AN EXAMINATION OF OCCUPANCY ON A COASTAL REFUGE AND MERCURY CONCENTRATIONS IN TEXAS BATS

by

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LIST OF ABBREVIATIONS

Abbreviation	Description
CBH	Columbia Bottomland Hardwood
PMDI	Modified Palmer Drought Index
SBNWR	San Bernard National Wildlife Refuge
Hg	Mercury
THg	Total Mercury
WNS	White-nose Syndrome

ABSTRACT

Texas bats are threatened by habitat loss, contaminants such as mercury (Hg), disease, and wind turbines. In east Texas, specifically along the Gulf Coast, much of the native landcover is fragmented from anthropogenic activities. East Texas also has 5 of the top 10 Hg emitters in the United States, which contribute to Texas producing more Hg pollution than any other state. Being consumers of prey connected to aquatic ecosystems, Hg bioaccumulates in bats and has been documented to reach toxic levels in some species. Knowledge gaps exist regarding critical foraging habitat for bats on the upper Texas Gulf Coast and Hg concentrations in bats throughout Texas. I identified habitats in which bats were active using multi-state occupancy models for 5 bat species: tri-colored (*Perimyotis subflavus*), evening (*Nycticeius humeralis*), eastern red (*Lasiurus borealis*), northern yellow (*Lasiurus intermedius*), and Brazilian free-tailed (*Tadarida brasiliensis*) surveyed with fixed acoustic detectors on the San Bernard National Wildlife Refuge (SBNWR) and measured total Hg (THg) concentrations in the fur of 7 bat species: cave myotis (*Myotis velifer*), hoary (*Lasiurus cinereus*), *P. subflavus, N. humeralis, L. borealis*, *L. intermedius*, and *T. brasiliensis* from eastern and central Texas. All bat species were widely distributed within the SBNWR; thus, I assessed high and low activity areas using multi-state occupancy models. Occupancy rates of *T. brasiliensis* were lower in areas with greater canopy cover whereas high activity rates of *N. humeralis* decreased in open habitats. Additionally, I collected 427 fur samples from 32 sites to investigate inter- and intraspecific variability in fur THg concentrations. Two species, *P.*

subflavus and *N. humeralis,* had greater mean THg concentrations (6.04 and 5.87 µg/g, respectively) than other species with several individuals exceeding the 10 µg/g threshold cited as having deleterious health effects in bats. *Nycticeius humeralis* was the only species that demonstrated intraspecific variation with adults having greater mean THg concentrations than juveniles (7.45 and 4.29 µg/g, respectively). *Nycticeius humeralis* fur THg concentrations were greater along the upper Gulf Coast than in central and southern Texas and were positively influenced by the density and distance to coal-fired power plants. My research concluded that Hg may be a greater threat to bat populations on the upper Gulf Coast than other areas in the state. To aid future conservation efforts of bats in Texas, I recommend maintaining a matrix of native habitats for bats to use in areas where bats are widely distributed, like SBNWR, and also facing multiple stressors including habitat loss and the accumulation of harmful contaminants like Hg.

I. DRIVERS OF BAT OCCUPANCY ON THE SAN BERNARD NATIONAL WILDLIFE REFUGE

Introduction

Bats, order *Chiroptera*, are a highly specialized taxon of mammal. Bats have evolved to be the only truly volant order in the mammalian class (Adams and Pedersen 2013). Globally, there are roughly 1,400 species of bats and they vary greatly in morphology and diet (Aldridge and Rautenbach 1987, Simmons 2005). Most species of bats use echolocation to maneuver through cluttered habitats and forage for insects while flying at night (Aldridge and Rautenbach 1987, Jones and Teeling 2006). Bats give birth to only 1 or 2 pups a year (Racey 1982) and have lifespans ~3.5 times longer than other taxon similar in size (Wilkinson and South 2002).

Bats provide several benefits to humans predominantly through the consumption of pestilential insects and pollination of economically important crops (Kunz et al. 2011). The consumption of crop-destroying insects by bats reduces the application of pesticides thereby decreasing human exposure (Pimentel and Zepp 1991) and cost. Further, bats aid the agriculture industry by reducing the amount of crop damage caused by insects (Cleveland et al. 2006, Boyles et al. 2011). Boyles et al. (2011) combined all the estimated costs bats save the North American agricultural industry and estimated this value to be approximately $22.9 billion annually. Bat pollination ("chiropterophily") benefits over 500 plant species including important food crops such as mango (*Mangifera indica*), cacao (*Erythroxylum coca*), and durian (*Durio spp.*) (Fleming et al. 2009). Bats also are the main pollinator of blue agave (*Agave tequilana*), which is used to

manufacture tequila, a multi-million dollar industry in the United States and Mexico (Molina-Freaner and Eguiarte 2003).

Although population trends are difficult to observe in cryptic animals like bats, the combination of threats impacting North American bat populations including fatalities from wind energy facilities, disease, exposure to contaminants and habitat loss, has resulted in an estimated 18–31% of species (based on NatureServe conservation status) to be at risk of declines (Hammerson et al. 2017). The development of wind farm facilities near foraging grounds and along migration routes may threaten the persistence of bat populations (Santos et al. 2013). There are several diseases negatively impacting bats, but at the forefront is white-nose syndrome (WNS) (Blehert et al. 2009), which has killed at least 6.7 million bats in eastern North America (Hopkins and Soileau 2018). White-nose syndrome is caused by the fungus (*Pseudogymnoascus destructans*) and is spreading across North America, endangering several species including the little brown bat (*Myotis lucifugus*) and Indiana bat (*Myotis sodalis*), which are experiencing population declines (Foley et al. 2011). Another threat to bats is the bioaccumulation of harmful pollutants from the diet (Becker et al. 2018), which can lead to negative health effects, mortality, and population declines (Bayat et al. 2014, Hernout et al. 2016). Habitat loss is one of the worst threats currently impacting wildlife and bats are negatively affected through the reduction of roosting and foraging habitat (Russo and Ancillotto 2015).

Most of the 44 regularly reoccurring bat species native to Canada and the US (Jones et al. 1986) are temperate species that share common traits like nocturnality and dietary preference for insects, and they can vary by ecological niches including roosting strategy, foraging technique, and prey preference (Patterson et al. 2003). The variation of

ecological niches that North American bats occupy allows for several species to co-occur in the same region (Patterson et al. 2003). For example, roosting strategies of North American bats differ among species with one example being the hoary bat (*Lasiurus cinereus*), which roosts solitarily in tree foliage, whereas other species, including the Brazilian free-tailed bat (*Tadarida brasiliensis*), roosts in caves with aggregations that can exceed 20 million individuals (Ammerman et al. 2012). Another example of variability among North American bats is the type of insects they prey upon and the foraging techniques used to capture prey. Some species such as members of the *Lasiurus* genus are considered 'specialists' and have an affinity for lepidopterans (moths) that they hunt in open habitats (Rolseth et al. 1994, Clare et al. 2009). Smaller bats such as the tri-colored bat (*Perimyotis subflavus*) are dietary 'generalists' as they forage on several orders of insects along forested and riparian habitats (Helms 2011). Several factors can influence the diets of bat species among populations including occupancy of different geographical regions, season, local habitat quality, and habitat structure (Johnston and Fenton 2001, Lasso 2005, Clare et al. 2014).

Texas plays an important role in the ecology of North American bats, in part because of the high levels of species diversity and large populations of cave-roosting species. Texas has the greatest species richness of bats in the US, totaling 33 species, and the largest colony of roosting bats in the world (Ammerman et al. 2012, BCI 2019). The karst limestone geography in Texas creates a proliferation of cave systems, many of which are utilized by bats (Scheel et al. 1996). Despite the richness of bat species, there are many gaps in the scientific literature concerning bats in Texas. The ecology of bats on

the upper Texas Gulf Coast and the species occupying this region have not been researched thoroughly.

The Gulf Coast of Texas is the most biologically rich and diverse region of the state (TPWD 2009). This region is comprised of a mosaic of coastal saltmarsh, wetland, and bottomland hardwood habitats, which are fragmented and shrinking due to human development and climate change (White et al. 2005, EPA 2017). In Texas, over 25% of the human population resides within the Gulf Coast (Hegar 2020), resulting in over half of Texas wetlands disappearing to urban development and agriculture (Moulton et al. 1997, TPWD 2003). Coastal saltmarsh and bottomland hardwood historically dominated this region, providing unique habitats to wildlife, including bats (Hoye 2002, Lamb 2009, Gonsalves et al. 2013, Clarke-Wood et al. 2016). However, information about the bats that occupy this region, including the species residing in this area and the ecological factors influencing their distribution across the landscape, is scant.

One factor that could be influencing the distribution of bats on the Texas Gulf Coast, considered a fertile region where significant crop production occurs, is the abundance of agricultural industry in the region, which is primarily focused on corn and cotton production (Gleaton and Anderson 2005). Bats use agricultural areas for foraging and save Texas cotton farmers an estimated $74 per acre by suppressing pest species (Cleveland et al. 2006). Agriculture is the second largest industry in Texas and economically important as the agricultural industry employs hundreds of thousands of workers (Sawe 2019). The ecosystem and economic services bats provide to the agricultural industry highlight the importance of researching and conserving the taxa.

The warm climate of the Texas Gulf Coast is expected to experience drastic changes due to climate change over the next century, which likely will influence the bats inhabiting the region (EPA 2016). Since 1975, Texas has experienced a gradual increase in average summertime monthly temperatures and is predicted to rise to 37.17 °C by 2036, which would be a 0.37 °C increase from the monthly summer average observed between 2000–2018 (Nielsen-Gammon et al. 2020). Precipitation in the Gulf Coast region has increased by 10–20% since 1895 (Nielsen-Gammon et al. 2020). The amount of annual precipitation on the Gulf Coast is predicted to increase accompanied by more frequent storm activity including extreme weather events like hurricanes (Scavia et al. 2002). Sea level rise has been documented along the Texas coastline varying from 3.05–6.49 mm/yr and is predicted to continue (Nielsen-Gammon et al. 2020). The long-term impacts of climate change on bats is unclear and likely site-specific (Sherwin et al. 2013, Nagy et al. 2017). Bats on the Gulf Coast are likely negatively affected by increased precipitation and extreme weather events, which reduce the amount of time bats can safely spend gathering food (Sherwin et al. 2013). Bat prey abundance may experience dramatic changes on the Gulf Coast due to climate change. Insect orders like Diptera, notably mosquitoes in family *Culicidae*, are expected to become more abundant with increasing temperatures and standing water (Ramasamy and Surendran 2012). However, other insect groups, including several members of Lepidoptera, are more susceptible to climate fluctuations and populations could be negatively impacted with climate change (Hunter et al. 2014). Extreme storm events and rising sea levels could negatively impact bats by damaging urban structures and stands of forests that provide suitable roosting habitat to bats in the Gulf Coast area. The threat of climate change to bats makes it

critical to obtain information on species that occupy habitats experiencing annual changes caused by climate change, such as the Texas Gulf Coast, in order to advance the conservation of bats in a rapidly changing climate.

The biology of bats including their volancy, echolocation, and nocturnality creates challenges for an effective study. Acoustic monitoring recently has evolved as a credible method for gathering information on bats by recording echolocation calls that often can be identified to the species level. Acoustic monitoring provides biologists with detection and non-detection data as well as activity levels at a given location. Occupancy models are an effective tool that biologists have utilized to study wildlife, including bats, where only detection and non-detection data are available (MacKenzie et al. 2002, Yates and Muzika 2006, Gorresen et al. 2008, Hein et al. 2009, Starbuck et al. 2014, Gorresen et al. 2018). Occupancy modeling identifies influential biological and environmental covariates, as well as spatial and temporal variations that influence a species' probability of occupying an area (MacKenzie et al. 2003). Occupancy models that use passive acoustic detection of bats have proven to be an effective method for identifying important patch and landscape factors, both of which influence bat presence within a specified area (Yates and Muzika 2006, Gorresen et al. 2008, Hein et al. 2009, Starbuck et. al 2015, Gorresen et al. 2018).

Objectives of the Thesis

The purpose of this study was to examine habitat characteristics, including prey, vegetation structure, and composition, that influence bat occupancy across the San Bernard National Wildlife Refuge (SBNWR). My specific objective was to estimate rates

of occupancy and detection probability for all bat species identified on the SBNWR and assess the influence of landscape composition, local habitat structure, and insect availability on species' occupancy rates. I predicted insect abundance would positively influence bat occupancy because sites with the most insect abundance will have more food resources (Tibbels and Kurta 2003). I also predicted sites with taller vegetation structure and denser canopy would have greater rates of occupancy because of the roosting potential of these locations (Yates and Muzika 2006). This research is warranted because it is important to identify which bat species are utilizing habitat on the SBNWR as well as to determine the factors that influence bat habitat use on the refuge so that managers can make more informed management decisions that benefit bat populations.

Methods

Study Area

The SBNWR (28.8626° N, 95.5407° E) was founded in 1968 and is 32 km south of the town of Sweeney on the upper Texas Gulf Coast (Figure 1). The refuge is 185.06 km^2 and dominated by saline prairie, open water, and Columbia bottomland hardwood (CBH) forests in Brazoria and Matagorda counties (White et al. 2005). The refuge is divided into a main refuge and 26 smaller land tracts the refuge has purchased over time. Among the smaller land tracts that comprise the refuge, most are dominated by CBH forest habitat and vary in size from 0.034 km^2 to 18.2 km^2. Annual precipitation is 145 cm with average temperatures of 33.2 °C in the summer and 6.5 °C in the winter (USFWS 2018). The refuge provides critical habitat to a rich array of wildlife species including the endangered piping plover (*Charadrius melodus*) and Kemp's Ridley Sea

Turtle (*Lepidochelys kempii*) as well as a diverse array of migratory bird, reptile, and amphibian species (USFWS 2018). Since bats that use the SBNWR have never been studied, the species which occupy the refuge are unknown. Preliminary capture data on the refuge confirms at least 3 species of bats including the eastern red bat (*Lasiurus borealis*), evening bat (*Nycticeius humeralis*), and *T. brasiliensis* are active at SBNWR.

Study Design

To assess occupancy of bats within the SBNWR, I surveyed 20 sites in both 2018 and 2019, and added 2 additional sites in 2019 (Figure 1). I systematically surveyed sites twice during the 2018 season and 3 times during 2019 season. I placed acoustic detectors within 20 m from the edge of a water source if one was present at a study site. I placed acoustic detectors in a pipeline or corridor on sites dominated by CBH to reduce the effects of clutter or vegetation that would negatively affect call quality. In sites with open habitat, I placed detectors 50–100 m into the interior of the habitat to avoid edge effects. I spaced the distance between detector deployments ≥ 0.1 km. Due to navigational constraints on the refuge, specific survey locations were ≤ 200 m from roads or trails across the refuge.

Acoustic Monitoring

I surveyed bats acoustically from sunset to sunrise at fixed points using D500x Pettersson acoustic detectors (Pettersson Elektronik AB, Uppsala, Sweden). I deployed 5 to 8 acoustic detectors simultaneously on a rotating schedule around the SBNWR for 3-day intervals in 2018 and 4-day intervals in 2019. I placed the microphone at a height of

3.5 m, angled at 45 degrees, and oriented it in a direction with minimal clutter. I programed all devices to have a 2 second trigger window and 15 second file length with a division ratio set at 8 to capture the frequencies of bats with call frequency ranges that overlapped within the SBNWR.

I defined a bat pass as a sequence with identifiable pulses. Each pass required a minimum of 5 search phase pulses to improve the ability of identifying the bat to species. I used Sonobat call analysis software (version 4.0, DNDesign, Arcata, CA, USA) to scrub noise files that did not contain bat call characteristics. I used Sonobat auto-identification software to classify and identify all calls with medium or better call quality to species for data sampled in 2018. I manually vetted all call files from 2019 to identify species when possible and more generalized classifications (such as low or high frequency) when calls were not of good quality.

Weather Data

I used nightly data from a Remote Automatic Weather Station (RAWS) located in the middle of the main refuge on the SBNWR to assess weather as observation-level covariates. I assembled hourly data of precipitation (mm), relative humidity (%), wind speed (mph) and temperature (°C) during each deployment and averaged the hourly data for each monitoring night. I assessed drought as an observation-level covariate and used the weekly Modified Palmer Drought Index (PMDI: scaled from -6–6 with negative values indicating drought) for the Upper Coast region from the Texas Water Development Board (TWDB 2020). I averaged the PMDI value for each site in 2018 and 2019 to assess PMDI as a site-level covariate as well.

Insect monitoring

In 2019, I surveyed insects at each acoustic deployment site using a modified Townes-style malaise trap, which is an effective trap that captures flying insects (Townes 1972). I paired a modified Townes-style malaise insect trap (ezMalaise, BugDorm LLC: L x W x H = 165 x 115 x 190 cm) with a randomly selected acoustic bat detector for a 2-night survey, using similar methods as Brooks et al. (2017). Due to a limited number of malaise traps, I was only able to survey invertebrates at 3 detector deployments during a rotation. I equipped each malaise trap with a small LED headlamp placed 5 m from the acoustic device. I modified each Townes-style malaise trap according to the methods specified by Brooks et al. (2017). I turned on a small LED light outside the trap at dusk and attached an insect collecting bottle that was 1/8th filled with 80% ethanol. Each morning, at approximately sunrise, I turned off the light and collected all insects from each trap. I stored all insect samples in plastic containers that contained an 80% ethanol solution. To estimate abundance and richness, I counted and identified insects to order and family for *Culicidae* (order: Diptera; mosquitoes) using a key developed by Johnson and Triplehorn (2004). I used the most abundant orders as site-level covariates in all 2019 models by averaging the abundance of each order for each 2-night survey.

