vegetation, seeds, and invertebrates for dabblers (White and James, 1978; Paulus, 1984) and nut-grass tubers (*Cyperus spp.*),and agricultural grains, wolfberry (*Lycium virginiana*) and insects for waders (Hunt and Slack, 1989; Ballard and Thompson, 2000). Given that the diets of dabbling and wading birds consist of almost exclusively primary producers, Hg concentrations within these prey items and therefore within these particular birds should be relatively low compared to omnivorous and piscivorous species.

Intraspecific Variation in THg Concentrations

Of the species investigated in this study with $n \geq 5$ ($n = 10$), the highest median THg concentrations were observed in the wing feather for gadwall, northern pintail, and sandhill crane, the liver for green-winged teal and red-breasted merganser, and in the breast feather for redhead ducks. These findings for gadwall, northern pintail, and sandhill crane coincide with Kenow et al., (2007a), where it was found that THg levels in Common Loon chicks vary by tissue with wing feathers having higher THg than liver > kidney > muscle, however, green-winged teal, red-breasted merganser, and redhead duck saw variations to this pattern. The tissue-specific variation in THg accumulation seen in this study has been repeatedly observed in various waterbird species throughout North America with the greatest THg concentrations being found in either wing feathers or the liver (Thompson et al., 1991; Kenow et al., 2007b; Eagles-Smith et al., 2008; Eagles-Smith et al., 2009; Ackerman et al., 2011; Fernández et al., 2018) and could be a result of: differences in Hg pharmacokinetics within each tissue, timing and frequency of molt/s, age, and habitat (Furness et al., 1986; Burger, 1993; Bearhop et al., 2000; Heinz and Hoffman, 2004; Evers et al., 2005; Eagles-Smith et al., 2009).

Of the tissues investigated in this study, Hg in waterbird muscle has the shortest turnover time (6 - 12 days in growing juveniles, 12 – 49 days in adults) largely because of remobilization of THg from muscle to blood during periods of physical stress such as migration or predation where lean tissue is catabolized to provide energy for the bird

(March et al., 1982; Seewagen et al., 2016; Wurtsbaugh et al., 2011). In comparison, Thg in the liver can take 8 – 10 weeks to turnover (March et al., 1982; Wurtsbaugh et al., 2011) and were observed in the current study having consistently higher values than other internal tissues regardless of species due to it being the organ responsible for xenobiotic detoxification of heavy metals such as the demethylation of MeHg into Hg^{2+} (Thompson and Furness, 1989; Kim et al., 1996; Eagles-Smith et al., 2008). Thresholds of demethylation of organic mercury within the liver can also vary among species (Thompson and Furness, 1989; Kim et al., 1996; Eagles-Smith et al., 2008, 2009). Eagles-Smith et al., (2008) found that Caspian terns (*Hydroprogne caspia)*, Forster's terns (*Sterna forsteri*), American avocets (*Recurvirostra americana*), and black-necked stilts (*Himantopus mexicanus*) have different percentages of MeHg (as a percentage of THg) in their liver suggesting a threshold limit by which demethylation of MeHg can occur. They observed a threshold concentration of 8.51 µg/g dw where the percentage of THg accounted for by MeHg began to decrease from between 5.5 – 8.2% depending on species, age, and guild (Eagles-Smith et al., 2008). In the present study, 100% of hooded merganser (n = 2) and 40% of red-breasted merganser (n = 10) liver samples exceeded 8.51 µg/g. However, due to the turnover time of Hg in the liver coupled with many species arriving on wintering grounds just prior to sampling, it is likely that several species investigated in this study have residual Hg that was accumulated outside of Texas.

Bird feathers, due to their ability to sequester THg mobilized from the blood and other tissues, as well as containing high concentration of sulfhydryl groups within keratin which Hg binds to, contain between 50 to 93% of a bird's body burden making them a phenomenal excretory pathway (Braune and Gaskin, 1987; Lewis and Furness, 1991; Ahmadpour et al., 2016). However, there were three species (green-winged teal, northern shoveler, and red-breasted merganser) in this study which had their highest THg concentration found in the liver which could be a result of a recent molt. Compared to the internal tissues mentioned, the half-life of Hg in bird feathers ranges from 0.9 days during a rapid distribution phase during early molting to 116 days towards the end of the molt process (Fournier et al., 2002). As a result, feathers developed during each molt are indicative of the blood THg burden at that time (Bottini et al., 2021). Additionally, wing feathers are known to vary in THg content on a feather-to-feather basis and therefore likely influenced the observed intraspecies differences between tissues seen in the current study (Braune, 1987; Dauwe et al., 2003).

Timing and frequency of molt can have a significant impact on the MeHg concentration of bird feathers. Birds exposed to elevated MeHg levels before molt face lower toxicological risk by sequestering dietary and tissue-stored MeHg into developing feathers which become chemically inert once molt is complete, whereas birds exposed prior to breeding are at elevated reproductive risk because females can maternally offload some of their body burden to developing eggs (Bearhop et al., 2000; Heinz and Hoffman, 2004). Therefore, species which recently experienced a molt would likely have lower internal THg concentrations which will increase over time until an additional molt occurs. Presence or absence of molt was not recorded in the current study; however, all 16 waterbird species undergo a partial or complete prebasic (younger birds lose and replace all feathers), basic (replacing post-breeding plumage), or alternative (replacing breeding plumage) molt while in the area. For example, both hooded and red-breasted mergansers undergo a complete prebasic molt rendering them flightless beginning in August and finishing in late October (Dugger et al, 2020; Craik et al., 2020), while mottled ducks experience a complete prebasic molt starting earlier in May and extending through September (Bielefeld et al., 2020).

Age can also impact tissue THg concentrations due to growth dilution (Monteiro and Furness, 1995) and increased rates of protein synthesis during early development (hatching to fledgling) (Hoffman and Curnow, 1979; Lewis, 1991; Tartu et al., 2014). Since age was not recorded in this study, it is possible that the intraspecific variation observed within the investigated species can be explained by differences in age with older birds having higher concentrations that juveniles.

As a result of seasonal dietary fluctuations observed in many waterbird species, THg found in various tissues can be affected by preferential changes in food sources or by seasonal availability of food sources. For example. species such as European wigeon and Northern pintail monitored in May, June, and August were documented preferring Chironomidae (nematoceran flies similar to mosquitos) found on the water surface during warmer months but switched to subsurface scavenging of Chironomidae larvae and emerging hydrophytes by August due to lack of Chironomidae availability (Danell and Sjöberg, 1982) and this dietary change which would likely result in different levels of THg accumulated.

Species at Risk of Adverse Biological Effects due to Hg Exposure

Several species including American coot, blue-winged teal, hooded merganser, lesser scaup, northern pintail, northern shoveler, and red-breasted merganser were observed having blood-equivalent muscle and or liver THg wet weight concentrations which place them at various levels of biological risk. Determining toxicological "risk" of THg accumulation in waterbirds has been widely studied in recent years with two large review papers on the subject having been published in 2004 and 2016 (Evers et al., 2004; Ackerman et al., 2016). Evers et al., (2004) reviewed over 4,700 records of Hg in freshwater birds from North America to assess patterns of Hg accumulation and found factors such as tissue, aquatic habitat, age class and gender, species, to be the factors that influence avian Hg concentrations the most. Ackerman et al., (2016) reviewed THg in 29,219 total tissue samples from 200 publications across western North America to arrive at three distinct (blood-equivalent) threshold levels by which various adverse effects begin to occur: >0.2 µg/g ww (lowest value for observable effects), >1.0 µg/g ww (impairment), and >2.0 µg/g ww (substantial impairment). In addition to the large reviews conducted by Ackerman and Evers, Whitney and Cristol, (2017a) sought to review avian Hg data to determine a concentration where Hg becomes sublethal across species and arrived at a value of 5.0 µg/g ww and additionally determined four categories, trace (≤ 0.5 µg/g), low (0.5 – 1.0 µg/g), medium (1.0 – 2.0 µg/g), and high (> 2.0 µg/g), which account for THg levels within common prey items such as fish and terrestrial arthropods.