Vegetation Structure

I assessed vegetation structure and groundcover composition surrounding each acoustic detector through surveys that were conducted every 5 m along 3 transects, each 25 m in length (Fritts et al. 2016). I used a Robel pole to assess vegetation height and classified vegetation (Robel et al. 1970). Vegetation classifications included grasses,

forbs, bare ground and woody plants. I estimated canopy cover using a concave densitometer at each 5 m mark on each transect. All vegetation surveys were conducted during the last 2 weeks of the 2018 and 2019 field season.

Landscape Composition

I used ArcGIS Pro (version 2.4, Environmental Systems Research Institute, Redlands, CA, USA) to plot all acoustic sites and assess landscape composition around each site. I imported a raster file containing landcover types in Texas (USGS 2016), converted the raster file into vector data, and used the model builder tool to estimate percentage of each habitat composition in 2-km buffers around each acoustic deployment site. I selected 2-km as a buffer to capture the local habitat scale similar to methods used in Starbuck et al. (2014). For landscape scale, I simplified the habitat classifications into 3 classifications including CBH, open, and urban. Columbia bottomland habitat included any habitat classified as CBH by the landcover type layer. Open habitat was dominated by coastal and saline prairies, and to a lesser extent agriculture, and barren habitat classifications. Additionally, I used ArcGIS to measure the distance from each detector to the nearest city using a shapefile layer containing all Texas cities (TDOT 2016). Preliminary research on roosting dynamics of *N. humeralis* within the SBNWR suggest urban areas may provide essential roosting habitat to bats in the area (Rogers 2020).

Statistical analysis

I created single-season single-species occupancy models to compare the rates of occupancy (Ψ) and detection probability (p) for 5 bat species identified on the SBNWR

including *P. subflavus*, *L. borealis*, *N. humeralis*, northern yellow bats (*L. intermedius*), and *T. brasiliensis* using package "unmarked" (Fiske and Chandler 2011) in program RStudio (version 1.1.463 R Core Team 2012). I elected to assess single-season occupancy models instead of combining both seasons into dynamic occupancy models due to variability between season duration and number of primary sampling occasions at each site. I evaluated detection histories of each site by classifying each species as non-detected if the species was never positively identified and detected if the species was positively identified during a monitoring night. I coded detection histories to represent this with each species (0: undetected, 1:detected). Most species identified within the SBNWR were widely distributed and detected at the majority of sites at least once. I scaled all covariates by subtracting the average and dividing by the standard deviation prior to including it in a model, but back-transformed values for model predictions.

To gain further inferences about factors potentially influencing rates of use by bats on the SBNWR, I created a single-season single-species multi-state model for each of the 4 most abundant species surveyed in 2019, which included *P. subflavus*, *L. borealis*, *N. humeralis*, and *L. intermedius*. I modified detection histories for each species to account for multiple states of activity (0: undetected, 1:detected with low activity, 2: detected with high activity). The multi-state occupancy model assesses: Ψ (probability of occupancy), R (probability of high activity given a site is occupied), *p1*(probability of detecting species given a site has low activity), *p2* (probability of detecting species given a site has high activity), and δ (probability of detecting high activity given a site was occupied) (MacKenzie et al. 2009). I examined the number of call files identified for each species and identified natural breaks that distinguished sites with high activity versus low

activity. *Perimyotis subflavus* were the most active and widely distributed species observed on the SBNWR. I defined high activity as a given night having ≥ 10 distinct manually identified *P. subflavus* bat passes and low activity as having 1–9 distinct passes. *Lasiurus borealis*, *N. humeralis*, and *L. intermedius* occupied most of the survey sites, but with less activity than *P. subflavus*, thus for these 3 species I defined high activity as a monitoring night having ≥ 5 distinct manually identified bat passes and low activity as having 1–4 distinct bat passes. *Tadarida brasiliensis* had the lowest rates of occupancy during summer 2019 and was only evaluated using single-season single-species occupancy models.

To select the best fitting single-season occupancy model for each species, I first determined the observation-level covariates that influenced the detection probability of each species. I assessed each observation-level covariate (Table 1) in a univariate model and examined additive combinations of the most competitive (≤ 2 AIC) covariates, which were included for all further occupancy models examining site-level covariates (Yates and Muzika 2006). Once the observation-level covariate(s) was determined for each species, I calculated occupancy models that assessed the influence of site-level covariates on Ψ. For all multi-state models, I assessed only site-level covariates to examine their impacts on activity. I assessed site-level covariates by first examining a univariate model of each site-level covariate and determined if the model had good fit by assessing the standard errors of the intercept and covariate. Models with inflated standard errors were removed. If more than one covariate was considered competitive (≤ 2 AIC), I assessed additive combinations of competitive covariates to determine if a more parsimonious occupancy model was possible for each species. I checked for

multicollinearity when 2 or more site-level covariates were included in a model by assessing the Variance Inflation Factors (VIF) (VIF > 4 would result in the model being omitted from analysis). I used the model selection tool in the unmarked package to select the best fitting models with the most influential drivers using the Akaike's Information Criteria (AIC) weights for each species. I assessed significance of each parameter by calculating 95% confidence intervals for all competitive models. I used the modavg tool in the "AICcModavg" package (Mazerolle 2020) to determine the coefficient values averaged among all competitive models for site-level covariates. I assessed the best fitting models for each species for both 2018 and 2019 and factored in multiple states of activity level for 4 bat species in 2019.

Results

In 2018, I monitored bats for 70 monitoring nights reflecting 3,442 monitoring hours and resulted in 97,045 sound files with 32,596 containing bat call characteristics. In 2019, I collected 574,886 sound files resulting in 15,499 call files with bat characteristics and 1,092 monitoring hours. I coded observation-level and site-level covariates with descriptions in Table 1. Parameters for all site-level covariates are reported in Table 2. All competitive occupancy models for 5 bat species are presented in Tables 3–4 (2018, 2019, respectively). Untransformed outputs for the top model for each species including site-level and observation-level covariates are in Tables 5–6 and model averaged site-level covariates for all models are in Tables 7–8 (2018, 2019, respectively). Back-transformed occupancy rates and *p* for all competitive models are reported in Tables 9–10 (2018, 2019, respectively).

Site-level covariates

Vegetation height ranged from 0.51 m to 1.71 m with an average of 0.99 m in 2018, and 0.64 m to 2.00 m with an average of 1.2 m in 2019. In 2018, canopy cover ranged from 0% to 96% among sites with the average equating to 25.1%, and 0% to 100% with an average of 38.9% in 2019. Within a 2-km buffer, average CBH habitat was 59.8%, the average amount of open habitat was 36.1%, and the average percent of urban habitat was 1.0%. The distance to the nearest city from detector locations averaged 19.9 km.

I collected 5,224 insects during 2019 surveys and the most abundant insect orders were Diptera (n = 2,614, 50%), Lepidoptera (n = 1,044, 20%), and Hemiptera (n = 925, 17.7%). The family *Culicidae* was the most abundant insect family surveyed and was extracted from Diptera to be analyzed separately as a covariate. Insect abundance ranged from 29 to 513 and averaged 233 insects across a 2-night survey for a given site in 2019. Insect order richness ranged from 4 to 8 and averaged 6.14 across a 2-night survey for a given site in 2019 (Table 2).

Brazilian free-tailed bat

I detected *T. brasiliensis* at 47.7% of surveyed points in 2018 with a naïve occupancy of 84%. In 2019, I detected *T. brasiliensis* on 25.1% of monitoring nights with a naïve occupancy of 77%. In 2018, the best model for estimating *p* included PMDI which negatively affected *p*. Site occupancy decreased from 0.92 to 0.09 when the amount of canopy cover increased from 0% to 96.1% (Figure 2). In 2019, precipitation

was included for assessing *p* but was not significant. The average PMDI was included in the selected model for Ψ, but the confidence interval overlapped with zero.

Evening bat

I detected *N. humeralis* on 32.9% of monitoring nights in 2018 with a naïve occupancy of 84%. In 2019, I detected *N. humeralis* on 38.2% of monitoring nights, with naïve occupancy of 82%. In 2018, the best model for estimating *p* included PMDI, which had a negative impact on detection rates and the null was selected for Ψ. In 2019, the null was selected for estimating *p*. Both open habitat and distance to nearest city were competitive for estimating R and had a negative impact on high rates of activity, but open habitat was the only significant site-level covariate. The mean site occupancy rate with high activity decreased from 0.51 to 0.14 when the amount of open habitat increased from 0.16% to 99.4% in a 2-km radius (Figure 3).

Eastern red bat

I detected *L. borealis* at 55.3% of surveyed points with a naïve occupancy of 95%. In 2019, I detected *L. borealis* on 30.7% of monitoring nights with a naïve occupancy of 86%. The best model for 2018 included PMDI, which negatively impacted estimating *p* and the null for estimating Ψ. In 2019, the most parsimonious multi-state occupancy model included the null for *p*, as well as Hemiptera abundance and open habitat for R. Hemiptera abundance was included in both competitive models and had a positive correlation with high levels of activity, while the amount of open habitat had a negative correlation with R, however neither were significant.

Northern yellow bat

I detected *L. intermedius* at 51.7% of monitoring nights in 2018 with a naïve occupancy of 95%. In 2019, I detected *L. intermedius* at 38.8% of monitoring nights, with a naïve occupancy of 91%. In 2018, the best model for estimating *p* included PMDI, which negatively impacted detection rates and the null was selected for Ψ. In 2019, the top multi-state model selected included Julian date for estimating *p2*, indicating that Julian date had a positive influence on detecting high activity levels of *L. intermedius*. The null was selected for estimating Ψ and R.

Tri-colored bat

I detected *P. subflavus* bats 51.7% of monitoring nights in 2018 with a naïve occupancy of 89%. I detected *P. subflavus* bats 49.6% of monitoring nights in 2019 with a naïve occupancy of 96%. In 2018, the best model included PMDI for *p* and veg structure for Ψ, but the null model was considered competitive for Ψ and confidence intervals for all covariates overlapped zero. In 2019, the best multi-state model for estimating *p* included Julian date. Julian date had a negative impact on detection probability. Open habitat and abundance of Coleopterans was selected for R, but the confidence interval overlapped zero. Open habitat had a negative correlation with high *P. subflavus* bat activity, but the abundance of Coleopterans had a positive correlation with high activity.

Discussion

Results suggest the SBNWR experienced high rates of use for the 5 bat species observed across the summers of 2018 and 2019 in all habitat types. The bat species occupying SBNWR were unknown prior to this study, therefore my hypothesis applied to bats as a collective and were not species specific. Despite only detecting 15% of the bat species that occur in Texas, all bat species observed on the SBNWR were detected at the majority of survey sites at least once. I detected greater naïve occupancy rates for *P. subflavus*, *N. humeralis*, *L. borealis*, and *T. brasiliensis* on the SBNWR than Weinkoaf (2015) detected across 2 summers of surveying bats in hardwood forests in east Texas. Further, Debelica-Lee and Wilkins (2014) assessed bat assemblages using live capture methods in forested habitats on the Sam Houston National Forest in southeastern Texas and captured *N. humeralis* and *L. borealis* more frequently than any of the other 8 species captured, which included *T. brasiliensis* and *P. subflavus* bats. My research shows that the bat species on SBNWR, with the exception of *L. intermedius* have been observed in other bat studies performed in east Texas (Debelica-Lee and Wilkins 2014, Weinkoaf 2015). The range of *L. intermedius* is restricted to southeastern Atlantic and Gulf Coast habitats and prefer roosting habitat of Spanish moss and fan palms which are abundant on SBNWR (Ammerman et al. 2012). Although *P. subflavus* bats are experiencing population declines in northeastern US and are a species of special concern in Texas, they were the most active and detected species on SBNWR. Thus, SBNWR may be providing important habitat to *P. subflavus* and future management efforts need to consider the impact various refuge management strategies may have on this species of concern prior to them being implemented.

I did not find supporting evidence to corroborate my prediction that insect abundance would positively influence occupancy rates. This suggests insect abundance is not driving rates of occupancy or activity on SBNWR. Becker et al. (2017) found similar results when they examined the relationship between insect abundance and activity among 5 species/groups of bats including several that overlap with this study such as *N. humeralis*, *L. borealis*, *P. subflavus*. However, Becker et al. (2017) did observe a positive relationship between insect abundance and *L. cinereus* activity. Similarly, high activity rates for the Hawaiian hoary bat (*L. cinereus semotus*) have been documented to be positively associated with beetle biomass (Gorreson et al. 2018). Conversely, another study in Texas suggested occupancy and activity of southeastern myotis (*Myotis auriculus*), silver-haired bats (*Lasionycteris noctivagans*), and *N. humeralis* were negatively correlated with insect biomass, but the entire study was within bottomland hardwood habitat (Weinkoaf et al. 2015). A possible explanation for the observed lack of significance is because insect hatches fluctuate and therefore may not be properly observed during a 2-day survey with a single insect trap. Another possibility is the number of sites surveyed was not enough to obtain a clear assessment of insect abundance and the relationship to rates of occupancy and high use among bats.

I did not find supporting evidence to support my prediction that sites with taller vegetation structure and denser canopy cover would have greater occupancy rates. The top 2018 model for *T. brasiliensis* had a negative correlation with canopy cover, which makes biological sense since *T. brasiliensis* have longer, narrower wings than other species in this study resulting in a high wing aspect ratio. Bats with high wing aspect ratios have more difficultly maneuvering through cluttered landscapes (Findley et al.

1972). Both *N. humeralis* and *P. subflavus* have lower wing aspect ratios compared to the other species in this study and prefer cluttered habitat in other areas of their distribution (Findley et al. 1972, Ammerman et al. 2012). However, Loeb and O'Keefe (2006) found that sparse vegetation density was the best predictor for habitat use by several bat species including *L. borealis* and *P. subflavus* sampled at various forested habitats in northwestern South Carolina. Reducing canopy cover on SBNWR could improve site use by *T. brasiliensis* and should be considered as a possible management strategy if increasing *T. brasiliensis* use on the refuge is desired.

Although open habitat at the local scale of 2-km has more influence over activity and site use by *L. borealis*, *N. humeralis*, and *P. subflavus* on SBNWR when compared to site-level habitat covariates such as vegetation structure or canopy cover, the generalized classification of open habitat makes it difficult to determine what is driving this observation. The cues used for habitat selection may change for some species depending on scale. For example, *N. humeralis* had greater activity in open habitats than cluttered forests, but when examining clutter within one habitat type (riparian areas), *N. humeralis* had greater activity in cluttered riparian habitats versus open riparian habitats (Menzel et al. 2005). Starbuck et. al (2015) found that site occupancy for *N. humeralis* and *P. subflavus* decreased with an increase in forest and urban habitat in a 16-km radius but increased for *L. borealis* in the Missouri Ozark Highlands, although the effect of open habitat was not assessed for these species. The composition of open habitat varies greatly geographically and future studies should take strides to decipher specific open habitat classifications when assessing bat activity.

There could be several reasons for *N. humeralis* to avoid open habitats on the SBNWR including predator aversion and prey preference in non-open habitats. Barred owls (*Strix varia*) were commonly observed on SBNWR during both field seasons and have been documented predating on bats (Bergstrom and Smith 2017). The literature on predator avoidance and bats is scant, but Baxter et al. (2006) did observe lower activity among bats when an owl call was played at a site compared to a matched control site where no owl call was played. Foraging behavior in closed habitats could also be a factor. The diet of *N. humeralis* is often described as 'generalist' with Coleopterans being cited as the primary order in the diet (Feldhamer et al. 1995, Geluso et al. 2008, Wilson 2017). Weinkauf (2015) assessed the diet of *N. humeralis* using fecal samples in northeast Texas and found evidence of Dipterans, Coleopterans, and Lepidopterans. I observed greater Coleopteran and Lepidopteran abundance in open habitats rather than forested, but greater Dipteran abundance in forested habitat. Future research should examine fecal composition of bats on the SBNWR to assess what *N. humeralis* on SBNWR are consuming and whether Dipterans are a staple in the diets of *N. humeralis* on the refuge.

In 2018, the Texas upper Gulf Coast region and SBNWR experienced a drought that limited the availability of potable water to bats in the region and weekly PMDI influenced detection probability of all species. The PMDI value was negatively correlated to detection probability for all 2018 models which suggests that drought improved ability to detect bats on the SBNWR. This could mean bats are more likely to utilize SBNWR habitat during times of drought or that bats had grater activity during these times because they had to fly greater distances to water. The SBNWR has permanent water, which bats increase activity around during drought (Amorim et al. 2017). The placement of most

detectors was at or near available water sources that maintained water longer than other water sources in the area. In 2019, the upper Gulf Coast experienced considerably more rainfall during the field season and PMDI was not selected as an observation-level covariate for any species. However, the top model for *T. brasiliensis* included PMDI as a site-level covariate and precipitation as an observation-level covariate. The impact of drought on bats occupying SBNWR should be assessed through long-term monitoring over several years so trends can be observed over a longer time period.

This study had several limitations that could have influenced the results and lack of significance for site-level variables in most models. While manually identified calls were matched with auto-identified calls over 90% of the time, only 2019 data was manually identified which could have led to some misclassifications from auto identification software for 2018 data. Further, I had only 8 acoustic detectors which limited the number of sites I could survey concurrently and may have made it more difficult to observe variability among covariates. I experienced several limitations including the amount of dense CBH forest and significant amount of open water present on the refuge that restricted the number of locations I could safely deploy detectors. This resulted in some overlap of habitat characteristics at the local landscape level among sites. Another limitation was the duration of both field seasons overlapped the timeframe when most temperate bat species would be giving birth to pups thereby increasing the population of bats in the area (Ammerman et al. 2012). This is likely a violation of the assumption that the occupancy state is closed during the duration of the season. This could explain why Julian date had a positive effect on detection probability for *L. intermedius* because more bats would be entering the population thereby increasing the

likelihood of detection. It is interesting that Julian date had a negative correlation with detection probability and delta for *P. subflavus* in 2019. This species might be utilizing upper Gulf Coast habitat more frequently during late spring and summer and spreading out more during later months when pups would be entering the population.

Future research

Future monitoring efforts of bats on SBNWR should attempt to build a more robust sample size of sites with more replications. Long-term acoustic monitoring should continue on the SBNWR and occur year-round to observe possible changes that may occur as climate change continues to modify the Texas Gulf Coast and periods of drought arise. Further, the presence of *P. subflavus* and their frequent use of different habitats observed on SBNWR, creates an important opportunity to study this species and advance the understanding of detailed habitat use by *P. subflavus*. Finally, the dietary composition of bats utilizing the refuge should be explored to assess if specific prey species are driving use in certain areas.

Conclusion

This is one of the first studies to examine the bat species and their rates of occupancy and activity on the SBNWR and an upper Gulf Coast ecosystem. Several bat species use a range of areas and habitats on the SBNWR. Maintaining open water during years of drought on the refuge could be helping bats in the area by providing potable water sources when resources are limited. Additionally, *P. subflavus*, which are a species of special concern in Texas and have been petitioned to be listed under the Endangered

Species Act, were using refuge habitat more frequently than other areas in east Texas. The SBNWR has been acquiring small land tracts surrounding the main refuge since its founding in 1968 and future acquisitions should prioritize forested habitats to protect areas that *P. subflavus* and other bat species are more likely to use. Management efforts for bats on the SBNWR can utilize this research to make more informed decisions in the future.

II. INTER- AND INTRASPECIFIC VARIABILITY IN MERCURY CONCENTRATIONS IN TEXAS BATS

Introduction

Mercury as a global pollutant

Mercury (Hg) is a nonessential trace element and considered to be a global pollutant (Boening 2000). In the environment, Hg occurs in 3 forms: elemental (Hg^0), inorganic (Hg^{2+}), and organic [also known as methylmercury (CH_3Hg^+); hereafter referred to as MeHg]. Mercury naturally occurs in the environment, primarily due to volcanic eruptions, wildfires, and the erosion of cinnabar deposits (Boening 2000, Futsaeter and Wilson 2013). However, humans have doubled the amount of Hg in the environment since pre-industrial times, primarily through the combustion of coal and its use in small artisanal gold mining operations (Wang et al. 2004, USGS 2014). Other anthropogenic sources of Hg pollution include, but are not limited to, ferrous metal smelting, oil refining, cement production, chlor-alkali production, and waste incineration (Mason et al. 1994, Futsaeter and Wilson 2013, EPA 2018). Mercury cycles through the environment beginning when Hg^0 and Hg^{2+} enters the atmosphere (where Hg^0 can be photo-oxidized to Hg^{2+}), gets mobilized through air currents, and deposited over land and water via wet and dry deposition (Morel et al. 1998, Boening 2000). In aquatic systems, Hg^{2+} can be converted to MeHg, the most bioavailable form of Hg, primarily by sulfate-reducing bacteria in sediment and the overlying water column (Ullrich et al. 2001, Lin et al. 2012).

Methylmercury is the most toxic form of Hg and is capable of causing neurological, cardiovascular, renal, and respiratory damage to wildlife and humans at low concentrations (Burton et al. 1977, Clarkson and Magos 2006, Lin et al. 2012, Nam et al.

2012). The uptake of MeHg into the food web begins when phytoplankton take up MeHg from the water (Pickhardt and Fisher 2007, Luengen and Flegal 2009). Methylmercury is then biomagnified as it is trophically transferred up the aquatic food web (Mason et al. 2000, Lin et al. 2012); as a result, species at the top of aquatic food webs, such as predatory fishes (e.g. striped bass (*Morone saxatilis*) and largemouth bass (*Micropterus salmoides*)) (Cizdziel et al. 2003, Chumchal et al. 2008), ospreys (*Pandion haliaetus*) (Grove et al. 2009), and river otters (*Lontra canadensis*) (Halbrook et al. 1994), have the greatest tissue Hg body burdens.

Mercury in bats

The accumulation of Hg in bats has been documented by researchers across the world, including in North America (Hickey et al. 2001, Wada et al. 2010, Nam et al. 2012, Yates et al. 2014, Little et al. 2015, Chételat et al. 2016, Korstian et al. 2017, Edwards et al. 2019), Central America (Becker et al. 2017, Becker et al. 2018), South America (Kumar et al. 2018, Moreno-Brush et al. 2018, Carrasco-Rueda et al. 2020), Europe (Åkerblom and de Jong 2017, Lisón et al. 2017, Ferrante et al. 2018), and Asia (Miura et al. 1978, Syaripuddin et al. 2014, Heiker et al. 2018, Costantini et al. 2019). Several studies focused on fur Hg concentrations in bats sampled near locations of point source Hg pollution (Nam et al. 2012, Yates et al. 2014, Little et al. 2015, Ferrante et al. 2018, Kumar et al. 2018). For example, in Peru, researchers examined total Hg (THg) concentrations in fur from bats captured downriver from several artisanal gold mining sites and documented THg concentrations at least 2 times greater than concentrations in bats captured ~170 km away from mining sites (Kumar et al. 2018). In Italy, several

greater mouse-eared bats (*Myotis myotis*) were captured at a cave one km from a petrochemical plant and had greater fur THg concentrations than individuals sampled at a control site ~15 km from the petrochemical plant (Ferrante et al. 2018). Most of the bat species described in the scientific literature are insectivorous, however Becker et al. (2018) assessed fur THg concentrations among dietary guilds in tropical bats from Belize and found piscivorous species had the greatest THg concentrations while frugivorous bats had the lowest. Additionally, Kumar et al. (2018) reported similar findings in Peru and documented fur THg concentrations increased in bats as trophic level, as determined through stable isotopes, increased.

The accumulation of Hg in North American bats has predominantly been examined in northeastern populations of little brown bats (*Myotis lucifugus)*, big brown bats (*Eptesicus fuscus*), and other members of the Myotis genus in the US and Canada (Nam et al. 2012, Yates et al. 2014, Little et al. 2015, Hernout et al. 2016, Chételat et al. 2018). Several previous studies observed interspecific variation in fur THg concentrations among captured bat species (Syaripuddin et al. 2014, Yates et al. 2014, Becker et. al 2018, Heiker et al. 2018, Korstian et al. 2018, Kumar et al. 2018). Bats captured near point sources of Hg pollution accumulated significantly greater THg concentrations compared to bats of the same species captured further from the point source (Nam et al. 2012, Yates et al. 2014).

Mammals have the ability to excrete trace elements, including Hg, into growing hair via the bloodstream, which allows metal cations to bind with the keratin in hair follicles (Beernaert et al. 2007). Fur is an effective proxy for measuring Hg concentrations in bats because it provides an opportunity to use a non-lethal and

minimally invasive sampling technique to investigate the body burden of THg (Hernout et al. 2016). In addition to fur, other tissues including liver, brain, and blood have been used to assess Hg concentrations in bats and previous studies suggest THg in fur is a good predictor of THg concentrations in blood (Yates et al. 2014), and THg concentrations in liver and brain tissues (Nam et al. 2012). The concentration of MeHg in fur was examined in 5 bat species in Yates et al. (2014) and the percentage of MeHg composing THg levels ranged from 71% to 95% with an average of 86%. This study also reported a positive correlation, with a nearly perfect linear relationship of 1:1, between MeHg and THg concentrations implying fur THg is a good representation of the MeHg concentration in bats.

The negative health effects associated with Hg exposure are poorly understood in bats; yet, several studies have cited 10 μg/g for THg in fur as a threshold associated with Hg toxicity based on research undertaken in common white-footed deer mice (*Peromyscus maniculatus*), mink (*Neovison vison*), and little brown bats (*Myotis lucifugus*) (Wobeser et al. 1975, Burton et al. 1977, Nam et al. 2012). Nam et al. (2012) reported *M. lucifugus* with fur THg levels of ≥10 μg/g experienced neurochemical changes in the brain. A previous study observed negative behavioral changes including decreased ambulatory responses in rodents with average fur THg levels of 7.8 μg/g (Burton et al. 1977). Hg toxicity was also noted in an experimental study with *N. vison* in which lethargy, anorexia, and death were all observed in animals that received a MeHg dose ≥ 1.1 μg/g (Wobeser et al. 1976).

The primary uptake route of Hg into bats is through their diet, which mainly consists of insects that have connectivity to aquatic food webs (Becker et al. 2018). Bats

consume both terrestrial and aquatic insects (Hickey et al. 1996); however, bats that prey primarily on terrestrial insects, such as members of the *Lasiurus* genus, have been reported to have lower concentrations of THg compared to other insectivorous species (Clare et al. 2009, Yates et al. 2014, Korstian et al. 2018). By preying upon emerging aquatic insects as well as terrestrial insects that spend part of their lifecycle in water or consume aquatic insects, bats bioaccumulate Hg from insects linked to aquatic ecosystems (Anthony and Kunz 1977, Lee et al. 2005, Becker et al. 2018).

According to Yates et al. (2014) bats may experience greater rates of accumulation compared to other species and are appropriate bioindicators of Hg exposure because they have: 1) wide distributions and occurrence in a variety of habitats; 2) long life-spans with some species capable of living over 30 years in the wild; 3) accumulate higher concentrations of contaminants due to their higher trophic position; and 4) consumption of large amounts of insects that occur in both terrestrial and aquatic food webs (Wilkinson and South 2002, Wickramasinghe et al. 2004, Simmons 2005, Fukai et al. 2006, Becker et al. 2018). These attributes have promoted insectivorous bats as bioindicators of heavy metal pollution (Cd, Cu, Cr, Hg, Pb, Zn) in the scientific literature (Yates et al. 2014, Zukal et al. 2015, Hernout et al. 2016).

Bats provide several ecosystem services including pollination and insect consumption, which are critical to the functions of healthy ecosystems and benefit humans (Kunz et al. 2011). Bats pollinate several plant species that provide food to a plethora of wildlife and humans (Fleming et al. 2009). Bats consume a variety of insects that prey on crops and as a result they reduce the need for applications of pesticides that can harm wildlife especially amphibians (Pimentel and Zepp 1991, Davidson et al. 2002).

The negative health effects of Hg toxicity in bats has never been fully assessed, but high concentrations of Hg in bats could impede these services resulting in harm to wildlife and humans.

Mercury in Texas bats

Currently, there is limited information on THg concentrations in Texas bats. To date, the one study that has examined Hg accumulation in 8 species of Texas bats reported that THg concentration in bat fur varied significantly among species and most concentrations were below the previously reported toxicity threshold level of 10 μg/g (Korstian et al. 2018). These fur samples (n = 406) were collected at 2 remote wind energy facilities with one located near the northern border and the other placed at the southern border of the state (Korstian et al. 2018). Further, these wind energy facilities are not near any known point sources of Hg pollution such as coal-fired power plants or cement factories; thus, there is a critical need to assess THg concentrations in Texas bats at more locations across the state to determine their THg body burdens and if certain species are at greater risk than others for Hg toxicity.

Texas has the greatest diversity of bats (33 species) of any state in North America (Manning et al. 2008). The diversity of Texas bats is currently under threat by the detrimental disease white-nose syndrome (WNS), caused by the fungus *Pseudogymnoascus destructans* (Pd). Among the diverse number of bat species that occur in Texas, 8 species including the cave myotis (*Myotis velifer*), Rafinesque's big-eared bat (*Coryhorhinus rafinesquii*), big brown bat (*Eptesicus fuscus*), silver-haired bat (*Lasionycteris noctivagans*), eastern red bat (*Lasiurus borealis*), southeastern bat (*Myotis*

austroriparius), tricolored bat (*Perimyotis subflavus*), and Brazilian free-tailed bat (*Tadarida brasiliensis*) have been observed with the fungus Pd in Texas and/or other states. Among the Texas species detected with Pd, 2 species (*P. subflavus* and *E. fuscus*) currently are experiencing population declines in the northeastern US from WNS (White-nose Syndrome Occurrence Map 2018). In March 2020, the first case of WNS was observed in *M. velifer* (TPWD 2020). In the northeastern US, *P. subflavus* accumulated the greatest levels of THg in their fur (average range 4–8 μg/g) compared to 6 other bat species (Yates et al. 2014) and the greatest average concentrations among bats sampled in Texas (Korstian et al. 2018). Further, Yates et al. (2014) proposed that the risk of immunosuppression caused by Hg toxicity could potentially exacerbate the contraction and signs of WNS. Thus, the presence of *P. subflavus* in Texas provides an important opportunity to collect fur samples from this species and examine factors that could influence their accumulation of greater Hg concentrations.

In addition to the greatest diversity of bat species, Texas also produces more Hg pollution than any other state in the US (Bolate 2017). In 2014, Texas emitted 6,160 kg of Hg into the atmosphere, which constituted 12.3% of all Hg emissions from the US that year (Bolate 2017). Most Hg is emitted from the 13 coal-fired power plants which are mostly located in east Texas, as well as historic gold and mercury mining efforts, and emissions from oil and gas refineries, which are mainly located along the Gulf Coast (TCEQ 2006, EIP 2010). The Hg pollution occurring in the state is predominantly in east Texas where 5 of the 10 greatest Hg polluting coal-fired powerplants in the US occur (EIP 2010). Due to the diversity of bat species, considerable amount of Hg pollution, and limited data on THg concentrations among bats in Texas a detailed study is warranted.

Objectives of the thesis

The overall goal of this study was to examine THg concentrations in fur from 7 common bat species [*L. borealis*; *N. humeralis*; *L. cinereus*; *L. intermedius*; *M. velifer*; *T. brasiliensis*; and *P. subflavus*] distributed throughout eastern and central Texas. Specifically, this research addressed the following 3 objectives:

1. Examined interspecific variability of THg in fur among all species collected. I predicted there would be significant variation in the THg accumulation among bat species because of the varied diets that different bat species consume (Carter et al. 2003).
2. Investigated intraspecific variability of THg in fur by comparing THg concentrations between sex and age classes. I predicted adults would have greater concentrations of THg compared to juveniles because older bats would be able to bioaccumulate Hg over a longer period of time (Becker et al. 2018).
3. Assessed the concentration of THg in fur from bats sampled at various sites across Texas. I predicted fur THg concentrations would vary among sites because sites nearer to point source Hg emitters would have a greater rate of atmospheric fallout (Yates et al. 2014).

This study is warranted because results will provide managers with current information about THg concentrations among several bat species in central and east Texas and identify species that may require additional management focus due to high THg concentrations. Further, results from this study can be used by managers to identify localized areas of Hg contamination that could impact other wildlife species.

Methods

Sample collection

I collected fur samples using live capture techniques and collaborations with bat researchers and rehabilitators across central and eastern Texas (Figure 4). In total, I collected fur samples from 83 locations, which were pooled, when feasible, to describe a general site location (hereafter referred to as 'site'). Austin had the most pooled sampled locations (n = 42). Other sites that included pooled sample locations are North Hays County (n = 3), Bell County (n = 3), Round Rock (n = 3), San Marcos (n = 3), Mineral Wells (n = 2), Tom Green County (n = 2), and the San Bernard National Wildlife Refuge (SBNWR) office (n = 2). This resulted in 32 sites across eastern and central Texas. These sites were selected based on where collaborators had permission to capture live bats or collect deceased individuals as well as where I had permission to survey. I was not able to procure any samples in west Texas because I did not find contacts in the region. The Greater Austin and San Antonio Metropolitan Areas encompassed the largest subset of sites (Austin, Bell County, Dripping Springs, Georgetown, Granger, Lockhart, N Hays County, Johnson City, Comal County, Round Rock, and Largo Vista). Several sites were scattered along the upper Gulf Coast region (Houston, Lake Jackson, Brazoria, SBNWR office, SBNWR BP, and West Columbia). I sampled several sites in east Texas region (N and S Freestone County, N and S Leon County, N and S Walker County, Lavender, and Palestine). Other sampled areas include the most western site (Tom Green County), the most northern site (Mineral Wells) and 2 sites in South Texas (Starr and Hidalgo County).

I collected fur samples from live bats captured using mist nets as well as by hand in roosts at various locations across the state. For the collaborations, I provided a kit containing tools to collect fur samples from captured bats to multiple biologists and organizations. I visually identified each bat to species and recorded life stage (juvenile or adult) based on the epiphysial cartilage method (Ammerman et al. 2012), sex, and collection date and location. Using stainless steel scissors, I collected ~4 mg of fur from the dorsum of all bats captured or sampled for this study. I placed each fur sample in a clean polypropylene vial and stored them at room temperature (approximately 21°C) until further processing and analysis. I included fur from any bat species that was not federally protected, but predominantly focused on using samples from 7 species including *N. humeralis*, *L. cinereus*, *L. intermedius*, *L. borealis*, *M. velifer*, *T. brasiliensis*, and *P. subflavus*. A complete sample size breakdown by species and collection location is shown in Table 11.

Fur cleaning experiment

Prior to starting the THg analysis, I undertook a cleaning experiment to determine whether the fur samples needed to be cleaned prior to analysis by comparing the THg concentration in fur from 4 different cleaning treatments to an untreated control. My objective was to confirm that cleaning fur samples was not necessary, which previous studies have demonstrated (Little et al. 2015, Chételat et al. 2018), because Hg contamination from external sources would significantly impact my results. Thus, I compared 5 cleaning treatments by collecting fur from the dorsum of 15 frozen bats, divided each fur sample into 5 subsamples weighing ~4 mg, and exposed each subsample

to 5 ml of a cleaning treatment: 2:1 chloroform:methanol solution, 1:30 deionized water:acetone solution, versa-clean detergent (Fisher Scientific, Canada) rinse, deionized water rinse, and untreated control. Treatments were selected based on methods in previous experiments (Little et al. 2015, Chételat et al. 2018, Korstian et al. 2018). After adding fur to the vial containing the treatment, I shook the sample for one minute, emptied the sample onto filter paper and repeated 3 times, after which the fur was dried overnight on filter paper in a fume hood. The mean THg concentration among the 5 treatments did not differ (one-way ANOVA; $F = 0.197_{4,70}$; $P = 0.939$; Table 12); therefore, I did not clean samples with organic solvents prior to THg analysis. Instead, I rinsed all samples with Milli-Q water and dried samples overnight under a fume hood. Prior to analysis, I visually inspected samples for exogenous material, which, if present, was removed with a Kimwipe (Kimberly-Clark Professional, Roswell, GA).