Hooded merganser, red-breasted merganser, and Northern shoveler were the only species whose blood-equivalent muscle THg concentrations exceeded 0.2 – 1.0 µg/g ww. However, seven species: American coot, blue-winged teal, hooded merganser, lesser scaup, Northern pintail, Northern shoveler, and red-breasted merganser, had blood-equivalent THg liver concentrations which exceeded the observable effects threshold of

0.2 μg/g ww. Of the species whose liver blood-equivalent concentrations exceeded 0.2 μg/g ww, only the merganser species had samples which exceeded 1.0 – 2.0 μg/g ww. The observed discrepancy between THg accumulation in the muscle versus liver is likely a result of different pharmacokinetics within each tissue with muscle having consistently lower THg values compared to liver (regardless of species), in addition to Hg being remobilized from muscle to the blood and other organs thereby reducing concentration. As a result of the observed differences in THg accumulation within muscle versus liver, liver appears to be the superior tissue to use when determining if a species is at risk of experiencing deleterious biological effects due to Hg exposure because it is consistently elevated compared to other internal tissues.

Unfortunately, lesser scaup is the only investigated species which currently has published data on deleterious effects resulting from Hg exposure and it was determined that at liver blood-equivalent THg concentrations below 0.2 μg/g ww, there is a negative relationship between Hg and thiobarbituric acid (TBA), a common measure of oxidative stress (Custer et al., 2000).

Species Exceeding Hg Advisory Levels

Several species were identified as having muscle or liver MeHg wet weight concentrations which exceed federal and/or state advisory levels. Total mercury (THg) was used as a proxy for MeHg in muscle (98% THg is MeHg) and liver (88% THg is MeHg (Thompson et al., 1991; Eagles-Smith et al., 2009). All waterbird species included in this study are recreationally hunted in Texas, however, the only available THg data for any species included in this study which was also collected in Texas was conducted 25 years ago (Gamble and Woodin, 1993). Between the two internal tissues analyzed, THg concentrations in the liver were higher than muscle with eight species having liver THg samples that exceeded the EPA 0.3 µg/g ww advisory versus two species which had muscle THg exceeding the same standard. This suggests that people who are consuming duck are exposed to Hg concentrations above safe advisory levels when consuming liver, but not for muscle. Except for the two piscivorous merganser species, the remainder of species investigated had relatively low THg concentrations, especially granivorous and herbivorous species, indicating that consuming waterbirds hunted in Texas does not pose a serious risk to public health.

Stable Isotopes and Relationship with THg Concentrations

The present study contributes $\delta^{13}C$ and $\delta^{15}N$ values for 16 species of waterbird overwintering in Texas, however, significant variability in both $\delta^{13}C$ and $\delta^{15}N$ were found among species, and within foraging guilds and feeding strategies, suggesting that there are cofactors influencing these values which were outside the scope of this study. The time it takes for $\delta^{13}C$ and $\delta^{15}N$ to be reflective of the current diet can vary between species. Isotopic turnover times can be relatively fast, as observed in dunlin (*Calidris alpina pacifica*) (C: 11.2 ± 0.8 days and N: 10.0 ± 0.6 days, Ogden et al., 2004) and great skuas (*Catharacta skua*) (C: 15.7 days and N: 14.4 days, Bearhop et al., 2002), but take considerably more time in other species such as canvasback (C and N: 4.5 weeks, Haramis et al., 2001). Given the wide range of turnover times observed in different species coupled with the fact that nearly all 16 species are migratory and therefore have different baseline C and N levels and different arrival times on wintering grounds, it is likely that some of the variation in the current dataset could be accounted for by these variables. Therefore, interpreting results from this dataset without first better understanding the baseline $\delta^{13}C$ and $\delta^{15}N$ signatures of each sampling location, species specific isotope turnover rates, and migration history per species should be done with caution.

The range of $\delta^{13}C$ values for this dataset was large, however, significant differences were only found between the most enriched (redhead duck; mean = -15.9‰) and one of the most deplete (lesser scaup; mean = -24.3‰) which is likely a result of trophic fractionation between dietary sources caused by the enrichment of animals relative to their diet (DeNiro and Epstein, 1978; Tieszen et al., 1983). Additionally, samples were taken from 9 locations with spatial variation in baseline $\delta^{13}C$ which could account for the some of the inter- and intraspecific variation observed. The diet of redhead ducks wintering in Texas consists of almost exclusively seagrass (*Halodule wrightii*) (Cornelius, 1977), whereas the diet of lesser scaup is largely dominated by mollusks, clams, and small fish (Stroud et al., 2019). Past studies assessing $\delta^{13}C$ values in waterbirds wintering in the region are similar to those observed in the current dataset. $\delta^{13}C$ values of redhead muscle collected from Laguna Madre in 1988 (mean = -15.4 ± 4‰) and 1989 (mean = -11.3 ± 1.2‰) are similar to the mean $\delta^{13}C$ of redhead ducks in the current study (-15.9 ±1.7‰). For both guild and strategy, the highest THg levels were observed in piscivore and divers, respectively, yet the corresponding $\delta^{13}C$ values were among the most deplete within the dataset indicating that a relationship between the two variables is absent (Figure 7).

Unlike $\delta^{13}C$, significant interspecific differences in $\delta^{15}N$ values were observed among many species and were driven by the large difference in mean $\delta^{15}N$ values observed between trophically distinct piscivorous species (hooded merganser, red-breasted merganser, lesser scaup) and the remainder of herbivorous, granivorous, or omnivorous species. Overall, the most enriched species were the lesser scaup followed by both mergansers and the most deplete was the sandhill crane. As previously mentioned for $\delta^{13}C$, spatial differences in baseline $\delta^{15}N$ isotope signatures can have a significant impact on a stable isotope analysis due to unaccounted for variance which may have impacted the present dataset. Another factor which may have contributed to the interspecific differences in $\delta^{15}N$ that were observed includes anthropogenic inputs of NO_3^- into groundwater which can cause elevated enrichment at the base of the food web in primary producers that can then be tracked to higher trophic positions (Fry 2002; Wissel and Fry, 2005; Piola et al., 2006). Over 25 sites on the coastline of the Gulf of Mexico were determined by Martinez et al., (2006) to be vulnerable to anthropogenic disturbances and an additional 11 sites on the Tamaulipas shelf, Gulf of Mexico, had elevated levels of toxic heavy metals which were attributed to anthropogenic inputs (Celis-Hernandez et al., 2018). Lesser scaup were placed into the omnivore foraging guild based on DeGraaf et al., (1985), however, high mean THg tissue concentrations coupled with their high mean $\delta^{15}N$ suggest that lesser scaup overwintering in Texas may prefer a diet rich in fish or other prey species whose trophic niche is higher than previously thought (Rogers and Korshgen, 1966; Stroud et al., 2019). The combined effect of unknown baseline $\delta^{13}C$ and $\delta^{15}N$ values coupled with the potential for anthropogenic $\delta^{15}N$ enrichment are likely contributors to the high amount of interspecific variation in the dataset.

$\delta^{15}N$ values of piscivores were significantly different than both herbivore and omnivore values but were not statistically different than granivore species (Figure 6). This discrepancy may have been a result of an outlier value (14.9‰) found within the green-winged teal which, when removed, brings the mean $\delta^{15}N$ value for granivore from 10.8 ±2.0 ‰ down to 9.9 ± 0.7‰. $\delta^{15}N$ values varied significantly among feeding strategies with piscivorous diving species having significantly different $\delta^{15}N$ values versus the remaining guilds and between waders versus dabblers. These results agree with past studies indicating that $\delta^{15}N$ values of waterbirds species are closely related to their relative trophic positions within their respective food webs. The relationship between $\delta^{15}N$ and THg among foraging guilds and feeding strategies were similar to what was observed in $\delta^{13}C$, linear relationships were not significant and failed to account for 50% of the variance in the dataset. Piscivore and divers were predicted to be the highest trophic feeding species and therefore should have equally high $\delta^{15}N$ values since $\delta^{15}N$ values are indicative of trophic position. However, among foraging guilds, granivore and omnivore had some of the lowest THg values recorded yet the highest $\delta^{15}N$ values observed (Figure 7). A similar result was found for feeding strategies whereby both dabblers and dabbler/divers had THg concentrations below 1 µg/g yet had among the highest $\delta^{15}N$ values observed indicating that the relationship between the two variables is not present among guild and strategy. For both $\delta^{13}C$ and $\delta^{15}N$, past studies focusing on these isotopes within various avian foraging guilds or feeding strategies either focused on several species of one guild type (Cherel et al., 2007; Hahn et al., 2012) or the guilds chosen are significantly different than those of the current study thereby making comparisons between the studies unhelpful (Lei et al., 2021). Lastly, it should be reiterated that these species are all migratory and as a result, likely have significant variation among stable isotope signatures (Bearhop et al., 2004).

Predicting Internal Tissue THg Concentrations using Wing and Breast Feathers

Significant relationships between THg concentrations in the muscle versus wing feather and between THg concentrations in the liver versus the wing feather did not exist for both redhead and gadwall regardless of which internal tissue wing feather concentrations were predicted against. Wing feathers have been used to successfully predict muscle, liver, and kidney THg concentrations (Vermeer and Armstrong, 1972; Heinz and Hoffman, 2003; Espín et al., 2012; Zabala et al., 2019), so the lack of a predictive relationship between wing feathers of gadwall and redhead ducks and their internal tissues was likely a result of significant intraspecific differences in tissue THg concentrations coupled with significant variability in wing feather THg depending on which primary feather was used for THg analysis. With no relationship between THg concentrations in wing feather and internal tissues for these two species, using wing feather samples to infer information on internal THg concentrations should be done only when you are able to account for factors that influence THg in bird feathers such as age, timing and sequence of molt, and sampling location.

A significant relationship was found between the muscle and breast feathers of redhead ducks. However, similar to what was seen in models generated for the stable isotope analysis, the R^2 value was extremely low (0.149) suggesting that there is a lot of variance that is not accounted for with this model.

Conclusions

This is the first detailed study on THg accumulation across a wide range of waterbirds and will increase the understanding of how Hg accumulates in waterbirds overwintering in Texas. Total Hg concentrations varied significantly by species, foraging guild, and feeding strategy. Based on the results of this study, piscivorous diving waterbird species overwintering in Texas are accumulating THg concentrations in muscle and liver which place them at risk for deleterious biological effects and are high enough in the liver of piscivorous species that they pose a health risk to those which hunt and eat these species. In addition, the mergansers, American coot, blue-winged teal, lesser scaup, northern pintail, northern shoveler, and redhead ducks all had individual liver samples that exceed the EPA (0.3 µg/g ww) safe advisory level in fish and should be consumed with caution. $\delta^{13}C$ and $\delta^{15}N$ values were determined to vary significantly by species, foraging guild, and strategy, and this data can be used in future studies which aim to compare $\delta^{13}C$ and $\delta^{15}N$ values in species migrating from wintering and breeding grounds. This study provides important data for TPWD who are currently focusing on writing conservation, management, and recovery plans for Texas waterbirds.

Future Research

Allocating species to specific foraging guilds (Eagles-Smith et al., 2009) and to trophic levels (Chumchal et al., 2010) can be extremely useful predictors of THg, however, THg accumulation in waterbirds can be influenced by additional factors which were outside the scope of this thesis. Due to the opportunistic nature of this study whereby all samples were provided by hunters before the experiment was designed, several factors recently shown to impact waterbird THg were left out but should be accounted for in future studies of similar interest. As previously mentioned, significant intraspecific variation in wing feather THg concentrations have been determined to exist among waterbird species and therefore determining specifically which feathers will be harvested (primary or secondary and which of the ~10) should be determined in advance (Furness et al., 1986). Related to feather selection, molt timing and patterns are one of the most significant factors influencing waterbird THg concentrations because as much as 90% of the body burden is depurated into feathers (Burger, 1993) and all species undergo at least one yearly molt. The presence or absence of geographic habitat features such as marshes can impact THg content in waterbird tissues (Eagles-Smith et al., 2009) to an extent that non-piscivorous waterbirds can have THg concentrations as high as piscivorous species due to increased methylation rates in the sediment (Robinson et al., 1999). Differences in THg caused by sex have been determined showing that female ducks which just laid eggs have significantly less THg compared to males as a result of depuration into eggs (Eagles-Smith et al., 2009), however, this occurs during the breeding season and therefore may not have an impact on the current dataset but should be considered for samples collected during migrations or breeding seasons.

A limiting factor in several areas of this study was sample size. Many species, due to the nature which samples were provided, had sample sizes as low as 1-2 while species like gadwall and redhead duck had 28 and 30 individual samples. Uneven sample sizes limited the interspecies analysis to only be ran on species whose $n \geq 10$, decreasing the number of species in this area of the study from 16 to only 6. Another sample size dependent issue which could be alleviated in future studies is the selection of an even number of waterbird species to be included within each guild to limit statistical bias resulting from differences in sample size. Limiting sampling locations may also reduce the variability of THg and stable isotope ranges in the dataset caused by significant spatial distances between locations. Also related to location, including more sampling sites in East Texas where anthropogenic Hg emissions are elevated could help to better understand what impacts humans have on Hg accumulation in waterbirds.

Lastly, incorporating selenium (Se) concentrations into future analysis on Hg accumulation in waterbirds should be included when evaluating piscivorous species because recent studies have shown that with high enough Hg concentrations in the liver, Se may have a protective effect on waterbirds by forming a toxicologically inert Hg-Se complex (Stoewsand et al., 1974; Scheuhammer et al., 2009; Eagles-Smith et al., 2009).

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Table 1. Species investigated in this study with their sample size (n), weight (minimum and maximum), foraging guild and feeding strategy according to DeGraaf et al. (1985), and percent muscle and liver moisture content (mean ± standard deviation). ND = not determined due to low sample size.

Family	Common name	Scientific name	Weight (kg)	n	Feeding guild	Feeding strategy	Muscle % moisture	Liver % moisture
Anatidae	American Wigeon	*Mareca americana*	0.652 - 0.793	6	Herbivore	Dabbler	75 ± 3.1	71 ± 0.6
	Blue-winged Teal	*Spatula discors*	0.026 - 0.510	5	Omnivore	Dabbler	74 ± 1.2	71 ± 1.2
	Canvasback	*Aythya valisineria*	0.960 - 0.960	1	Omnivore	Diver	75 ± ND	69 ± ND
	Gadwall	*Mareca strepera*	0.652 - 1.02	30	Herbivore	Dabbler	74 ± 1.3	72 ± 2.9
	Green-winged Teal	*Anas carolinensis*	0.255 - 0.397	11	Granivore	Dabbler	74 ± 1.1	71 ± 0.9
	Hooded Merganser	*Lophodytes cucullatus*	0.675 - 0.735	2	Piscivore	Diver	72 ± 0.4	70 ± 0.1
	Lesser Scaup	*Aythya affinis*	0.652 - 0.709	4	Omnivore	Diver	73 ± 2.8	72 ± 1.0
	Mottled Duck	*Anas fulvigula*	0.272 - 0.963	3	Omnivore	Dabbler	73 ± 0.9	72 ± 1.5
	Northern pintail	*Anas acuta*	0.652 - 1.05	10	Omnivore	Dabbler	74 ± 1.4	72 ± 1.2
	Northern Shoveler	*Anas clypeata*	0.340 - 0.595	9	Omnivore	Dabbler	75 ± 1.0	71 ± 1.0
	Redhead	*Aythya americana*	0.513 - 1.36	28	Herbivore	Dabbler/Diver	74 ± 1.5	73 ± 2.4
	Red-Breasted Merganser	*Mergus serrator*	0.822 - 1.30	10	Piscivore	Diver	73 ± 1.2	70 ± 1.2
	Ring-necked Duck	*Aythya collaris*	0.555 - 0.720	7	Omnivore	Diver	74 ± 0.7	72 ± 1.9

	Wood Duck	*Aix sponsa*	0.640 - 0.640	1	Granivore	Dabbler	76 ± ND	71 ± ND
Gruidae	Sandhill Crane	*Antigone canadensis*	2.94 - 4.08	15	Omnivore	Wader	75 ± 1.5	73 ± 1.8
Rallidae	American Coot	*Fulica americana*	0.482 - 0.567	7	Herbivore	Dabbler/Diver	74 ± 1.0	74 ± 0.5

Table 2. Median, mean, standard deviation (SD), minimum, and maximum THg concentrations in muscle, liver, breast feather, and wing feather of each species investigated. ND = not determined due to small sample size (n ≤ 2). Muscle and liver data is reported as µg/g dw and ww, whereas all feather data is in µg/g dw. Sample sizes for each species are reported in Table 1.