THg Analysis

To measure the concentration of THg in each fur sample, I used a Direct Mercury Analyzer (DMA-80, Milestone Inc., Shelton, CT), which uses thermal combustion, gold amalgamation, and atomic absorption spectrometry as described in EPA method 7473 (EPA 2007). The DMA-80 was calibrated as needed using certified reference materials (CRM) from the National Research Council Canada [NRCC; MESS-4 marine sediment (0.08 µg/g THg), TORT-3 lobster hepatopancreas (0.292 µg/g THg), and PACS-3 marine sediment (2.98 µg/g THg).

To confirm the validity of the data, quality assurance/quality control included empty quartz boats (blanks), CRMs, and duplicate samples. The blanks (n = 40) had a

mean THg concentration < 0.0000 µg/g and the duplicate samples had a mean relative percentage difference of 5.23% (range = 0.04–22.6%). The 2 CRMs used were DORM-4 (fish protein; NRCC; 0.412 µg/g THg; n = 39) and ERM-CE464 (tuna; European Reference Materials; 5.24 µg/g THg; n = 12), which had a mean percentage recovery of 98.4% (range = 93.5–105.0%) and 98.4% (range = 93.0–109.8%), respectively.

Geospatial analysis

I used GIS in ArcMap Pro (version 2.4, Environmental Systems Research Institute, Redlands, CA) to compare collection locations to the distance to the nearest coal-fired powerplant and other sources of Hg pollution. I used information from the EPA Toxic Release Inventory (TRI) Program to identify potential point sources of Hg pollution that had documented their amount of Hg emissions to the EPA in 2018 (TRI 2019). The type of Hg pollution sources is classified by industry sector and include hazardous waste control, electric utilities, primary metal production, petroleum refining, and chemical production. I used the near tool in ArcMap Pro to estimate the distance to the nearest potential point source of Hg pollution for coal-fired powerplants and all other Hg sources (Figure 4). I constructed buffers of 50, 100, 150 and 200-km to examine the amount of Hg pollution (lbs.) released around sampling sites.

I imported a raster file containing landcover types in Texas to assess percent of each of 5 habitat types: forest, grassland/prairie, urban, open water and other surrounding all fur collection locations (USGS 2016). I converted the raster file into vector data and used the model builder tool in ArcPro to assess habitat composition in 5-km buffers around each collection site. Sample collection sites included roosts, foraging sites, and

areas around wind energy facilities where bat fatalities occurred. I used a 5-km buffer to assess landscape composition around sampling sites because this area is large enough to encompass habitat that many species sampled would utilize for roosting and foraging in a given night. There are knowledge gaps regarding the nightly foraging bout distances for the species sampled in this study. Several species including *L. borealis*, *N. humeralis*, and *P. subflavus* have all been documented to forage, on average, within 5 km^2 of the roost each night (Clem 1993, Krishon et al. 1997, Helms 2011). I selected the percentage of forest surrounding a site as a covariate because forests sequester Hg primarily deposited from atmospheric deposition (Grigal 2003). I used the percentage of urban landscape surrounding each site to classify the surrounding landscape categorically as urban (> 50% urban within 5-km buffer) or rural (< 50% urban within 5-km buffer).

Statistical analysis

All statistical analyses were performed in RStudio (version 1.1.463; R Core Team, 2012) and significance was assessed at $\alpha \leq 0.05$. Sample sizes for *M. auriculus* and Seminole bat (*Lasiurus seminolus*) were small (n = 1 and n = 2, respectively) and omitted from all statistical analyses. Sampling locations with low sample sizes (n = 1) and near other sampling sites were pooled, resulting in 32 fur collection locations. In some instances, such as the majority of *P. subflavus* samples, I was unable to acquire life stage or sex data and only obtained species and collection location. Due to the variability of season, site, and data collected for each fur sample, I pooled samples by species and utilized every sample available to assess variability of THg concentrations among species. I first tested a one-way analysis of variance (ANOVA), but the assumptions of

homogeneity and normality were violated with untransformed and log-transformed data. I therefore used a Kruskal-Wallace ANOVA on Ranks followed by a Dunn's pairwise test comparison to examine variability in THg concentration among species. I further examined interspecific variability in THg concentrations by comparing the THg concentrations in males, females, juveniles, and adults among species. I first tested a one-way ANOVA with untransformed and log transformed data, however, the models violated assumptions, thus I used a Kruskal-Wallace ANOVA on Ranks followed by a Dunn's pairwise comparison test.

I assessed intraspecific variability of THg concentrations using a 2-tailed t-test for each biological between sex and life stage (juvenile/adult). I investigated the interaction of life stage and sex using a 2-factor ANOVA followed by a Tukey post-hoc test for each species. Total mercury concentrations were log-transformed to meet the assumptions of normality.

I assessed the variation in THg concentrations among sites for all bat species that had viable sample sizes at 3 or more sites. I collected adequate sample sizes to assess variation among sites for *T. brasiliensis* (n = 84), *N. humeralis* (n = 91) and *P. subflavus* (n = 67). I applied a one-way ANOVA with untransformed and log-transformed data for each species, but the assumptions of homogeneity and normality were violated for *T. brasiliensis* and *N. humeralis* data. Thus, I used a Kruskal-Wallace ANOVA on Ranks followed by a Dunn's pairwise comparison to assess variation of THg concentrations among sites for these species.

I used a hierarchical linear mixed effect model (LMER) using package "lme4" (Bates et al. 2007) to assess site level variation on THg concentrations for all species that

exhibited variation among sites. I assumed minimal variation of THg in fur samples collected across years and pooled samples across 2017–2019 because I collected the majority of *T. brasiliensis* and *N. humeralis* samples in 2018 and *P. subflavus* samples in 2019. I pooled samples across spring and fall seasons because I did not have enough samples per species to compare between seasons. I used a minimum of 5 samples of a single species per site as criteria for inclusion in a LMER. I tested additional LMER models for each species by assigning age and sex as fixed effects if data was collected at more than 2 sites. The random effect was site for all models. The response variable was THg concentration (μg/g) in bat fur, which I log-transformed to meet the assumption of heteroscedasticity. I tested a global model assessing fixed effects which included the percent of forest within a 5-km buffer around sampling site, landscape (urban or rural), sum of Hg emissions and distance to the nearest coal-fired powerplant and other Hg point sources. Before testing the global model, I first assessed which sum of Hg emission buffer (50, 100, 150, 200-km) to use in the global model and included that buffer as a fixed effect in the global model. In cases where age and sex data were collected for a species, the global model included age and sex as fixed effects. The final model used was:

$$Global.model = lmer(Hg \sim 1 + Hg\_emissions + coal + Hg\_source + landscape + forest\_5km + (1|site))$$

I used backwards selection to determine the best model for each species. I then compared the selected model against the global and null model and used the lowest AIC value to select the best model. I used package "MuMin" (Barton and Barton 2019) to calculate marginal and conditional R^2 values to examine how well the model explained the

variation in the data. I tested significance of the fixed effects by calculating 95% confidence intervals using a bootstrap method with 1,000 simulations.

Results

I collected 427 fur samples from 9 species and 32 sites across central and eastern Texas during 2017–2019 (Table 11). The majority of samples were collected from 3 species: *T. brasiliensis* (n = 115, 26.9%), *N. humeralis* (n = 101, 23.6%) and *P. subflavus* (n = 69, 16.1%). The Austin and San Antonio Metropolitan Areas had the most samples collected across 11 sites (n = 121, 28% of the total samples). Other regions with large sample sizes include 2 sites in south Texas (n = 96, 22.5% of the total samples), the upper Gulf Coast with 6 sites (n = 82, 19.2% of the total samples), and east Texas with 8 sites (n = 63, 14.7% of the total samples).

Interspecies variation in THg concentration

Total Hg concentrations differed among species ($H = 254.22_6$, $P = <0.001$) with *N. humeralis* and *P. subflavus* having greater concentrations than all other species. For all sites combined, mean THg concentrations (± standard deviation) were greatest in *P. subflavus* (6.04 ± 3.15 μg/g), followed by *N. humeralis* (5.87 ± 4.31 μg/g), *M. velifer* (2.17 ± 0.921 μg/g), *L. intermedius* (1.73 ± 1.58 μg/g), *T. brasiliensis* (1.02 ± 0.756 μg/g), *L. borealis* (0.971 ± 1.46 μg/g), and lowest in *L. cinereus* (0.812 ± 0.469 μg/g) (Table 11 and Figure 5). Total Hg concentrations assessed for all samples ranged from 0.067 to 20.1 μg/g. Two species had individuals with THg concentrations above the 10 μg/g threshold, which included 17% of *N. humeralis* (n = 16) and 9% of *P. subflavus* (n =

6). All *N. humeralis* samples > 10 μg/g, were collected on the upper Texas Gulf Coast at 3 sites including West Columbia (n = 8), Lake Jackson (n = 7), and SBNWR BP (n = 1). The *P. subflavus* samples > 10 μg/g, were all collected in east Texas at 4 sites including N Walker County (n = 3), S Walker County (n = 1), S Freestone County (n = 1), and S Leon County (n = 1).

Total Hg concentrations differed among juveniles from 5 species ($H = 41.628_4$, $P = <0.001$) as well as adults ($H = 117.79_4$, $P = <0.001$). Among juveniles, *N. humeralis* had the greatest THg concentration (4.29 ± 3.76 μg/g), followed by *M. velifer* (2.02 ± 0.840 μg/g), *L. intermedius* (1.88 ± 1.57 μg/g), *T. brasiliensis* (0.944 ± 0.519 μg/g), and *L. borealis* (0.753 ± 0.379 μg/g). Among adults, post-hoc tests revealed *N. humeralis* (7.45 ± 4.30 μg/g) had the greatest THg concentrations, then *M. velifer* (2.1 ± 0.857 μg/g), *L. intermedius* (1.65 ± 1.66 μg/g), *T. brasiliensis* (0.992 ± 0.668 μg/g), and *L. borealis* (0.551 ± 0.378 μg/g) had the lowest mean THg concentrations (Table 14). Interspecific variation was also observed among THg concentrations for females of 5 species ($H = 84.98_4$, $P = <0.001$) as well as males ($H = 71.356_4$, $P = <0.001$). Among females, *N. humeralis* (7.13 ± 4.94 μg/g) had the greatest THg concentrations, followed by *M. velifer* (2.03 ± 0.81 μg/g), *L. intermedius* (1.53 ± 1.29 μg/g), *T. brasiliensis* (0.827 ± 0.936 μg/g), and *L. borealis* (0.712 ± 0.440 μg/g) had the lowest THg mean concentrations. Among all males, *N. humeralis* (5.78 ± 3.72 μg/g) had the greatest THg concentrations, then *M. velifer* (2.15 ± 0.899 μg/g), *L. intermedius* (1.89 ± 2.03 μg/g), *T. brasiliensis* (1.02 ± 0.720 μg/g), and *L. borealis* (0.584 ± 0.224 μg/g) had the lowest mean THg concentrations (Table 15).

Intraspecies variation in THg concentrations

All sites were pooled to compare fur THg concentrations between life stages (Table 14) and sex (Table 15) for 5 bat species. Intraspecific variation in THg concentrations was observed between adults and juveniles for *N. humeralis* ($F = 9.842_{1,76}$, $P = 0.002$) (Figure 6); a post-hoc analysis revealed adult females had 3.5 times greater fur THg concentrations than juvenile females ($P = 0.013$) and 1.6 times greater than juvenile males ($P = 0.041$). Intraspecific variation in THg concentrations between life stage did not differ for the other 4 investigated species. Intraspecific variation between sex was not observed for any species.

Influence of site level factors to THg concentrations in three bat species

I compared mean THg concentrations among sites for 3 bat species (Figure 7). Total mercury concentrations differed among sites for *P. subflavus* ($F = 2.592_{6,60}$, $P = 0.0267$), *N. humeralis* ($H = 39.78_{5}$, $P = <0.001$), and *T. brasiliensis* ($H = 15.41_{4}$, $P = 0.004$). The results of the best candidate LMER models examining site level factors influencing THg concentrations for *P. subflavus, T. brasiliensis,* and *N. humeralis* are listed in Table 16. The selected LMER model for *N. humeralis* included the fixed effects of sum of Hg emissions within a 200-km buffer and distance to coal-fired power plant (AIC = 168.1; $w_i = 0.761$). Log-transformed THg concentrations increased as the sum of Hg emissions within a 200-km buffer increased and as the distance to coal-fired power plants increased. The sum of Hg emissions within a 200-km buffer was the most influential covariate ($F = 16.525_{1,88}$, $P < 0.001$) (Table 17). When age and sex were included as fixed effects for *N. humeralis,* the reduced model revealed age as a significant

fixed effect along with sum of Hg emissions within a 200-km buffer (AIC = 149.4; w_i = 0.216). Juvenile life stage had a negative effect on log-transformed THg concentrations and the sum of Hg emissions within a 200-km buffer continued to have a positive effect. The sum of Hg emissions within a 200-km buffer continued to be the most influential fixed effect ($F = 53.361_{1,4.19}$, $P = 0.0016$) (Table 17). The null model was the best candidate model for *P. subflavus* (AIC = 108.6; w_i = 0.540) with site location accounting for 14.1% of THg concentration variation observed among samples. The null model was selected for *T. brasiliensis* (AIC = 130.6; w_i = 0.998) with site accounting for 24.8% of the observed variation in THg concentrations among samples. When age and sex were included as fixed effects the global model (AIC = 126.8; w_i = 0.61) was ranked above the null (AIC = 127.7; w_i = 0.39), but the null model was within 2 AIC units.

Discussion

Interspecific variation in THg concentrations in Texas bats

Results demonstrated that Texas bats experienced interspecific variation in fur THg concentrations with *P. subflavus* and *N. humeralis* having significantly greater mean concentrations than *M. velifer*, *L. intermedius, T. brasiliensis, L. borealis,* and *L. cinereus*. Although the mean fur THg concentrations for all species assessed in this study were lower than the 10 μg/g threshold for negative health effects in bats (Nam et al. 2012), several individuals of *P. subflavus* (n = 6, 9% of samples) and *N. humeralis* (n = 16, 17% of samples) had fur THg concentrations > 10 μg/g. This suggests that within both species, some individuals could be experiencing negative health effects associated with Hg toxicity.

Similar to the results of Korstian et al. (2017) and Yates et al. (2014) *P. subflavus* had one of the greatest mean THg concentrations compared to other bat species. The mean fur THg concentrations of *P. subflavus* in this study was ~1.5 times greater than concentrations observed in a previous Texas study (Korstian et al. 2017), but substantially lower by ~6.5 times than the mean fur THg concentration observed in the northeastern US in Yates et al. (2014). However, Yates et al. (2014) combined samples collected at pollution point sources as well as non-point sources to calculate the mean THg concentration and samples taken from point sources were nearly 8 times greater than samples collected at non-point sources. The *P. subflavus* samples collected for this study were taken from roost sites that were not directly down stream of any known Hg pollution point sources, but all samples were collected within 75 km from an active coal-fired powerplant.

The negative health effects bats experience as a result of Hg toxicity have never been evaluated for *P. subflavus*. *Perimyotis subflavus* is one of the smallest bats in North America (Ammerman et al. 2012) resulting in less tissue mass to secrete and detoxify heavy metals. *Perimyotis subflavus* was petitioned to be federally listed under the Endangered Species Act (ESA) in June 2016 and is of special concern to Texas wildlife managers because of the recent discovery of Pd spreading in central Texas. The recent petition for *P. subflavus* to be listed under the ESA highlights the dangers this species is already experiencing from multiple stressors, even before THg concentrations were discovered to be high in this species compared to others, which subsequently may be responsible, in part, for deleterious health effects in some individuals with THg concentrations ≥ 10 μg/g.

The mean THg concentration reported in this study for *N. humeralis* is ~1.7 times greater than concentrations reported another study in Texas (Korstian et al. 2017). The greater concentrations observed in *N. humeralis* in this study compared to Korstian et al. (2017) could be explained by the larger sample size in this study (n = 101 vs n = 56) and the greater number of sites that I sampled (10 compared to 2), which are nearer to point sources of Hg pollution than the sites that Korstian et al. (2017) surveyed. Most of the fur samples for *N. humeralis* (74%) were collected from the upper Gulf Coast region which has numerous Hg pollution point sources including, but not limited to, coal-fired powerplants and petroleum refineries.

The 5 species sampled in this study that did not have any individuals exceeding the THg threshold of 10 μg/g followed similar patterns of being among the lowest mean THg concentrations observed when compared to other published studies. Similar to results from other Texas sites, mean THg concentrations for *T. brasiliensis*, *L. borealis*, and *L. cinereus* were among the lowest sampled (Korstian et al. 2017). Among the 10 species sampled in northeastern US by Yates et al. (2014), the lowest THg concentrations were observed in *L. borealis* and *L. cinereus*, although the mean concentration for *L. borealis* was nearly 4 times greater than the mean concentration observed in this study. The mean THg concentrations for *L. intermedius* in this study was ~2 times lower than the observed mean THg concentration at a site in south Texas by Korstian et al. (2017). Based on an extensive search, there is no record of fur THg concentrations in *M. velifer* in any published study. Land et al. (2019) examined Hg in guano from *M. velifer* in 5 central Texas caves and the greatest observed concentration was 0.41 μg/g. Other species in the *Myotis* genus, including *M. lucifugus* sampled in northeastern states by Yates et al.

(2014) and Minnesota by Korstian et al. (2017), and the Northern long-eared bat (*Myotis septentrionalis*) sampled in northeast US by Yates et al. (2014) and across Canada by Chételat et al. (2016), have some of the greatest mean THg concentrations among species and values that range 2 to 13 times greater than mean THg in fur observed for *M. velifer* in this study. It is unclear if *M. velifer* is less susceptible to accumulating dangerous concentrations compared to other *Myotis* species. The small number of sites with more than one sample (n = 2) and number of total fur samples (n = 49) should be considered when assessing risk of Hg toxicity for this species.

Although the majority of the variation in THg concentrations observed in this study occurred at the species level rather than the site level for *P. subflavus, N. humeralis,* and *T. brasiliensis,* each of these species exhibited variation of THg concentrations among sites. This suggests the factors involved with a bat species biology may be more influential on THg concentrations than the location of where the bat was sampled. Diet likely is driving a significant amount of the variation in THg concentrations among species (Becker et al. 2018). In 2 studies examining dietary guilds in tropical ecosystems, piscivorous bat species had the greatest THg concentrations, followed by insectivorous species, and frugivorous species had the lowest (Becker et al. 2018, Kumar et al. 2018). All the bats sampled in this study are insectivorous and display variation in prey preference. The diets of the 3 bats in the *Lasiurus* genus that were sampled in this study are primarily composed of moths from the order Lepidoptera (Rolseth et al. 1994, Clare et al. 2009). The life cycles of most moths occur in terrestrial habitats and are typically not connected to aquatic food webs. There is considerable overlap described by the diets of the other bats surveyed for this study. The diets of *P.*

subflavus, N. humeralis, M. velifer and *T. brasiliensis* are more diverse, including, but not limited to, the insect orders Coleoptera, Lepidoptera, Hymenoptera, Diptera, and Hemiptera (Griffith and Gates 1985, Kurta 2001, Carter et al. 2003, McWilliams 2005, Marquardt and Choate 2009). Due to the overlap in diets for these bats, it is possible other biological and behavioral factors such as foraging behavior, migration, metabolic rates, molting patterns, and life stage could be influencing the THg concentration variation observed among species.

Foraging range and non-migratory seasonal home range size could play a role in interspecific THg concentration variation because bats with larger home and foraging ranges could be consuming insects across a larger area including those further from Hg point sources. Foraging range refers to the distance a bat travels to forage during a nightly bout whereas non-migratory seasonal home range size is larger and describes the area a bat will occupy over the duration of its summer or winter non-migratory season. These factors are difficult to study effectively in bats and few studies have attempted to measure them for the species of interest in this study. Published foraging ranges for *P. subflavus* and *N. humeralis* are smaller compared to the other species sampled in this study. Studies that examined foraging range in *P. subflavus* estimated an area of 3.22–3.89 km^2 (Krishon et al. 1997, Helms 2011). The foraging range for *N. humeralis* has been described as less than 2.5 km^2 from the roost in an Indiana population (Clem 1993). The literature describing *N. humeralis* home range is scant, but one study estimated a home range of 0.15 km^2 for a population in Georgia (Krishon et al. 1997). The foraging range for *T. brasiliensis* is larger with the minimum distance in a foraging bout ranging from 15–56 km and encompassing an area as large as 4,000 km^2 (Ammerman et al. 2012). The

foraging range of *M. velifer* has been described as greater than other *Myotis* species owing to the larger body size and flight power. The home range of *M. velifer* was estimated to be 932–1619 km^2 in an Arizona population (Hayward 1970). Further research is needed to assess whether there is a relationship between THg concentration and foraging range and non-migratory seasonal home range size.

The variability in the migration patterns of bats could play a role in the differences observed for THg concentrations among species. All the bat species sampled in this study undergo some form of winter migration although the range and distances vary among species and populations. The general classifications of bat migration patterns include sedentary (year-round resident), regional migration (100–500 km) and long-distance migrations where species travel up to 2000 km between winter and summer ranges (Fleming and Eby 2003). Members of the *Lasiurus* genus and *T. brasiliensis* are classified as long-distance migrators with North American populations documented overwintering in regions of Mexico (Villa and Cockrum 1962, Glass 1982, Weller et al. 2016, Fleming 2019). *Perimyotis subflavus* is considered a regional migrant but demonstrates considerable intraspecific variability in the distances traveled among populations (Fraser et al. 2012). Members of the *Myotis* genus are often classified as regional migrants (Fleming 2019); *M. velifer* has documented populations that are regional migrants in Texas (Ammerman et al. 2012). Few published records exist that have documented *N. humeralis* migrations, but they indicate that *N. humeralis* would a regional migrant (Humphrey and Cope 1968). This pattern suggests that certain species that undergo a regional migration, including *N. humeralis, P. subflavus,* and members of the *Myotis* genus (such as *M. lucifugus* and *M. septentrionalis*), could be accumulating

greater THg concentrations than long-distance migrants such as *T. brasiliensis*, and members of the *Lasiurus* genus (Yates et al. 2014, Korstian et al. 2017).

Another consideration to explore regarding the interspecific variation observed in Texas bats is variability in metabolic rates and energetics among species. Metabolic rates measure the total energy metabolized over a unit of time and are typically lower in mammalian groups with larger body masses such as Primate and Carnivora compared to smaller groups like Insectivora (Hayssen and Lacy 1985, Elgar and Harvey 1987). Rodríguez-Durán (1995) provided evidence that diet, body mass, and roost microclimate are all important factors when assessing basal metabolic rate (BMR) in bats. Between 2 insectivorous species, the sooty mustached bat *(Pteronototus quadridens)* and the Antillean ghost-faced bat (*Mormoops blainvilli*) surveyed in Rodríguez-Durán (1995), BMR was ~1.3 times lower in *M. blainvilli*, which had nearly 2 times greater body mass. This could help explain why *P. subflavus* and *N. humeralis*, which have the lowest average body mass of the species sampled, had significantly higher THg concentrations than the other species. Metabolic rates are complex in bats since bats undergo a daily torpor with low metabolic activity followed by nocturnal volant movement, which requires a large amount of energy (Thomas and Suthers 1972, Speakman and Thomas 2003). Thus, cost of transport (COT) or the energy required to move a unit of mass a certain distance is likely a better method to compare energetics among species. The COT in small bats is typically greater than COT in larger bats (Norburg 1986, Speakman and Thomas 2003). Smaller bats have shorter muscles and wings which store less kinetic energy and require a higher COT to create enough inertia to overcome the drag experienced in flight (Speakman and Thomas 2003). Smaller bats, like *P. subflavus*,

could be exposing themselves to more Hg by ingesting a higher proportion of insects relative to their mass than larger species. There is a need for more research comparing the metabolic rate among insectivorous temperate bat species and the relationship with THg accumulation.

The timing of the molt, or period of earliest cellular activity before new hair growth and shedding of dead hair as defined by Ling (1972), could help explain interspecific variation of THg concentrations for the sampled individuals. The amount of scientific research on bat moulting patterns is scant, but it has been observed and widely accepted that most bats, with exceptions, undergo at least one annual moult during the summer and fall (Quay 1970, Fraser et al. 2013). This suggests THg concentrations from fur samples collected in late summer and fall are going to be representative of the Hg accumulated through the diet of the individual in the area the individual spent the summer. In comparison, fur samples collected in spring and early summer months are less representative of that region since the THg concentrations will be influenced by the migration patterns and location of the overwintering individual. The opportunistic method of fur sample collection for this study resulted in fur samples being collected across a range of years and seasons. Most fur samples were collected between summer 2018 and spring/summer 2019, however some fur samples from Starr and Hidalgo counties were collected in the fall of 2017. I did not assess season or year due to low sample sizes.

The relatively long lifespans of bats could be a factor influencing interspecific variability of THg concentrations. Bats have long lifespans for their size when compared to other taxon such as rodents (Austad and Fischer 1991). The age of a bat could be a

factor in THg accumulation because old bats would have a longer amount of time to accumulate THg resulting in a greater body burden of THg than younger bats. However, there is wide disparity among the average lifespans reported for the species sampled in this study and the mean THg concentrations observed. *Lasiurus cinereus* has a life span of 2 years which is the shortest average lifespan among the species sampled in this study, followed by *N. humeralis* with a reported life span of 2 to 5 years in the wild (Ammerman et al. 2012). There is no reported age for *L. intermedius,* but it is estimated that its life span is relatively short for an insectivorous bat species. In contrast, species including *P. subflavus, L. borealis, M. velifer* and *T. brasiliensis* all have documented lifespans exceeding 10 years in the wild (Ammerman et al. 2012). There is no effective way to age bats in the field beyond life stage at this time and age would be difficult to examine without a long-term study with permanently marked or banded individuals. While lifespan could be influencing some of the variability concerning THg concentrations among species, it is probably more influential for intra-specific variability when life stages are compared.

Intraspecific variation in THg concentrations in Texas bats

The results comparing intraspecific variability in THg concentrations for *N. humeralis* supported my prediction that adults have greater THg concentrations than juveniles and may be at greater risk of Hg toxicity, but it is unclear why this was not observed for other species. Adult bats among several species have been identified as having greater THg concentrations than juveniles (Yates et al. 2014, Chételat et al. 2016, Korstian et al. 2018). In Texas bats, Korstian et al. (2018) observed greater THg

concentrations in adults for all species sampled, but only adult *L. borealis* and *L. cinereus* were statistically different.

The discrepancy between studies observing variation between sexes and those that do not is unclear. Variation between fur THg concentrations and gender was not observed in this research similar to other studies (Chételat et al. 2016, Korstian et al. 2018). In contrast, Yates et al. (2014) reported female bats have greater THg concentrations than males. In 2 tropical studies, sex had a weak correlation with fur THg concentration (Becker et al. 2017, Becker et al. 2018). Location and seasonality of sampling in these studies could play a role in THg variations between sexes and requires further investigation. Gender related differences in diets could also be a factor, which Mata et al. (2016) observed in the insectivorous European free-tailed bat (*Tadarida teniotis*), but research is lacking for gender related dietary differences in North American species.

A possible explanation for not observing intraspecific variation in THg concentrations for any species other than *N. humeralis* in this study could be explained by the small sample sizes obtained for other species. The seasonality of sampling could also be an issue, because juveniles are captured in the mid-late summer and fall, which is the time frame when most of the *N. humeralis* samples were collected. This allowed me to acquire samples from newly volant *N. humeralis* juveniles that were unable to consume insects over a long period of time and exhibited a contrast to sampled adults that had the ability to accumulate Hg over a longer period of time. The age data collected for other species spanned longer time periods and could have included older juveniles capable of accumulating enough Hg to be indistinguishable from adults.

Several bat species including *P. subflavus*, *L. cinereus*, *M. velifer*, and *T. brasiliensis* have been documented displaying some intra-specific variability with moulting patterns (Fraser et al. 2013). Females have been recorded growing new fur later than males in *L. cinereus* (Cryan et al. 2004), *M. velifer* (Constantine 1957, Kunz. 1974), and *T. brasiliensis* (Constantine 1957). Fraser et al. (2012) used stable isotopes to assess moulting patterns in *P. subflavus* and was able to identify a time frame for male molting between June 23 and October 16 but was unable to identify a clear molting time for females, however they assumed the time frame was identical for both sexes. Variation in molting patterns between life stages have been observed in a few bat species such as the eastern water bat (*Myotis petax*) (Tiunov and Makarkiova 2007), and the little bent-wing bat (*Miniotperus australis*) (Dwyer 1968), whereas no difference was observed between life stages of other species like *M. myotis* (Mazak 1965). The literature on bat moulting patterns among different species is sparse and needs more research, but moulting patterns should be considered for future comparisons of fur THg concentrations among species, especially when bats are sampled near the timing of a known moult typically observed in late summer for most neo-tropical species (Fraser et al. 2013).

Assessing THg concentrations between life stages of bats could be biased by the age of the sampled individual. As noted previously, the lifespan of bats can have a broad range depending on species. Bats classified as adults could vary by several years in some species including *P. subflavus*, which can live up to 15 years in wild populations (Ammerman et al. 2012). While I was unable to collect data on life stage for this species, it would make a relevant candidate to assess the influence of age on THg concentrations

in a long-term monitoring study if individuals could be marked and roosts could be sampled on an annual basis.

Drivers of variation in THg concentration at the site level

The amount of Hg pollution released from point sources had a positive effect on THg concentrations in *N. humeralis*, suggesting that individuals occupying habitats in areas where more Hg pollution is released from point sources accumulate greater THg concentrations than bats residing in habitats with less pollution is released from point sources. This makes sense because the 200-km buffer I created to assess Hg pollution, overlaps with Harris county for 4 of the 6 sites assessed for *N. humeralis*, which is where the first and third greatest Hg polluting facilities in the state are located. Additionally, for *N. humeralis* distance to the nearest coal-fired powerplant had a positive impact suggesting that as the distance from coal-fired powerplants increases THg concentrations increases, which does not support my prediction that the distance to coal-fired powerplants would have a negative impact on THg concentrations. The low sample size of sites for *N. humeralis* and other factors occurring at the site level could be influencing this finding because bats captured at Hg pollution sources have been documented repeatedly with greater THg concentrations (Nam et al. 2012, Yates et al. 2014, Ferrante et al. 2018, Kumar et al. 2018). When I included life stage as a fixed effect in the *N. humeralis* LMER model, juvenile *N. humeralis* had a negative impact on THg concentrations, which suggests that populations of adult *N. humeralis* residing in areas with large amounts of Hg pollution are at the greatest risk for experiencing Hg toxicity for this species when concentrations exceed 10 μg/g threshold.

Although I observed variation of THg concentrations among sites for *N. humeralis, P. subflavus,* and *T. brasiliensis*; I only discovered site-level covariates for *N. humeralis* that explained some of the THg variation among sites. While my prediction that THg concentrations would vary among sites is supported by this finding, it is not clear why I did not discover any site-level covariates for *P. subflavus,* and *T. brasiliensis.* The most logical explanation is the small number of sites I could include in each model (n = 7 for *P. subflavus,* and n = 5 for *T. brasiliensis*). Another possible explanation is factors not assessed in the LMER models were contributing to the variation in THg concentration observed among sites. One factor not assessed is the amount of atmospheric deposition occurring at the sites, which has been reported to influence bat THg fur concentrations in Canada (Chételat et al. 2016). Future studies examining site-level factors to explain THg concentration variability should strive to have a larger number of sites sampled.

Future research

Future research should attempt to gain larger sample sizes, including samples in west Texas, and from more species to gain further insight into the current state of THg concentration in Texas bats. Future studies should assess stable isotopes ratios in Texas bats and their prey to gain insight into differences in dietary carbon source ($\delta^{13}C$) and trophic position ($\delta^{15}N$). The THg concentrations of the insects in foraging areas near sampling sites should also be examined to further the understanding of THg concentrations in bats and the relationship with diet. Diet is likely driving the accumulation of THg in Texas bats, therefore assessing the diets via fecal analysis and

trophic position of each bat sampled would provide more information about the specific diets that sampled bats are consuming. The foraging and non-migratory home range of bats may be influencing the THg concentrations among species as well, thus future research should evaluate whether bats with larger home and foraging ranges have lower concentrations of THg. Understanding the distances of a foraging bout for a sampled individual would improve our understanding on how foraging behavior might influence THg concentrations. Migration patterns in Texas bats is another area where more information is needed, specifically whether THg varies by season and if bats are accumulating greater concentrations in Texas summer locations or at their winter migration locations. Selenium, an essential element, binds to Hg making it biologically inert; however, Se:Hg molar ratios have never been assessed in bats but should be examined in future studies to provide insight on potential risk of toxic effects. The 10 μg/g threshold most bat Hg studies reference is based on a single study (Nam et al. 2012) and more research is needed to thoroughly understand how Hg effects the health of bats and whether the effects vary by species. Further, the impacts of the devastating disease WNS could be exacerbated through the immunosuppressive effects of Hg toxicity, which is why understanding the specific health effects of Hg toxicity in bats is so important.

Conclusion

This study assessed THg concentrations in fur from 7 species of Texas bats and is the first study to measure the THg concentration in fur samples collected from several regions in central and eastern parts of Texas where no data has been previously reported. This research illustrated that Texas bats exhibit interspecific variability in fur THg

concentrations. Results suggest 2 common bat species in Texas are at a greater risk of experiencing negative health effects related to Hg toxicity. Furthermore, I have demonstrated intraspecific variability in THg concentrations of *N. humeralis* and assessed that the amount of Hg pollution within a 200-km radius of a sampling location can impact the THg concentrations in this species. Managers should aim to monitor populations of *P. subflavus* and *N. humeralis* since these species are at greater risk of Hg toxicity. The findings of this study can be incorporated and utilized in future management and recovery plans for Texas bats.

Table 1. Observation-level covariates, site-level covariates, and the codes used in each occupancy model for a given year for 5 bat species on the San Bernard National Wildlife Refuge. Insects were only surveyed in 2019.

Covariate	Code	Year
Observation-level Covariates		
Julian Date	J.date	2018/2019
Precipitation (mm)	Precip	2018/2019
Relative humidity (%)	RH	2018/2019
Wind (mph)	Wind	2018/2019
Temperature (°C)	Temp	2018/2019
Modified Palmer Drought Severity Index	PMDI	2018/2019
Site-level Covariates		
Canopy cover (%)	Canopy	2018/2019
Vegetation height (m)	Veg	2018/2019
Columbia bottomland hardwood forest in 2-km buffer (%)	CBH	2018/2019
Open habitat in 2-km buffer (%)	Open	2018/2019
Urban habitat in 2-km buffer (%)	Urban	2018/2019
Distance to nearest city (km)	City	2018/2019
Insect abundance	Insect.A	2019
Insect richness	Insect.R	2019
Average abundance of Coleoptera	Coleoptera	2019
Average abundance of Culicidae	Culicidae	2019
Average abundance of Lepidoptera	Lepidoptera	2019
Average abundance of Diptera	Diptera	2019
Average abundance of Hemiptera	Hemiptera	2019

Table 2. Minimum (Min), maximum (Max), mean, standard deviation (SD), and year that each continuous site-level covariate was sampled for an assessment of bat occupancy on the San Bernard National Wildlife Refuge. ND = not determined due to data unavailable for several sites. Descriptions for each covariate are reported in Table 1.