	Tissue	Median	Mean	SD	Min	Max
American Coot	Muscle (dry)	0.0224	0.0688	0.122	0.0197	0.346
	Muscle (wet)	0.0060	0.0171	0.0293	0.0053	0.0836
	Liver (dry)	0.162	0.361	0.508	0.123	1.50
	Liver (wet)	0.0417	0.0925	0.128	0.0317	0.382
	Breast Feather	0.346	0.355	0.138	0.217	0.566
	Wing Feather	0.487	0.526	0.240	0.279	0.927
American Wigeon	Muscle (dry)	0.0232	0.0247	0.0112	0.0090	0.0425
	Muscle (wet)	0.0058	0.0064	0.0033	0.0024	0.0117
	Liver (dry)	0.135	0.139	0.0669	0.0474	0.245
	Liver (wet)	0.0417	0.0390	0.0192	0.0141	0.0705
	Breast Feather	0.227	0.198	0.102	0.0748	0.302
	Wing Feather	0.149	0.347	0.240	0.279	0.927
Blue-winged Teal	Muscle (dry)	0.231	0.222	0.120	0.105	0.388
	Muscle (wet)	0.0591	0.0574	0.0310	0.0264	0.0972
	Liver (dry)	1.20	1.26	0.583	0.414	1.81
	Liver (wet)	0.334	0.373	0.177	0.121	0.543
	Breast Feather	0.949	0.976	0.321	0.613	1.32
	Wing Feather	1.44	1.54	0.439	1.02	2.23
Canvasback	Muscle (dry)	0.0706	0.0706	ND	0.0706	0.0706
	Muscle (wet)	0.177	0.177	ND	0.177	0.177
	Liver (dry)	0.292	0.292	ND	0.292	0.292
	Liver (wet)	0.0914	0.0914	ND	0.0914	0.0914
	Breast Feather	0.193	0.193	ND	0.193	0.193
	Wing Feather	0.170	0.170	ND	0.170	0.170
Gadwall	Muscle (dry)	0.0470	0.0480	0.0157	0.0204	0.0820
	Muscle (wet)	0.0126	0.0125	0.0039	0.0051	0.0210
	Liver (dry)	0.284	0.289	0.106	0.127	0.616
	Liver (wet)	0.0806	0.0807	0.0317	0.0220	0.181
	Breast Feather	0.237	0.304	0.229	0.0585	1.05
	Wing Feather	0.402	0.423	0.291	0.0864	1.72
Green-winged Teal	Muscle (dry)	0.126	0.127	0.0770	0.0277	0.269
	Muscle (wet)	0.861	0.946	0.343	0.486	1.69
	Liver (dry)	0.405	0.423	0.214	0.117	0.724
	Liver (wet)	0.116	0.123	0.0619	0.0329	0.211
	Breast Feather	0.191	0.269	0.208	0.0541	0.619
	Wing Feather	0.380	0.650	0.557	0.198	2.01

Hooded Merganser	Muscle (dry)	3.42	3.42	1.75	2.18	4.65
	Muscle (wet)	0.944	0.944	0.473	0.610	1.28
	Liver (dry)	11.0	11.0	1.65	9.82	12.2
	Liver (wet)	3.25	3.25	0.499	2.90	3.60
	Breast Feather	7.85	7.85	1.47	6.81	8.89
	Wing Feather	10.1	10.1	2.29	8.44	11.7
Lesser Scaup	Muscle (dry)	0.244	0.246	0.211	0.0276	0.469
	Muscle (wet)	0.107	0.106	0.0812	0.0073	0.203
	Liver (dry)	1.19	1.16	0.935	0.204	2.06
	Liver (wet)	0.345	0.328	0.263	0.0545	0.568
	Breast Feather	0.587	0.611	0.122	0.498	0.775
	Wing Feather	0.775	0.822	0.265	0.573	1.17
Mottled Duck	Muscle (dry)	0.0413	0.0441	0.0262	0.0212	0.0727
	Muscle (wet)	1.93	1.99	0.453	1.52	2.60
	Liver (dry)	0.376	0.637	0.613	0.245	1.55
	Liver (wet)	0.110	0.187	0.185	0.0659	0.462
	Breast Feather	0.324	0.468	0.368	0.213	1.01
	Wing Feather	1.32	1.27	0.371	0.828	1.60
Northern Pintail	Muscle (dry)	0.136	0.158	0.0922	0.0419	0.348
	Muscle (wet)	0.0358	0.0407	0.0252	0.0112	0.0944
	Liver (dry)	0.588	0.796	0.577	0.234	2.14
	Liver (wet)	0.167	0.226	0.162	0.0678	0.584
	Breast Feather	0.490	0.218	0.702	0.0633	2.41
	Wing Feather	0.869	1.55	1.65	0.291	5.37
Northern Shoveler	Muscle (dry)	0.593	0.604	0.151	0.404	0.845
	Muscle (wet)	0.147	0.154	0.0405	0.103	0.228
	Liver (dry)	3.37	3.37	1.42	1.82	5.62
	Liver (wet)	0.988	0.978	0.415	0.497	1.60
	Breast Feather	2.43	2.31	0.606	1.22	3.06
	Wing Feather	2.52	2.28	1.01	1.10	4.13
Redhead	Muscle (dry)	0.0206	0.0428	0.0740	0.0111	0.380
	Muscle (wet)	0.0056	0.0110	0.0186	0.0028	0.0938
	Liver (dry)	0.106	0.200	0.264	0.0683	1.32
	Liver (wet)	0.0296	0.0535	0.0694	0.0140	0.348
	Breast Feather	0.199	0.280	0.252	0.0477	1.25
	Wing Feather	0.181	0.268	0.231	0.0324	0.848
Red-breasted Merganser	Muscle (dry)	1.08	1.27	0.565	0.852	2.73
	Muscle (wet)	0.292	0.340	0.141	0.219	0.693
	Liver (dry)	9.20	7.79	5.68	2.81	20.2
	Liver (wet)	2.30	2.69	1.58	0.819	5.76
	Breast Feather	4.32	5.65	4.06	0.751	14.7
	Wing Feather	6.46	7.19	5.52	0.876	16.2

Ring-necked Duck	Muscle (dry)	0.0577	0.0507	0.0328	0.0162	0.108
	Muscle (wet)	0.0151	0.0133	0.0085	0.0042	0.0277
	Liver (dry)	0.240	0.315	0.261	0.0881	0.845
	Liver (wet)	0.0647	0.0865	0.0653	0.0266	0.212
	Breast Feather	0.227	0.271	0.164	0.0974	0.561
	Wing Feather	0.402	0.496	0.365	0.202	1.28
Sandhill Crane	Muscle (dry)	0.0087	0.0157	0.0147	0.0047	0.0498
	Muscle (wet)	0.0021	0.0040	0.0037	0.0011	0.0121
	Liver (dry)	0.0335	0.0800	0.0949	0.0173	0.311
	Liver (wet)	0.0085	0.0215	0.0255	0.0047	0.0827
	Breast Feather	0.341	0.465	0.424	0.0502	1.42
	Wing Feather	0.608	0.903	0.736	0.0689	2.37
Wood Duck	Muscle (dry)	0.0161	0.0161	ND	0.0161	0.0161
	Muscle (wet)	0.0190	0.0190	ND	0.0190	0.0190
	Liver (dry)	0.154	0.154	ND	0.154	0.154
	Liver (wet)	0.0444	0.0444	ND	0.0444	0.0444
	Breast Feather	0.252	0.252	ND	0.252	0.252
	Wing Feather	0.344	0.344	ND	0.344	0.344

Table 3. Species which had muscle and liver THg concentrations that exceeded adverse biological effects threshold levels determined by Ackerman et al. (2016). Muscle and liver THg concentrations were converted to blood-equivalent THg concentrations for comparison. Values displayed are percentage of individuals that exceeded each threshold level out of the total number of individuals investigated in this study (n).

		Muscle Blood Equivalent Concentration (µg/g ww)			**Liver Blood Equivalent Concentration (µg/g ww)**		
Common name	**n**	**Observable effects (≥ 0.2 µg/g)**	**Impairment (≥ 1.0 µg/g)**	**Substantial Impairment (≥ 2.0 µg/g)**	**Observable effects (≥ 0.2 µg/g)**	**Impairment (≥ 1.0 µg/g)**	**Substantial Impairment (≥ 2.0 µg/g)**
American Coot	7	0	0	0	14	0	0
Blue-winged Teal	5	0	0	0	40	0	0
Hooded Merganser	2	100	50	0	100	100	0
Lesser Scaup	4	0	0	0	50	0	0
Northern Pintail	10	0	0	0	30	0	0
Northern Shoveler	9	100	0	0	100	0	0
Red-breasted Merganser	10	100	10	0	100	60	20

Table 4. Percentage of individuals within a species that had muscle and liver MeHg concentrations that exceeded the EPA human health criterion (0.3 µg/g ww) and TDSHS human health-based standard (0.7 µg/g ww) advisory levels. Values displayed are percentage of individuals that exceeded each threshold level out of the total number of individuals investigated in this study (n).