	2018				2019			
Covariate	**Min.**	**Max.**	**Mean**	**SD**	**Min.**	**Max.**	**Mean**	**SD**
Canopy	0.00	96.11	25.06	31.69	0.00	100	38.92	42.16
Veg	0.51	1.71	0.99	3.56	0.64	2.00	1.22	3.63
PMDI	-0.55	-0.99	-0.72	0.15	2.30	2.91	2.72	0.20
City	4.94	31.28	19.94	6.59	4.94	31.28	19.94	6.59
CBH	0.00	98.69	59.81	38.25	0.00	98.69	59.81	38.25
Open	0.16	99.35	36.14	37.72	0.16	99.35	36.14	37.72
Urban	0.00	6.98	1.03	1.76	0.00	6.98	1.03	1.76
Insect Richness	ND	ND	ND	ND	4.00	8.00	6.14	0.99
Insect Abundance	ND	ND	ND	ND	29.00	513.00	232.68	120.40
Coleoptera	ND	ND	ND	ND	0.00	18.00	4.76	4.11
Culicidae	ND	ND	ND	ND	5.50	159.5	49.99	38.38
Diptera	ND	ND	ND	ND	0.00	16.00	3.97	4.32
Hemiptera	ND	ND	ND	ND	0.00	85.00	18.55	22.81
Lepidoptera	ND	ND	ND	ND	2.00	53.00	20.08	12.97

Table 3. The most supported single-season single-species occupancy models for 5 bat species detected on the San Bernard National Wildlife Refuge in 2018 including the number of parameters (K), Akaike's Information Criterion (AIC), ΔAIC, and the AIC weight (w_i). The most supported models illustrate the observation-level covariates that influence detection probability (*p)* and site-level covariates that impact the probability of occupancy (Ψ).

Models by Species	**K**	**AIC**	**ΔAIC**	w_i
T. brasiliensis				
Ψ(Canopy), *p*(PMDI)	4	183.56	0.00	0.61
Ψ(Canopy, City), *p*(PMDI)	5	184.49	0.93	1.00
Ψ(.), *p*(.)	2	213.48	29.91	1.00
L. borealis				
Ψ(.), *p*(PMDI)	3	224.53	0.00	0.95
Ψ(.), *p*(.)	2	230.48	5.95	1.00
N. humeralis				
Ψ(.), *p*(PMDI)	3	210.06	0.00	0.89
Ψ(.), *p*(.)	2	214.33	4.27	1.00
L. intermedius				
Ψ(.), *p*(PMDI)	3	220.99	0.00	1.00
Ψ(.), *p*(.)	2	237.92	16.93	1.00
P. subflavus				
Ψ(Veg), *p*(PMDI)	4	226.15	0.00	0.65
Ψ(.), *p*(PMDI)	3	228.10	1.95	0.90
Ψ(.), *p*(.)	2	229.90	3.75	1.00

Table 4. The most supported single-season single-species occupancy models for *T. brasiliensis* and single-season single-species multi-state occupancy models for *L. borealis*, *N. humeralis*, *L. intermedius*, and *P. subflavus* detected on the San Bernard National Wildlife Refuge in 2019 including the number of parameters (K), Akaike's Information Criterion (AIC), ΔAIC, and the AIC weight (w_i). The most supported models illustrate the observation-level covariates that influence detection probability (*p)* and site-level covariates that impact the probability of occupancy (Ψ) and high activity (R).

Models by Species	**K**	**AIC**	**ΔAIC**	w_i
T. brasiliensis				
Ψ(PMDI), *p*(Precip)	4	219.13	0.00	1.00
Ψ(.), *p*(.)	2	290.10	70.97	1.00
L. borealis				
Ψ(.), R(Hemiptera, Open), *p1*(.), *p2*(.)	7	247.90	0.00	0.72
Ψ(.), R(Hemiptera), *p1*(.), *p2*(.)	6	250.03	2.13	0.97
Ψ(.), R(.), *p1*(.), *p2*(.)	5	254.44	6.54	1.00
N. humeralis				
Ψ(.), R(City), *p1*(.), *p2*(.)	6	297.96	0.00	0.74
Ψ(.), R(Open), *p1*(.), *p2*(.)	6	300.10	2.15	0.99
Ψ(.), R(.), *p1*(.), *p2*(.)	5	306.36	8.40	1.00
L. intermedius				
Ψ(.), R(.), *p1*(.), *p2*(J.date)	6	314.44	0.00	0.93
Ψ(.), R(.), *p1*(.), *p2*(.)	5	319.77	5.33	1.00
P. subflavus				
Ψ(.), R(Coleoptera, Open), *p1*(J.date), *p2*(J.date)	10	298.59	0.00	0.71
Ψ(.), R(Coleoptera, Open, Urban), *p1*(J.date), *p2*(J.date)	11	300.40	1.80	0.99
Ψ(.), R(.), *p1*(.), *p2*(.)	5	307.60	9.00	1.00

Table 5. The untransformed coefficient values (Coeff.), standard errors (SE) and lower and upper 95% confidence intervals (LCI, UCI, respectfully) for all site-level coefficients included in the most supported single-season single-species occupancy model of each species in 2018.

Variables by Species	Coeff.	SE	LCI	UCI
T. brasiliensis				
Ψ (Intercept)	2.42	1.17	0.14	4.71
Ψ (Canopy)	-2.47	1.19	-4.79	-0.14
p (Intercept)	0.34	0.19	-0.03	0.71
p (PMDI)	-0.82	0.22	-1.26	-0.38
L. borealis				
Ψ (Intercept)	2.90	1.04	0.87	4.93
p (Intercept)	0.30	0.16	-0.02	0.62
p (PMDI)	-0.42	0.18	-0.77	-0.06
N. humeralis				
Ψ (Intercept)	1.82	0.72	0.41	3.22
p (Intercept)	-0.53	0.19	-0.89	-0.17
p (PMDI)	-0.46	0.18	-0.81	-0.10
L. intermedius				
Ψ (Intercept)	2.90	1.03	0.87	4.92
p (Intercept)	0.19	0.17	-0.14	0.52
p (PMDI)	-0.66	0.20	-1.05	-0.27
P. subflavus				
Ψ (Intercept)	4.16	2.97	-1.66	9.99
Ψ (Veg)	2.80	2.64	-2.37	7.97
p (Intercept)	0.27	0.17	-0.06	0.59
p (PMDI)	-0.34	0.18	-0.69	0.02

Table 6. The untransformed coefficient values (Coeff), standard errors (SE) and lower and upper 95% confidence intervals (LCI, UCI, respectfully) for all coefficients included for the most supported single-season single-species occupancy models of 5 bat species surveyed on San Bernard National Wildlife Refuge in 2019.

Variables by Species	Coeff.	SE	LCI	UCI
T. brasiliensis				
Ψ (Intercept)	2.21	1.33	-0.39	4.81
Ψ (PMDI)	-2.62	1.64	-5.84	0.59
p (Intercept)	-0.50	0.18	-0.85	-0.16
p (Precip)	-0.14	0.21	-0.55	0.28
L. borealis				
Ψ (Intercept)	2.41	1.39	-0.33	5.13
R (Intercept)	-3.07	2.14	-7.26	1.13
R (Hemiptera)	4.04	5.13	-6.01	14.08
R (Open)	-3.42	3.22	-9.74	2.89
p1 (Intercept)	-1.36	0.26	-1.87	-0.85
p2 (Intercept)	0.60	0.39	-0.17	1.38
δ (Intercept)	-1.38	0.51	-2.39	-0.38
N. humeralis				
Ψ (Intercept)	1.56	0.66	0.26	2.85
R (Intercept)	1.15	0.97	-0.75	3.05
R (City)	-7.87	4.36	-16.42	0.67
p1 (Intercept)	-1.43	0.32	-2.05	-0.81
p2 (Intercept)	0.66	0.24	0.18	1.13
δ (Intercept)	-0.20	0.28	-0.76	0.35
L. intermedius				
Ψ (Intercept)	2.83	1.27	0.35	5.32
R (Intercept)	-0.85	0.50	-1.82	0.13
p1 (Intercept)	-0.89	0.22	1.31	-0.46
p2 (Intercept)	0.73	0.34	0.06	1.40
p2 (J.date)	0.75	0.32	0.13	1.37
δ (Intercept)	-0.12	0.36	-0.83	0.60
P. subflavus				
Ψ (Intercept)	1.717	0.66	0.42	3.02
R (Intercept)	-0.45	0.77	-1.96	1.05
R (Open)	-2.53	1.36	-5.18	0.13
R (Coleoptera)	2.86	1.68	-0.43	6.15
p1 (Intercept)	-1.15	0.35	-1.83	-0.46
p1 (J.date)	-0.67	0.32	-1.29	-0.05
p2 (Intercept)	1.19	0.39	1.11	2.64
p2 (J.date)	-0.55	0.35	-1.23	0.12
δ (Intercept)	-0.37	0.29	-0.93	0.19
δ (J.date)	-1.04	0.26	-1.74	-0.33

Table 7. Model averaged coefficients (Coeff), standard errors (SE), and lower and upper 95% confidence intervals (LCI, UCI, respectfully) for all site-level covariates included in competitive occupancy models for bats surveyed on the San Bernard National Wildlife Refuge in 2018.

Variables by Species	**Coeff.**	**SE**	**LCI**	**UCI**
T. brasiliensis				
Canopy	-2.45	1.22	-4.85	-0.06
City	0.98	1.04	-1.05	3.02
P. subflavus				
Veg	2.80	2.64	-2.37	7.97

Table 8. Model averaged coefficients (Coeff), standard errors (SE), and lower and upper 95% confidence intervals (LCI, UCI, respectfully) for all site-level covariates included in competitive occupancy models for bats surveyed on the San Bernard National Wildlife Refuge in 2019.

Variables by Species	Coeff.	SE	LCI	UCI
T. brasiliensis				
PMDI	-2.62	1.64	-5.84	0.59
L. borealis				
Hemiptera	2.71	3.77	-4.68	10.09
Open	-3.42	3.22	-9.74	2.90
N. humeralis				
City	-7.87	4.36	-16.42	0.67
Open	-1.81	0.82	-3.42	-0.19
P. subflavus				
Coleoptera	2.85	1.68	-0.44	6.14
Open	-2.52	1.36	-5.18	0.14
Urban	0.54	1.44	-2.28	3.35

Table 9. Back-transformed mean and standard errors (SE) estimates for detection probability (*p*) and occupancy probability (Ψ) for all parameters contained in competitive and null models for 5 bat species surveyed on San Bernard National Wildlife Refuge in 2018.

Species	Model	Parameter	Mean	SE
T. brasiliensis	Ψ(.), *p*(.)	Ψ (Intercept)	0.79	0.09
		p (Intercept)	0.59	0.04
	Ψ(Canopy), *p*(PMDI)	Ψ (Intercept)	0.92	0.09
		Ψ (Canopy)	0.08	0.09
		p (Intercept)	0.11	0.06
		p (PMDI)	0.03	0.02
	Ψ(Canopy, City), *p*(PMDI)	Ψ (Intercept)	0.94	0.08
		Ψ (Canopy)	0.09	0.10
		Ψ (City)	0.73	0.21
		p (Intercept)	0.58	0.05
		p (PMDI)	0.31	0.05
L. borealis	Ψ(.), *p*(.)	Ψ (Intercept)	0.95	0.05
		p (Intercept)	0.58	0.04
	Ψ(.), *p*(PMDI)	Ψ (Intercept)	0.95	0.05
		p (Intercept)	0.28	0.10
		p (PMDI)	0.15	0.08
N. humeralis	Ψ(.), *p*(.)	Ψ (Intercept)	0.86	0.09
		p (Intercept)	0.38	0.04
	Ψ(.), *p*(PMDI)	Ψ (Intercept)	0.86	0.09
		p (Intercept)	0.37	0.04

		p (PMDI)	0.39	0.04
L. intermedius	Ψ(.), *p*(.)	Ψ (Intercept)	0.95	0.05
		p (Intercept)	0.54	0.04
	Ψ(.), *p*(PMDI)	Ψ (Intercept)	0.95	0.05
		p (Intercept)	0.55	0.04
		p (PMDI)	0.34	0.04
P. subflavus	Ψ(.), *p*(.)	Ψ (Intercept)	0.90	0.07
		p (Intercept)	0.57	0.04
	Ψ(Veg), *p*(PMDI)	Ψ (Intercept)	0.99	0.05
		Ψ (Veg)	0.94	0.14
		p (Intercept)	0.35	0.11
		p (PMDI)	0.22	0.11
	Ψ(.), *p*(PMDI)	Ψ (Intercept)	0.90	0.07
		p (Intercept)	0.35	0.11
		p (PMDI)	0.22	0.11

Table 10. Back-transformed mean and standard errors (SE) estimates for all parameters contained in competitive and null occupancy and multi-state occupancy models for 5 bat species surveyed on San Bernard National Wildlife Refuge in 2019. Parameters included detection probability (*p*), probability of detecting species in state 1 given true state was 1 (*p1*), probability of detecting species in state 2 given true state was 2 (*p2*), probability of correctly detecting state 2 given species was detected (δ), probability of occupancy rate (Ψ), and probability that high activity was observed given bat was detected (R).

Species	Model	Parameter	Mean	SE
T. brasiliensis	Ψ(.), *p*(.)	Ψ (Intercept)	0.78	0.09
		p (Intercept)	0.32	0.03
	Ψ(PMDI), *p*(Precip)	Ψ (Intercept)	0.90	0.12
		Ψ (PMDI)	0.07	0.10
		p (Intercept)	0.38	0.04
		p (Precip)	0.47	0.05
L. borealis	Ψ(.), R(.), *p1*(.), *p2*(.)	Ψ (Intercept)	0.92	0.11
		R (Intercept)	0.23	0.11
		p1 (Intercept)	0.20	0.05
		p2 (Intercept)	0.63	0.10
		δ (Intercept)	0.23	0.11
	Ψ(.), R(Hemiptera, Open), *p1*(.), *p2*(.)	Ψ (Intercept)	0.92	0.11
		R (Intercept)	0.05	0.09
		R (Hemiptera)	0.98	0.09
		R (Open)	0.03	0.10
		p1 (Intercept)	0.20	0.04
		p2 (Intercept)	0.65	0.09
		δ (Intercept)	0.20	0.08
	Ψ(.), R(Hemiptera), *p1*(.), *p2*(.)	Ψ (Intercept)	0.94	0.12

L. borealis		R (Intercept)	0.15	0.10
		R (Hemiptera)	0.82	0.12
		p1 (Intercept)	0.20	0.04
		p2 (Intercept)	0.66	0.08
		δ (Intercept)	0.20	0.08
N. humeralis	Ψ(.), R(.), *p1*(.), *p2*(.)	Ψ (Intercept)	0.84	0.11
		R (Intercept)	0.49	0.13
		p1 (Intercept)	0.19	0.05
		p2 (Intercept)	0.66	0.06
		δ (Intercept)	0.45	0.07
	Ψ(.), R(City), *p1*(.), *p2*(.)	Ψ (Intercept)	0.83	0.10
		R (Intercept)	0.76	0.18
		R (City)	4.0e-4	2.0e-3
		p1 (Intercept)	0.19	0.05
		p2 (Intercept)	0.66	0.06
		δ (Intercept)	0.45	0.07
	Ψ(.), R(Open), *p1*(.), *p2*(.)	Ψ (Intercept)	0.82	0.98
		R (Intercept)	0.51	0.18
		R (Open)	0.14	0.10
		p1 (Intercept)	0.19	0.05
		p2 (Intercept)	0.66	0.06
		δ (Intercept)	0.45	0.07
L. intermedius	Ψ(.), R(.), *p1*(.), *p2*(.)	Ψ (Intercept)	0.94	0.07
		R (Intercept)	0.30	0.11
		p1 (Intercept)	0.29	0.05
		p2 (Intercept)	0.60	0.07
		δ (Intercept)	0.47	0.09

L. intermedius	Ψ(.), R(.), *p1*(.), *p2*(J.date)	Ψ (Intercept)	0.94	0.07
		R (Intercept)	0.30	0.11
		p1 (Intercept)	0.29	0.05
		p2 (Intercept)	0.68	0.08
		p2 (J.date)	0.68	0.07
		δ (Intercept)	0.47	0.09
P. subflavus	Ψ(.), R(.), *p1*(.), *p2*(.)	Ψ (Intercept)	0.83	0.08
		R (Intercept)	0.41	0.12
		p1 (Intercept)	0.37	0.06
		p2 (Intercept)	0.87	0.04
		δ (Intercept)	0.40	0.07
	Ψ(.), R(Coleoptera, Open), *p1*(J.date), *p2*(J.date)	Ψ (Intercept)	0.85	0.09
		R (Intercept)	0.39	0.18
		R (Open)	0.07	0.09
		R (Coleoptera)	0.95	0.09
		p1 (Intercept)	0.24	0.06
		p1 (J.date)	0.39	0.07
		p2 (Intercept)	0.87	0.05
		p2 (J.date)	0.37	0.08
		δ (Intercept)	0.41	0.07
		δ (J.date)	0.26	0.07
	Ψ(.), R(Coleoptera, Open, Urban), *p1*(J.date), *p2*(J.date)	Ψ (Intercept)	0.85	0.09
		R (Intercept)	0.43	0.22
		R (Open)	0.10	0.13
		R (Coleoptera)	0.93	0.12
		R (Urban)	0.63	0.33
		p1 (Intercept)	0.24	0.06

P. subflavus	*p1* (J.date)	0.34	0.07
	p2 (Intercept)	0.87	0.05
	p2 (J.date)	0.37	0.08
	δ (Intercept)	0.41	0.07
	δ (J.date)	0.26	0.07

Table 11. Specific sampling locations ordered north to south, total bats sampled (n) by site and for all sites combined, and fur THg concentrations [median, mean, standard deviation (SD) and range; μg/g] for all bats sampled from 2017–2019 across central and eastern Texas, USA. ND = not determined due to small sample size; SBNWR BP = San Bernard National Wildlife Refuge Brothers Pond.

Species	Site	n	Median	Mean	SD	Min.	Max.
L. borealis	All sites combined	27	0.598	0.971	1.46	0.067	8.00
	Mineral Wells	3	0.793	0.673	0.113	0.591	0.802
	Palestine	1	8.00	8.00	ND	8.00	8.00
	Bell County	5	0.589	0.656	0.224	0.424	0.972
	Austin	6	0.492	0.472	0.121	0.295	0.598
	San Marcos	2	0.702	0.702	0.479	0.363	1.04
	SBNWR BP	3	1.10	0.788	0.626	0.067	1.20
	Lake Jackson	2	1.38	1.38	0.416	1.08	1.67
	Starr County	2	0.147	0.147	0.0660	0.101	0.194
	Unknown	3	1.10	1.115	0.0884	1.04	1.21
L. cinereus	Starr County	8	0.699	0.809	0.469	0.242	1.47
L. intermedius	All sites combined	54	1.27	1.73	1.58	0.240	9.56
	Austin	3	0.698	3.55	5.22	0.381	9.58
	Houston	3	0.567	1.21	1.13	0.552	2.51
	Starr County	22	1.17	1.32	0.806	0.244	2.76
	Hidalgo County	26	1.27	1.62	1.16	0.373	5.13
L. seminolus	All sites combined	2	2.17	2.17	0.104	2.09	2.24
	Houston	1	2.24	2.24	ND	2.24	2.24
	Unknown	1	2.09	2.09	ND	2.09	2.09
M. auriculus	S Walker County	1	8.95	8.95	ND	8.95	8.95
M. velifer	All sites combined	49	1.92	2.17	0.921	0.350	4.95

	Johnson City	21	1.83	2.11	0.956	0.788	4.68
	Austin	1	2.53	2.53	ND	2.53	2.53
	San Marcos	25	2.02	2.19	0.877	1.09	4.95
	Hidalgo County	1	0.346	0.346	ND	0.346	0.346
	Unknown	1	1.40	1.40	ND	1.40	1.40
N. humeralis	All sites combined	101	4.49	5.87	1.60	0.163	18.8
	Georgetown	1	0.757	0.757	ND	0.757	0.757
	San Marcos	1	3.48	3.48	ND	3.48	3.48
	West Columbia	41	4.75	6.18	3.90	1.90	15.8
	SBNWR BP	5	5.68	6.53	4.29	2.42	13.5
	Brazoria	12	6.70	6.69	1.60	4.12	9.3
	Lake Jackson	15	9.66	10.8	4.85	3.05	18.8
	SBNWR Office	2	3.47	3.47	1.99	2.07	4.87
	Houston	2	1.78	1.78	1.57	0.675	2.89
	Starr County	6	2.05	2.31	1.01	1.33	4.24
	Hidalgo County	12	1.81	2.00	1.22	0.466	3.91
	Unknown	4	1.43	2.97	3.99	0.165	8.83
P. subflavus	All sites combined	69	5.83	6.04	3.15	1.39	20.1
	N Freestone County	10	4.38	4.13	1.78	1.39	6.62
	S Freestone County	10	6.09	6.16	2.13	2.56	10.1
	N Leon County	10	6.14	5.38	1.82	1.78	7.39
	S Leon County	10	5.30	5.74	2.84	2.17	12.4
	N Walker County	10	7.34	8.78	5.17	3.53	20.1
	S Walker County	10	6.58	7.21	2.78	3.34	13.2
	Austin	7	4.21	4.85	2.69	1.43	8.82
	San Marcos	1	2.82	2.82	ND	2.82	2.82

	Unknown	1	5.83	5.83	ND	5.83	5.83
T. brasiliensis	All sites combined	115	0.827	1.02	0.756	0.320	5.82
	Mineral Wells	22	0.794	0.926	0.528	0.400	2.35
	Tom Green County	3	0.836	1.05	0.300	0.776	1.37
	Lavender	1	1.83	1.83	ND	1.83	1.83
	Bell County	1	1.35	1.35	ND	1.35	1.35
	Granger	1	0.679	0.679	ND	0.679	0.679
	Round Rock	3	0.918	0.784	0.218	0.532	0.918
	Largo Vista	1	1.45	1.45	ND	1.45	1.45
	Austin	31	0.832	1.03	0.580	0.403	2.53
	Johnson City	8	1.06	1.46	0.977	0.794	3.52
	Dripping Springs	1	0.450	0.450	ND	0.450	0.450
	N Hays County	2	0.732	0.802	0.409	0.541	1.12
	San Marcos	1	0.450	0.450	ND	0.450	0.450
	Lockhart	1	0.785	0.785	ND	0.785	0.785
	Comal County	5	0.840	0.778	0.267	0.496	1.08
	Brazoria	2	3.87	3.87	2.76	1.92	5.82
	Starr County	15	0.509	0.573	0.170	0.319	0.905
	Hidalgo County	3	1.47	1.38	0.383	0.966	1.72
	Unknown	13	0.843	1.12	0.889	0.374	3.69

Table 12. THg concentrations (µg/g) from the bat fur cleaning experiment comparing 5 cleaning methods ($F = 0.197$; $df = 4,70$; $P = 0.939$).

Treatment	Median	Mean	SD	Min.	Max.
Unclean control	1.06	1.06	0.504	0.394	2.09
Acetone	1.22	1.22	0.542	0.378	2.44
Chloroform	1.15	1.17	0.597	0.371	2.77
Detergent	1.21	1.22	0.571	0.279	2.56
DI water rinse	1.22	1.14	0.606	0.242	2.42

Table 13. Sample size and percent of each sex (male/female) and life stages (adult/juvenile) collected for 5 bat species from 2017–2019 across 32 sites in Texas, USA. ND = not determined because data was not collected in field.

		Sex						Life Stage					
Species	**Site**	**M**	**F**	**ND**	**%M**	**%F**	**%ND**	**J**	**A**	**ND**	**%J**	**%A**	**%ND**
L. borealis	All sites combined	8	15	4	30	55	15	13	9	5	48	33	19
	Mineral Wells	1	2	-	33	67	0	1	2	-	33	67	0
	Palestine	-	-	1	0	0	100	-	-	1	0	0	100
	Bell County	2	3	-	40	60	0	5	-	-	100	0	0
	Austin	3	3	-	50	50	0	4	2	-	67	33	0
	San Marcos	1	1	-	50	50	0	-	2	-	0	100	0
	SBNWR BP	-	3	-	0	100	0	1	2	-	33	67	0
	Lake Jackson	-	2	-	0	100	0	2	-	-	100	0	0
	Starr County	1	1	-	50	50	0	-	1	1	0	50	50
	Unknown	-	-	3	0	0	100	-	-	3	0	0	100
L. intermedius	All sites combined	22	26	6	41	28	11	10	38	6	19	70	11
	Austin	1	2	-	33	67	0	-	3	-	0	1.0	0
	Houston	2	1	-	67	33	0	3	-	-	1.0	0	0
	Starr County	10	10	2	45	45	10	1	19	2	4	86	10
	Hidalgo County	9	13	4	35	50	15	6	16	4	23	62	0.15
M. velifer	All sites combined	17	20	11	35	41	22	9	29	10	18	59	20
	Johnson City	2	9	10	10	42	48	-	11	10	0	53	48
	Austin	-	1	-	0	100	0	-	1	-	0	100	0
	San Marcos	15	10	-	60	40	0	9	16	-	36	64	0
	Hidalgo County	-	-	1	0	0	100	-	1	-	0	100	0
	Unknown	-	-	1	0	0	100	-	-	1	0	0	1
N. humeralis	All sites combined	42	38	21	42	38	21	26	54	21	26	53	21

	Georgetown	-	-	1	0	0	1.0	-	-	1	0	0	100
	San Marcos	-	-	1	0	0	100	-	-	1	0	0	100
	West Columbia	25	16	-	61	39	0	21	20	-	51	49	0
	SBNWR BP	2	2	1	4	4	2	1	3	1	20	60	20
	Brazoria	4	4	4	33	33	33	-	8	4	33	67	0
	Lake Jackson	5	10	-	33	67	0	-	15	-	0	100	0
	SBNWR Office	-	-	2	0	0	100	-	-	2	0	0	100
	Houston	-	-	2	0	0	100	-	-	2	0	0	100
	Starr County	2	2	2	33	33	33	-	4	2	0	67	33
	Hidalgo County	4	4	4	33	33	33	4	4	4	33	33	33
	Unknown	-	-	4	0	0	100	-	-	4	0	0	100
T. brasiliensis	All sites combined	34	39	42	30	33	37	24	49	42	21	42	37
	Austin	18	13	-	58	42	0	7	24	-	23	77	0
	Bell County	-	-	1	0	0	100	-	-	1	0	0	100
	Mineral Wells	5	17	-	23	77	0	16	6	-	73	27	0
	Tom Green County	-	-	3	0	0	100	-	-	3	0	0	100
	Lavender	-	-	1	0	0	100	-	-	1	0	0	100
	Bell County	-	-	1	0	0	100	-	-	1	0	0	100
	Granger	-	-	1	0	0	100	-	-	1	0	0	100
	Round Rock	-	-	3	0	0	100	-	-	1	0	0	100
	Largo Vista	-	-	1	0	0	100	-	-	1	0	0	100
	Austin	18	13	-	58	42	0	7	24	-	23	77	0
	Johnson City	1	4	3	1	50	37	-	5	3	0	62	38
	Dripping Springs	-	-	1	0	0	100	-	-	1	0	0	100
	N Hays County	-	-	2	0	0	100	-	-	2	0	0	100
	San Marcos	-	-	1	0	0	100	-	-	1	0	0	100

Lockhart	-	-	1	0	0	100	-	-	1	0	0	100
Comal County	-	-	5	0	0	100	-	-	5	0	0	100
Brazoria	-	-	2	0	0	100	-	-	2	0	0	100
Starr County	8	5	2	53	33	13	-	13	2	0	87	13
Hidalgo County	2	-	1	67	33	0	1	1	1	33	33	33
Unknown	-	-	13	0	0	100	-	-	13	0	0	100

Table 14. Fur THg concentrations broken down by life stages (juvenile/adult) for 5 bat species sampled across central and eastern Texas, USA from 2017–2019. Sample size of each life stage collected at a specific location are reported in Table 13.

	Juvenile						Adult					
	n	**Median**	**Mean**	**SD**	**Min.**	**Max.**	**n**	**Median**	**Mean**	**SD**	**Min.**	**Max.**
L. borealis	13	0.587	0.753	0.379	0.389	1.67	9	0.591	0.551	0.378	0.0674	1.10
L. intermedius	10	1.40	1.88	1.57	0.244	4.81	38	1.25	1.65	1.66	0.358	9.58
M. velifer	9	1.73	2.02	0.840	1.18	3.56	28	2.10	2.10	0.857	0.789	4.95
N. humeralis	26	3.14	4.29	3.76	0.467	14.1	54	7.10	7.45	4.30	1.33	18.8
T. brasiliensis	24	0.794	0.944	0.519	0.401	2.35	49	0.737	0.992	0.668	0.319	3.52

Table 15. Fur THg concentrations (µg/g) by sex for 5 bat species that were sampled across central and eastern Texas from 2017–2019. Sample size of each sex collected at a specific location are reported in Table 13.

	Male						Female					
	n	**Median**	**Mean**	**SD**	**Min.**	**Max.**	**n**	**Median**	**Mean**	**SD**	**Min.**	**Max.**
L. borealis	8	0.587	0.584	0.224	0.389	1.04	15	0.591	0.712	0.440	0.0674	1.67
L. intermedius	22	1.28	1.89	2.03	0.549	9.58	26	1.15	1.53	1.19	0.244	5.14
M. velifer	17	2.12	2.15	0.899	1.09	4.95	20	1.80	2.03	0.810	0.788	3.56
N. humeralis	42	4.62	5.78	3.72	0.467	14.1	38	5.86	7.13	4.94	0.902	18.8
T. brasiliensis	34	0.726	1.02	0.720	0.401	3.52	39	0.827	0.936	0.523	0.319	2.36

Table 16. Predictive linear mixed-effects regressions (LMER) models assessing the impact of site-level covariates on the THg concentrations for *P. subflavus***,** *N. humeralis and T. brasiliensis* sampled from 2017–2019 across central and eastern Texas, USA. Additional LMER models that included age and sex as fixed effects are included for *N. humeralis,* and *T. brasiliensis*. Models are ranked by corrected Akaike Information Criterion (AIC) value with the degrees of freedom (df), AIC weight (w_i), marginal ($R^2_{m)}$ and conditional (R^2_c) R^2.

Species	**Log THg models**	***df***	**AIC**	w_i	$\mathbf{R^2_m}$	$\mathbf{R^2_c}$
P. subflavus (n = 67)	~ (1|site.p)	3	108.6	0.54	0.00	0.14
	~ 1 + forest + (1|site.p)	4	109.7	0.32	0.11	0.14
	Global model	8	111.3	0.14	0.19	0.20
N. humeralis (n = 91)	~1 + coal + Hg_emissions + (1|site.n)	5	168.1	0.68	0.47	0.47
	~1 + coal+ Hg_source + Hg_emissions + (1|site.n)	6	169.9	0.27	0.47	0.48
	Global model	8	173.5	0.05	0.47	0.47
	~ (1|site.n)	3	178.2	0.00	0.00	0.57
N. humeralis + age + sex (n = 83)	~1 + age + emissions + (1|site.n)	5	149.4	0.22	0.51	0.52
	~1 + age + Hg_emissions + forest + (1|site.n)	6	149.4	0.21	0.54	0.54
	~1 + age + Hg_emissions + forest + Hg_source.n + sex + (1|site.n)	8	149.6	0.19	0.52	0.52
	~1 + age + Hg_emissions + forest + Hg_source.n + coal + sex + (1|site.n)	9	149.7	0.19	0.52	0.52
	Global model	10	150.8	0.12	0.15	0.86
	~1 + age + Hg_emissions + forest + Hg_source.n + (1|site.n)	7	151.1	0.09	0.54	0.54
	~ (1|site.n)	3	178.2	0.00	0.00	0.57
T. brasiliensis (n = 84)	~ 1 + (1|site.t)	3	130.6	0.99	0.00	0.25
	Global model	8	139.5	0.01	0.09	0.66
T. brasiliensis + age + sex (n = 80)	Global model	10	126.8	0.61	0.19	0.38
	~ 1 + (1|site.t)	3	127.7	0.39	0.00	0.32

Table 17. Significance for coefficients of the selected LMER models describing THg variation in *N. humeralis* with and without age and sex as fixed effects sampled from 2017–2019 across central and eastern Texas, USA.

Species	Variable	Coeff.	SE	LCI	UCI	T Value	P value
N. humeralis	intercept	1.57	0.06	1.45	1.68	26.57	<0.001
	coal	1.96	0.60	0.78	3.20	3.25	0.002
	Hg_emissions	2.46	0.60	1.27	3.72	4.07	<0.001
N. humeralis	intercept	1.73	0.08	1.57	1.89	21.72	<0.001
+ age + sex	Hg_emissions	0.52	0.07	0.39	0.66	7.31	0.002
	age.Juvenile	-0.60	0.13	-0.87	-0.34	-4.59	<0.001

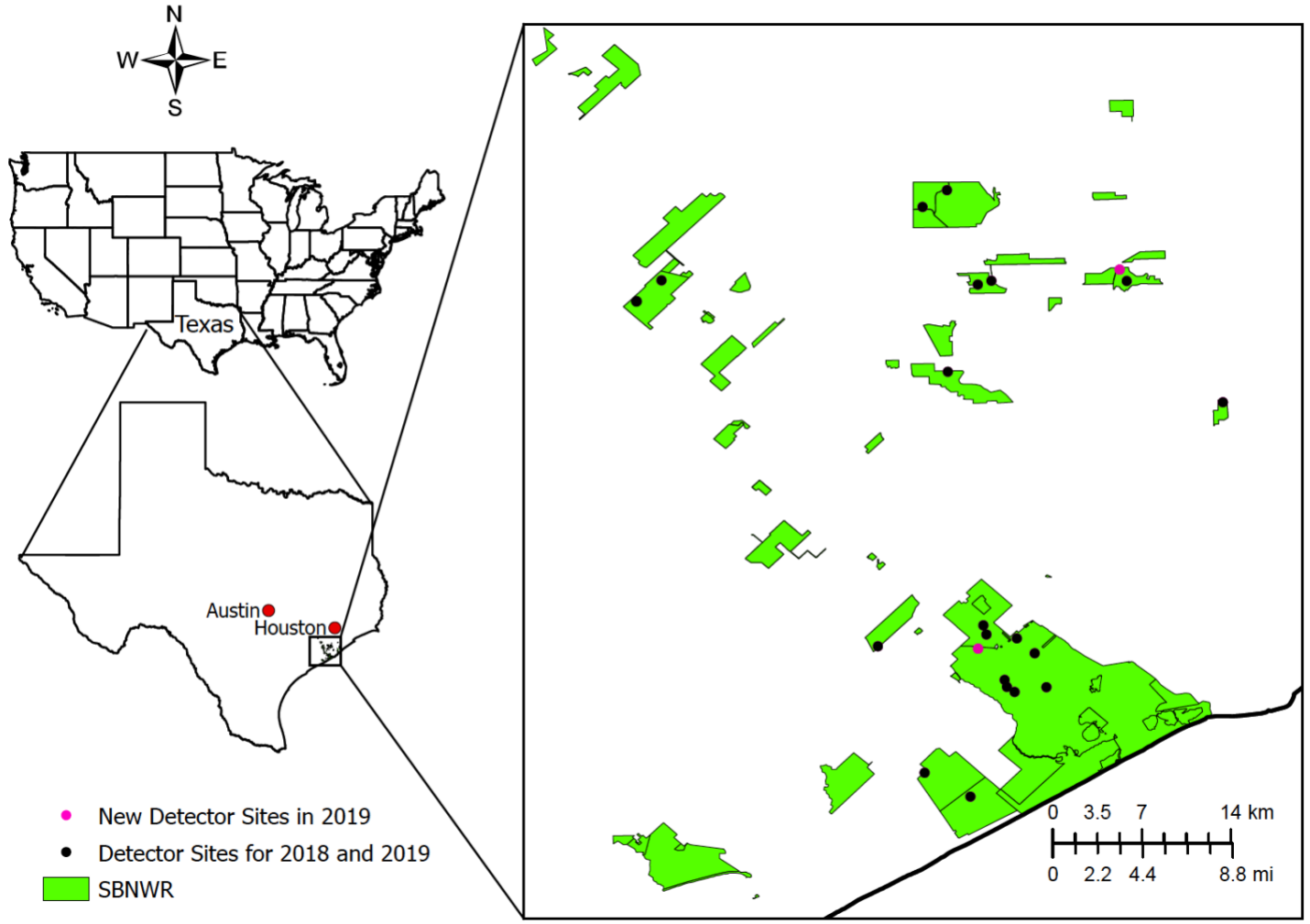

Figure 1. Fixed locations of acoustic bat detectors deployed summer 2018 and 2019 on the San Bernard National Wildlife Refuge.