		Muscle		Liver	
Common name	**n**	**EPA**	**TDSHS**	**EPA**	**TDSHS**
American Coot	7	0	0	14.3	0
Blue-winged Teal	5	0	0	80.0	0
Hooded Merganser	2	100	50.0	100	100
Lesser Scaup	4	0	0	50.0	0
Northern pintail	10	0	0	30.0	0
Northern Shoveler	9	0	0	100	55.6
Redhead	28	0	0	3.6	0
Red-Breasted Merganser	10	40.0	0	100	100

Table 5. Median, mean, standard deviation (SD), minimum, and maximum $\delta^{13}C$ and $\delta^{15}N$ values (‰) by species, foraging guild, and feeding strategy. ND = not determined due to low sample size (n < 2).

			Median	Mean	SD	Min	Max
Species	American Coot	$\delta^{13}C$	-17.99	-19.25	2.89	-25.59	-17.49
		$\delta^{15}N$	9.20	9.64	1.18	8.77	12.24
	American Wigeon	$\delta^{13}C$	-21.72	-21.32	3.70	-25.14	-16.05
		$\delta^{15}N$	8.41	8.81	1.05	7.84	10.71
	Blue-winged Teal	$\delta^{13}C$	-19.19	-19.46	1.68	-21.47	-17.23
		$\delta^{15}N$	9.06	9.12	1.25	8.03	11.11
	Canvasback	$\delta^{13}C$	-23.31	-23.31	ND	-23.31	-23.31
		$\delta^{15}N$	9.35	9.35	ND	9.35	9.35
	Gadwall	$\delta^{13}C$	-20.38	-21.16	2.49	-24.21	-18.18
		$\delta^{15}N$	9.43	9.81	1.88	8.56	13.94
	Green-winged Teal	$\delta^{13}C$	-19.43	-19.08	1.52	-20.53	-15.92
		$\delta^{15}N$	10.28	11.04	2.06	9.19	14.91
	Hooded Merganser	$\delta^{13}C$	-23.61	-23.61	0.95	-24.28	-22.94
		$\delta^{15}N$	12.11	12.11	1.84	10.81	13.41
	Lesser Scaup	$\delta^{13}C$	-24.70	-24.29	1.38	-25.45	-22.31
		$\delta^{15}N$	13.48	12.93	2.84	9.31	15.44
	Mottled duck	$\delta^{13}C$	-19.98	-20.30	0.81	-21.22	-19.71
		$\delta^{15}N$	11.01	9.68	2.98	6.26	11.75
	Northern Pintail	$\delta^{13}C$	-19.07	-18.73	2.09	-21.99	-16.27
		$\delta^{15}N$	10.32	10.90	1.70	9.13	12.85
	Northern shoveler	$\delta^{13}C$	-20.87	-20.58	2.35	-22.89	-16.46
		$\delta^{15}N$	10.59	10.44	1.40	8.26	11.90
	Redhead	$\delta^{13}C$	-15.90	-15.93	1.70	-18.81	-13.75
		$\delta^{15}N$	9.35	9.21	0.85	8.05	10.46
	Red-breasted Merganser	$\delta^{13}C$	-17.77	-19.26	3.78	-27.40	-16.58

		$\delta^{15}N$	12.86	12.94	0.50	12.18	13.86
	Ring-necked duck	$\delta^{13}C$	-21.22	-22.15	2.25	-26.24	-20.29
		$\delta^{15}N$	10.24	9.84	1.27	7.79	11.41
	Sandhill Crane	$\delta^{13}C$	-19.97	-20.59	1.22	-23.19	-19.80
		$\delta^{15}N$	6.80	6.98	0.59	6.38	8.09
	Wood duck	$\delta^{13}C$	-23.66	-23.66	ND	-23.66	-23.66
		$\delta^{15}N$	9.48	9.48	ND	9.48	9.48
Foraging Guild	Granivore	$\delta^{13}C$	-19.44	-19.66	2.14	-23.66	-15.92
		$\delta^{15}N$	10.10	10.84	1.98	9.19	14.91
	Herbivore	$\delta^{13}C$	-18.77	-19.34	3.40	-25.59	-13.75
		$\delta^{15}N$	9.30	9.39	1.29	7.84	13.94
	Omnivore	$\delta^{13}C$	-20.67	-20.71	2.36	-26.24	-16.27
		$\delta^{15}N$	9.49	9.82	2.24	6.26	15.44
	Piscivore	$\delta^{13}C$	-17.79	-20.23	3.81	-27.40	-16.58
		$\delta^{15}N$	12.86	12.75	0.86	10.81	13.86
Feeding Strategy	Dabbler	$\delta^{13}C$	-19.98	-20.16	2.41	-25.14	-15.92
		$\delta^{15}N$	9.61	10.05	1.78	6.26	14.91
	Diver	$\delta^{13}C$	-21.80	-21.77	3.22	-27.40	-16.58
		$\delta^{15}N$	12.10	11.74	2.06	7.79	15.44
	Dabbler/Diver	$\delta^{13}C$	-17.54	-17.59	2.86	-25.59	-13.75
		$\delta^{15}N$	9.31	9.42	1.01	8.05	12.24
	Wader	$\delta^{13}C$	-19.97	-20.59	1.22	-23.19	-19.80
		$\delta^{15}N$	6.80	6.98	0.59	6.38	8.09

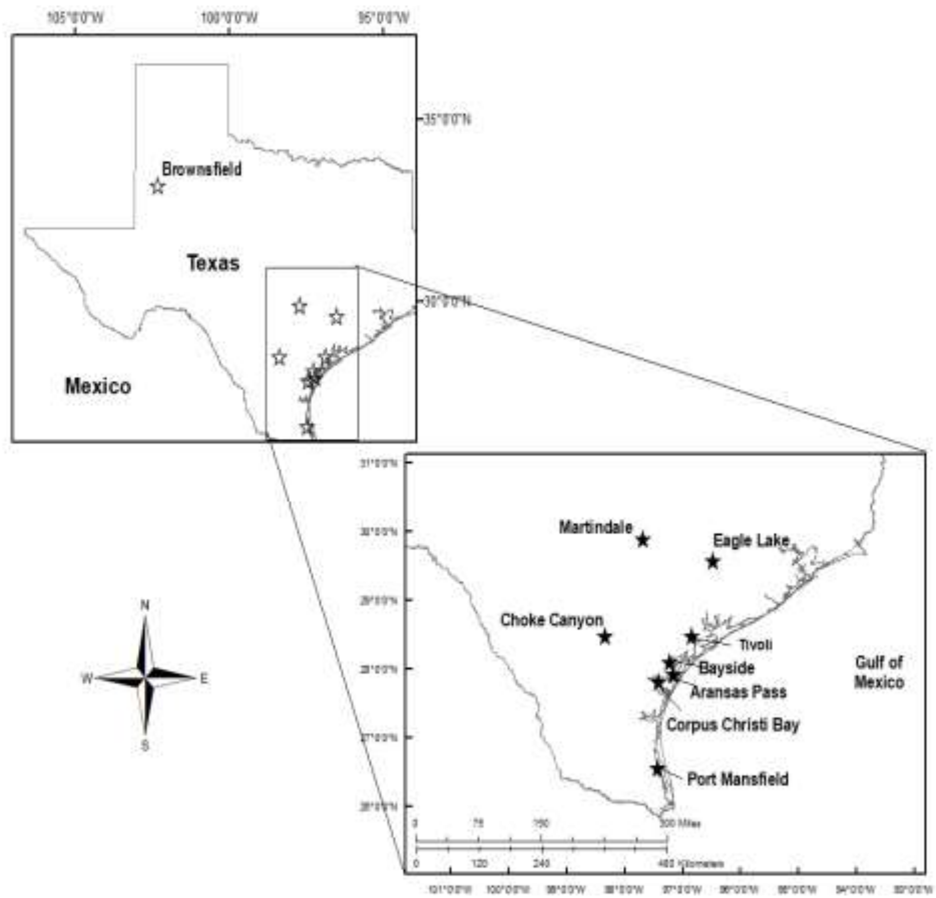

Figure 1. Waterbird sample collection locations across Texas. Locations and species sampled included: Brownsfield (sandhill crane), Choke Canyon (American wigeon, ring-necked duck), Eagle Lake (blue-winged teal, green-winged teal, lesser scaup, northern shoveler, red-breasted merganser), Martindale (American wigeon, canvasback, gadwall, green-winged teal, hooded merganser, ring-necked duck, wood duck), Aransas Pass (mottled duck, redhead), Bayside (American coot, American wigeon, blue-winged teal, lesser scaup, northern pintail, northern shoveler), Corpus Christi Bay (blue-winged teal, northern pintail, redhead), Port Mansfield (green-winged teal, lesser scaup, mottled duck, northern pintail, northern shoveler, redhead, red-breasted merganser), and Tivoli (American wigeon, blue-winged teal, gadwall, green-winged teal, lesser scaup, ring-necked duck). The sample size of each species collected at each location can be found in Appendix A.

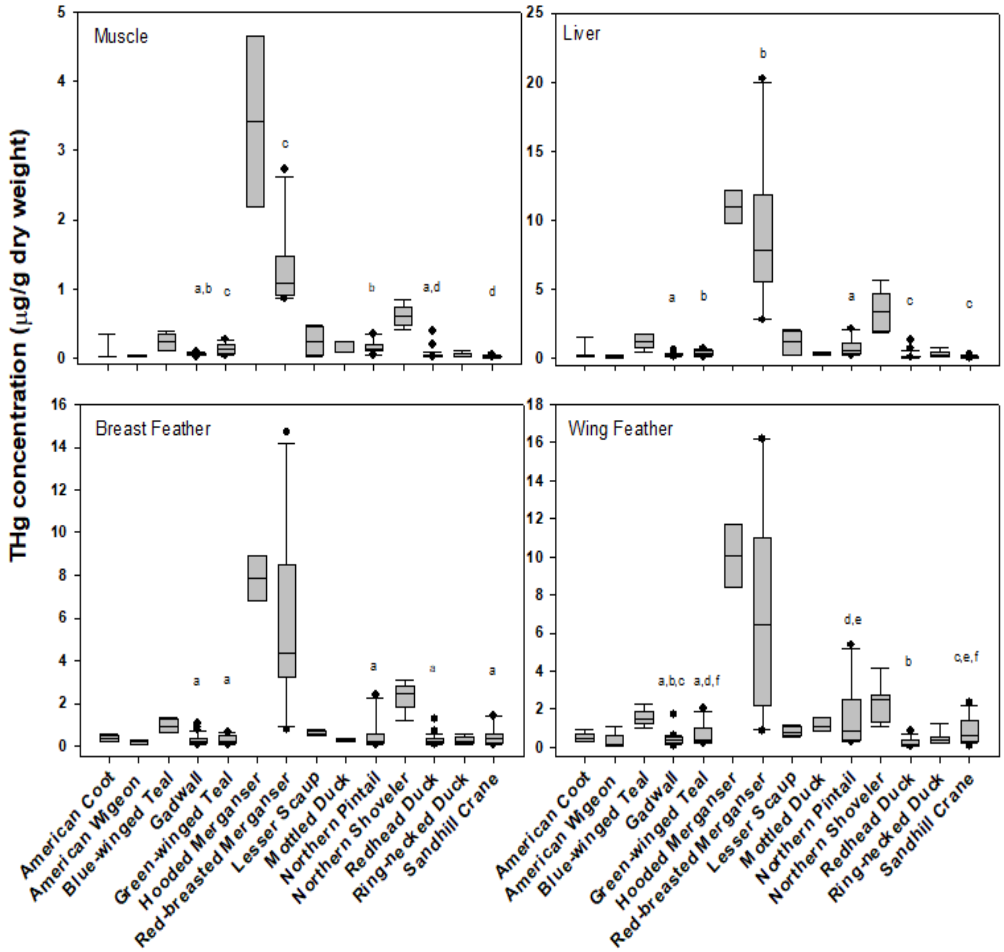

Figure 2. Mercury concentrations in muscle, liver, breast feather, and wing feather in 16 species of waterbird that overwinter in Texas. Lowercase letters above the error bars indicate species grouped by similar THg concentrations. The sample size for each species is shown in Table 1.

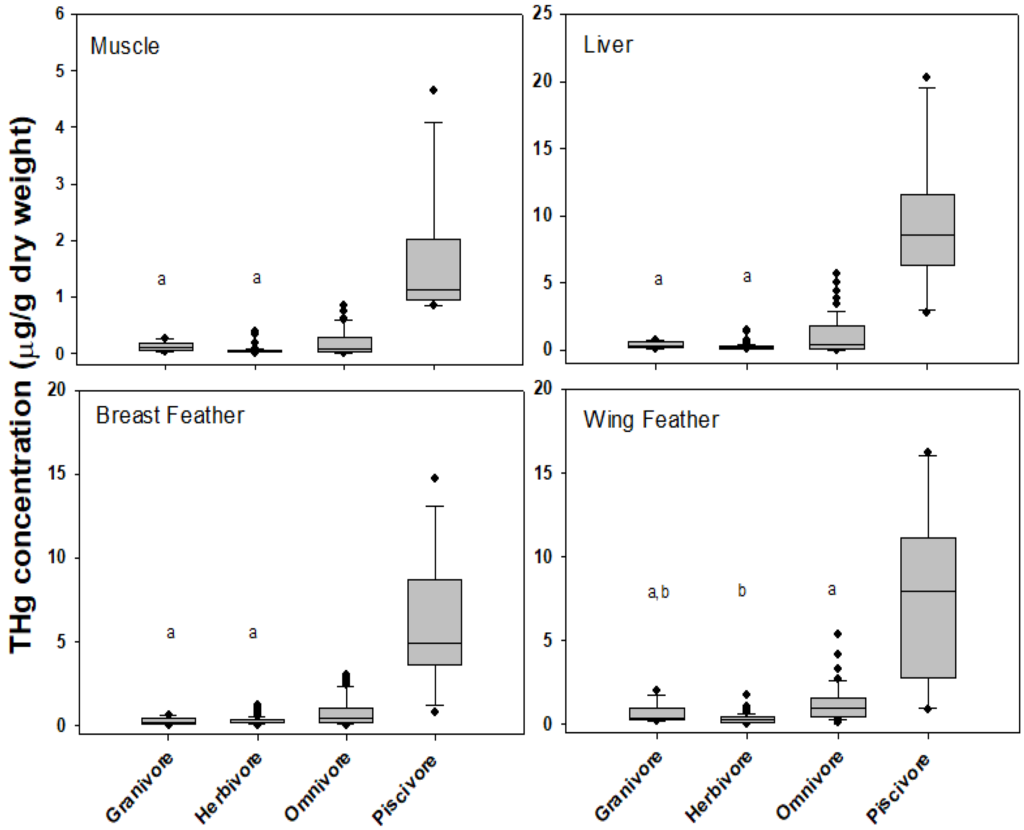

Figure 3. Mercury concentrations in muscle, liver, breast feather, and wing feather of waterbirds based on foraging guild (granivore: n = 12; herbivore: n = 71; omnivore: n = 55; and piscivore: n = 12). The species included in each foraging guild are shown in Table 1. Lowercase letters above the error bars indicate foraging guilds grouped by similar THg concentrations.

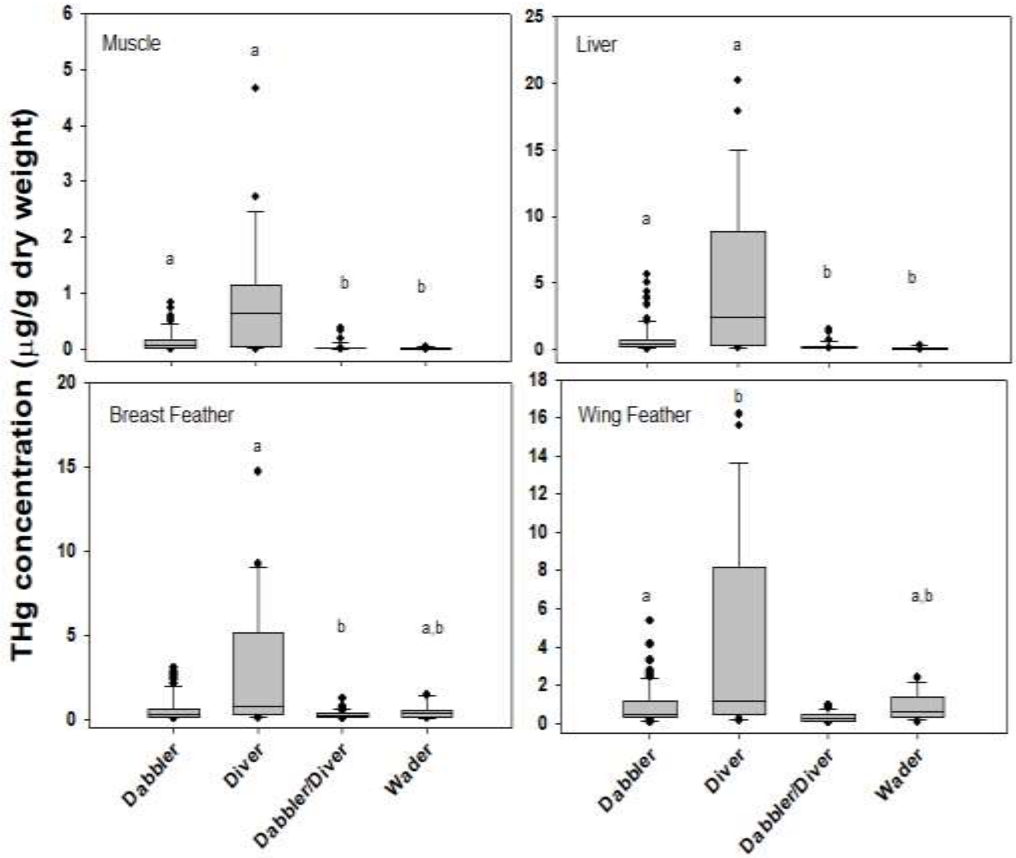

Figure 4. Mercury concentrations in muscle, liver, breast, and wing feather of waterbirds based on feeding strategy (dabbler: n = 75; diver: n = 24; dabbler/diver: n = 35; and wader: n = 15). The species included in each feeding strategy are shown in Table 1. Lowercase letters above the error bars indicate feeding strategies grouped by similar THg concentrations.

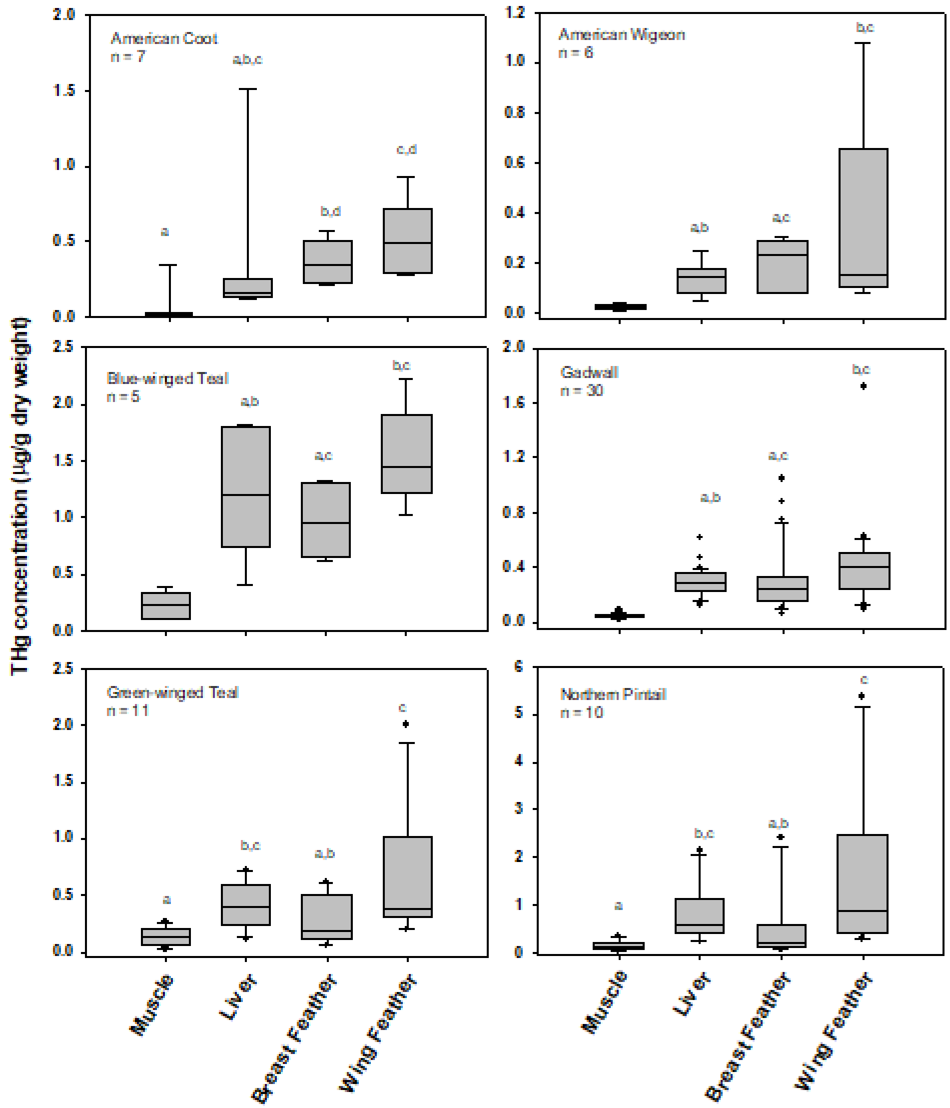

Figure 5. Mercury concentrations in muscle, liver, breast feather, and wing feather of waterbirds with a sample size n ≥ 5. Lowercase letters above the error bars indicate tissues grouped by similar THg concentrations.

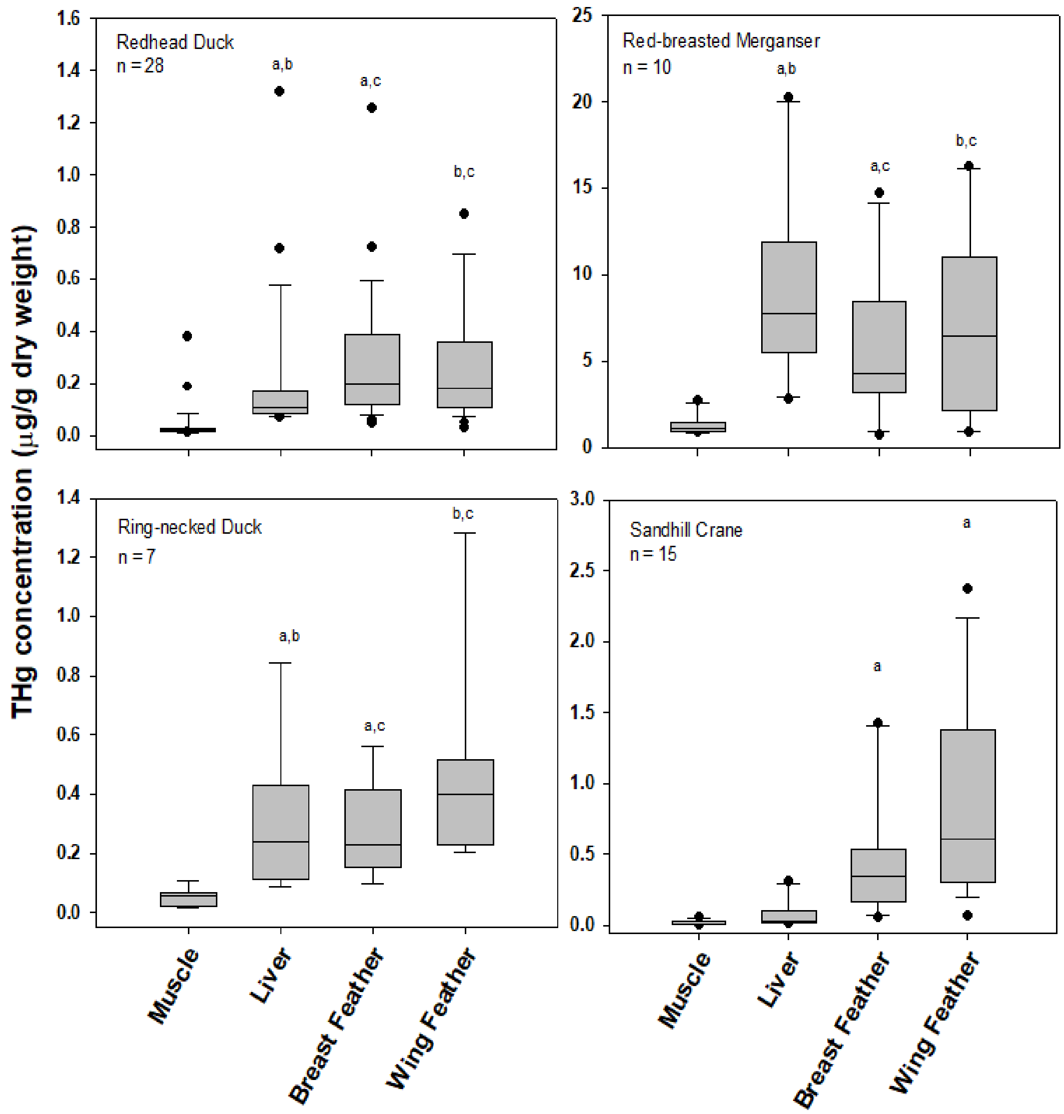

Figure 5 continued.

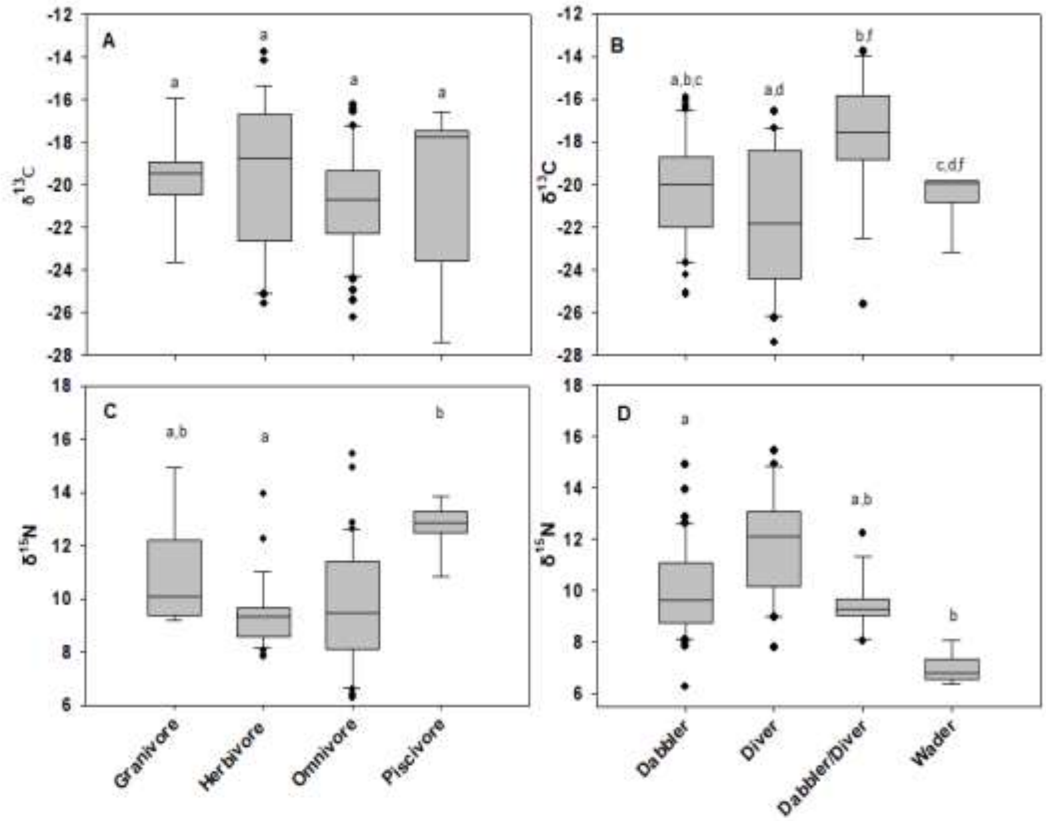

Figure 6. Muscle $\delta^{13}C$ and $\delta^{15}N$ values in waterbirds grouped by foraging guild [A and C (granivore: n = 12; herbivore: n = 71; omnivore: n = 55; piscivore: n = 12)] and feeding strategy [B and D (dabbler: n = 75; diver: n = 24; dabbler/diver: n = 35; wader: n = 15)]. Lowercase letters above the error bars indicate foraging guilds or feeding strategies grouped by similar $\delta^{13}C$ or $\delta^{15}N$ values.

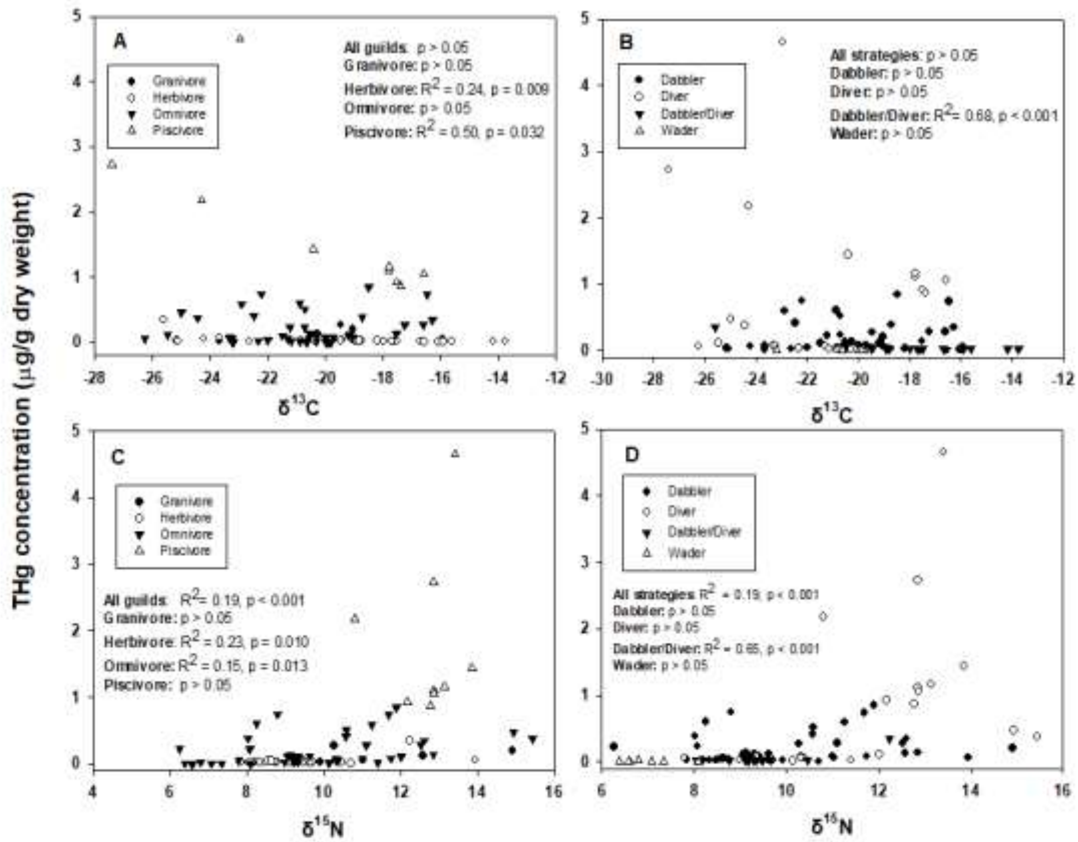

Figure 7. Relationship between muscle THg concentrations and $\delta^{13}C$ and $\delta^{15}N$ values for each foraging guild [A and C (granivore: n = 12; herbivore: n = 71; omnivore: n = 55; piscivore: n = 12)] and feeding strategy [B and D (dabbler: n = 75; diver: n = 24; dabbler/diver: n = 35; and wader: n = 15)].

APPENDIX SECTION

Appendix A. Sample size of each investigated species broken down by collection location.

Common name	Total n	Brownsfield	Choke Canyon	Eagle Lake	Martindale	Aransas Pass	Bayside	Corpus Christi Bay	Port Mansfield	Tivoli
American Coot	7		1				6			
American Wigeon	6				4		1			1
Blue-winged Teal	5			2			1	1		1
Canvasback	1				1					
Gadwall	30				15					15
Green-winged Teal	11			1	1				1	8
Hooded Merganser	2				2					
Lesser Scaup	4			1			1		1	1
Mottled Duck	3					1			2	
Northern pintail	10						1	1	8	
Northern Shoveler	9			2			1		6	
Redhead	28					3		2	23	
Red-Breasted Merganser	10			1					9	
Ring-necked Duck	7		1		5					1
Sandhill Crane	15	15								
Wood Duck	1				1					

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