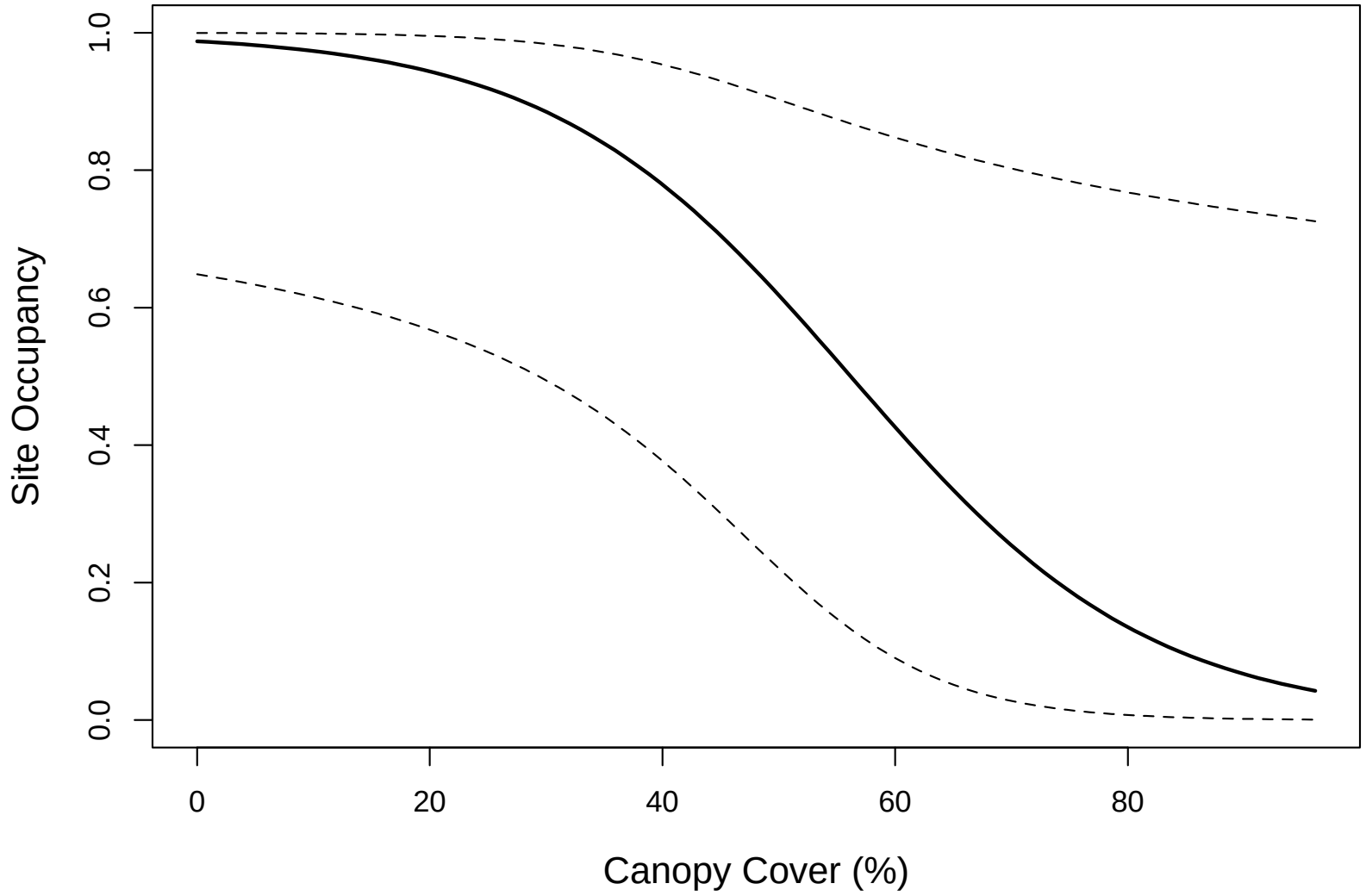

Figure 2. Probability of occupancy and 95% confidence intervals across canopy cover (%) for *T. brasiliensis* sampled during summer 2018 on the San Bernard National Wildlife Refuge, Texas USA.

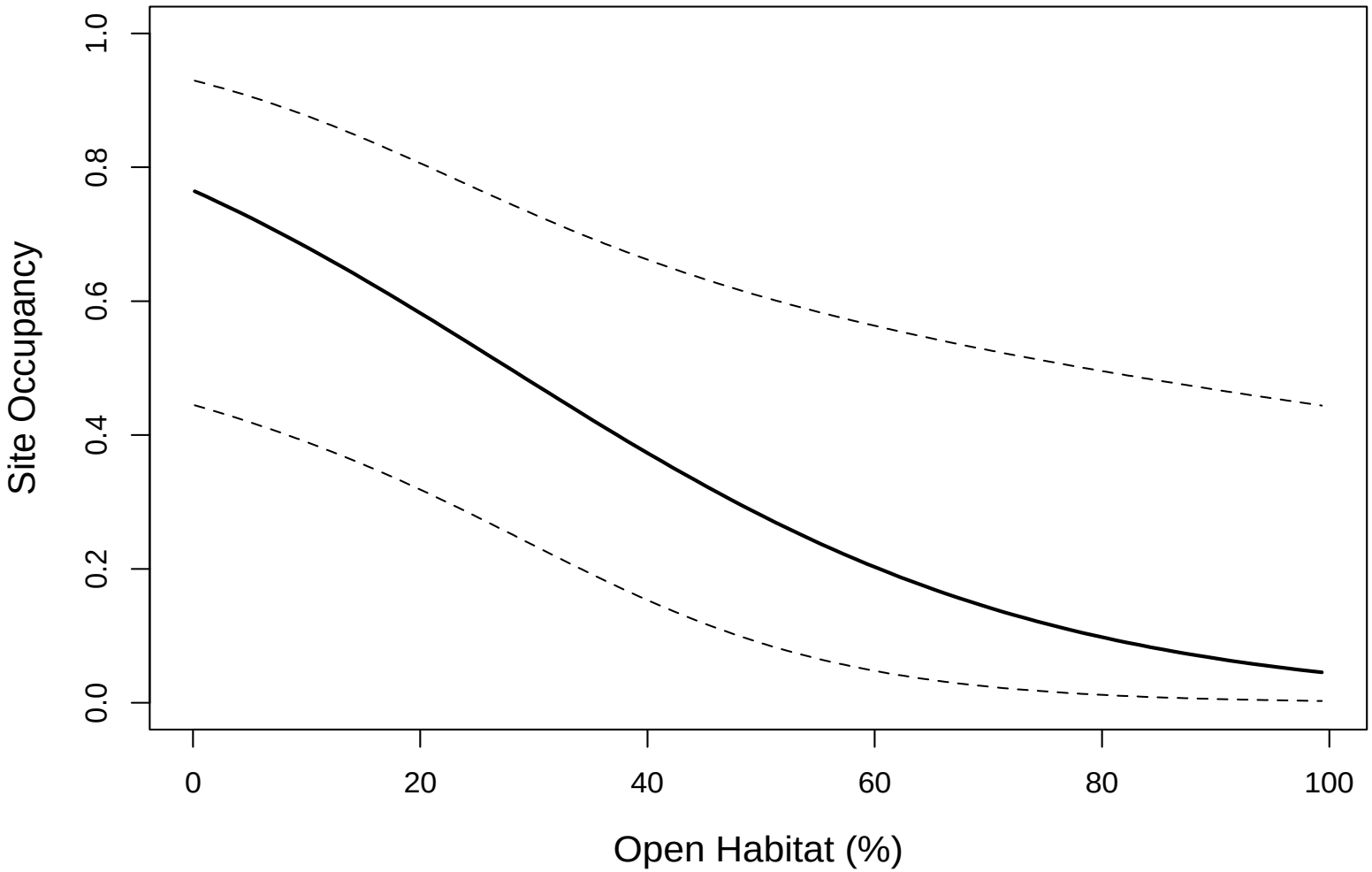

Figure 3. Probability of occupancy and 95% confidence intervals given a site was occupied with high activity across open habitat (%) within a 2-km radius for *N. humeralis* sampled during summer 2019 on the San Bernard National Wildlife Refuge, Texas USA.

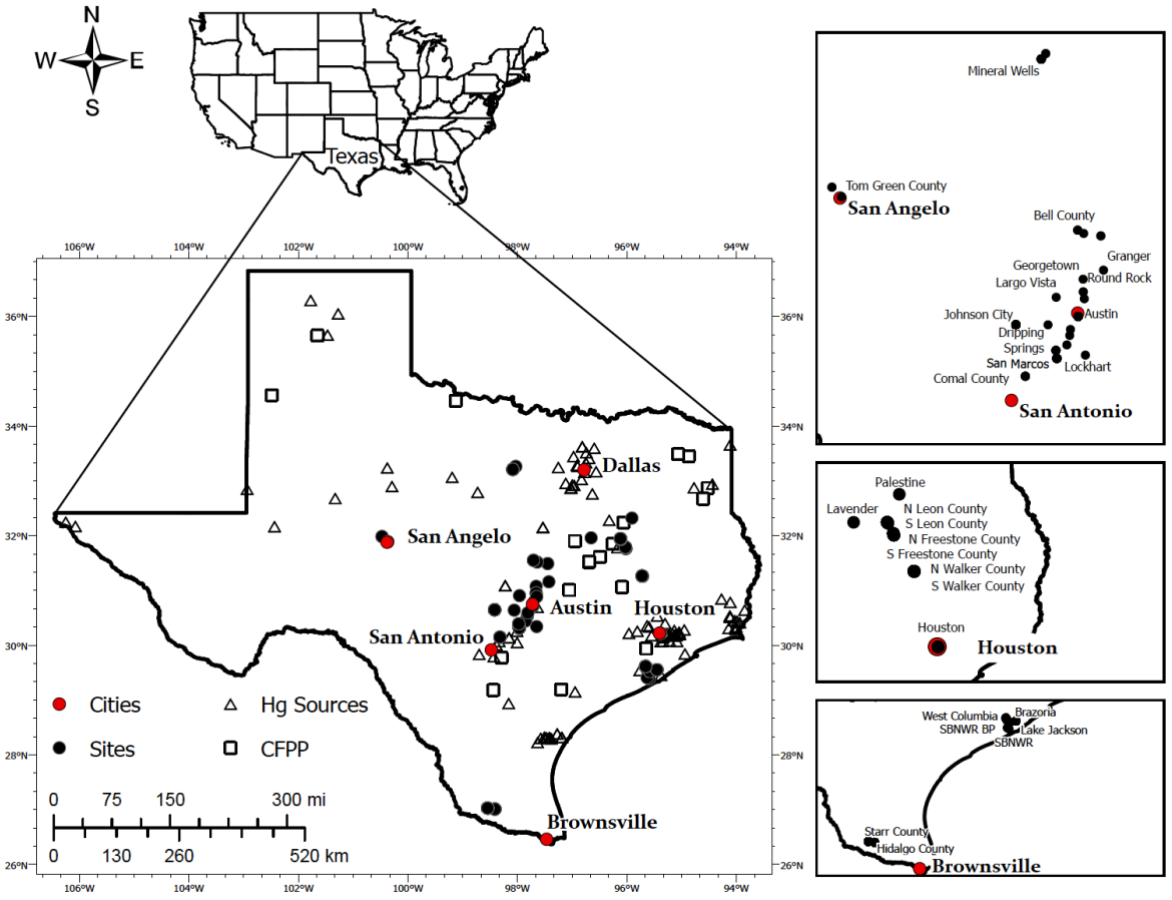

Figure 4. Locations of 32 bat fur sampling sites across eastern and central Texas along with all potential point sources of Hg pollution [coal-fired powerplants (CFPP) and other sources such as cement factories, refineries and other sources] that release Hg pollution data to the Environmental Protection Agency's Toxic Release Inventory program. Zoomed in maps on right include all fur sampling site names.

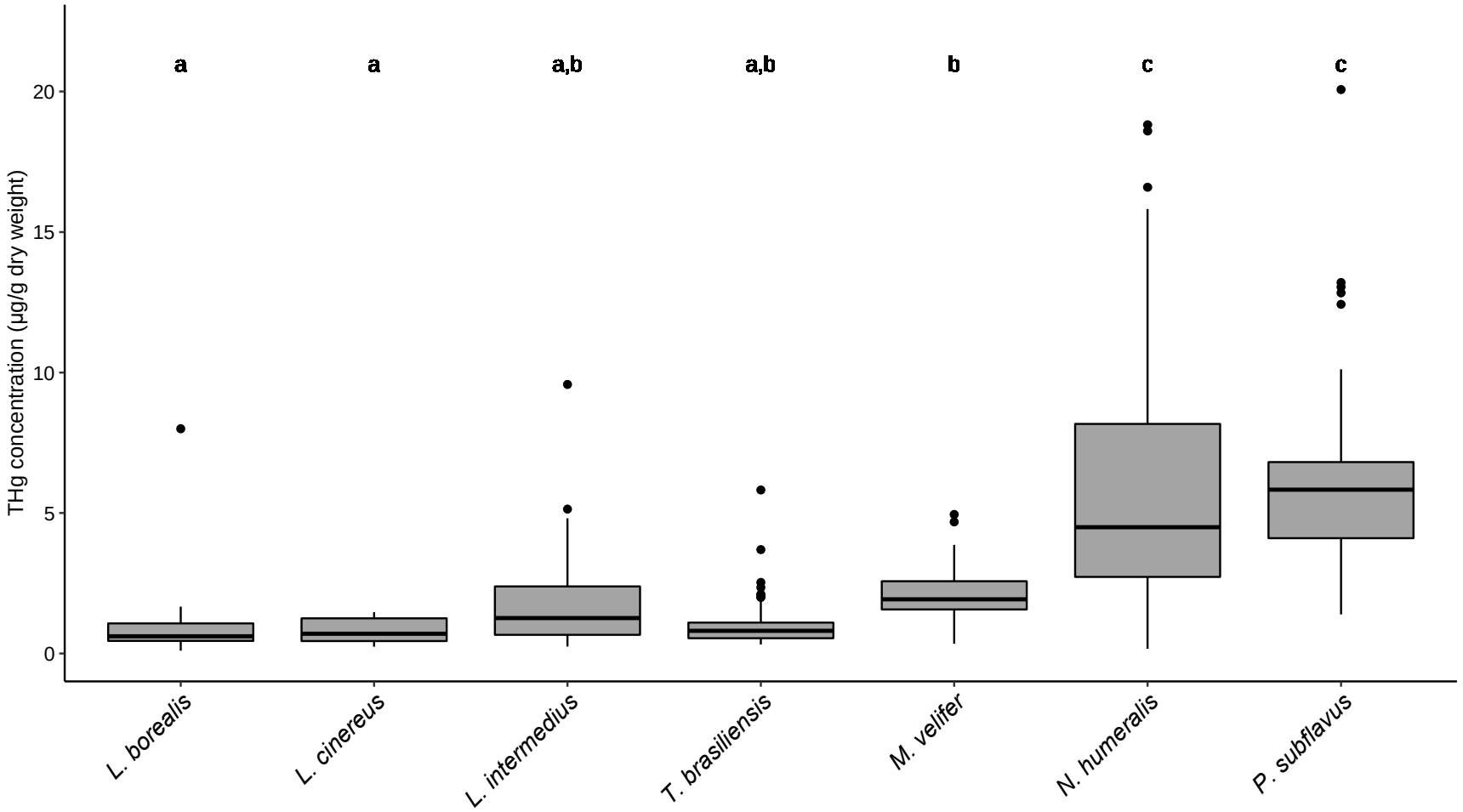

Figure 5. Bat fur THg concentrations for all species studied and all sites combined among 427 samples collected throughout central and eastern Texas, USA. Whiskers = ± 95% confidence intervals. Dots above whiskers are outliers. Letters indicate species with similar THg concentrations based on Dunn's pairwise comparison results (P <0.05). Total n values for each species are reported in Table 11.

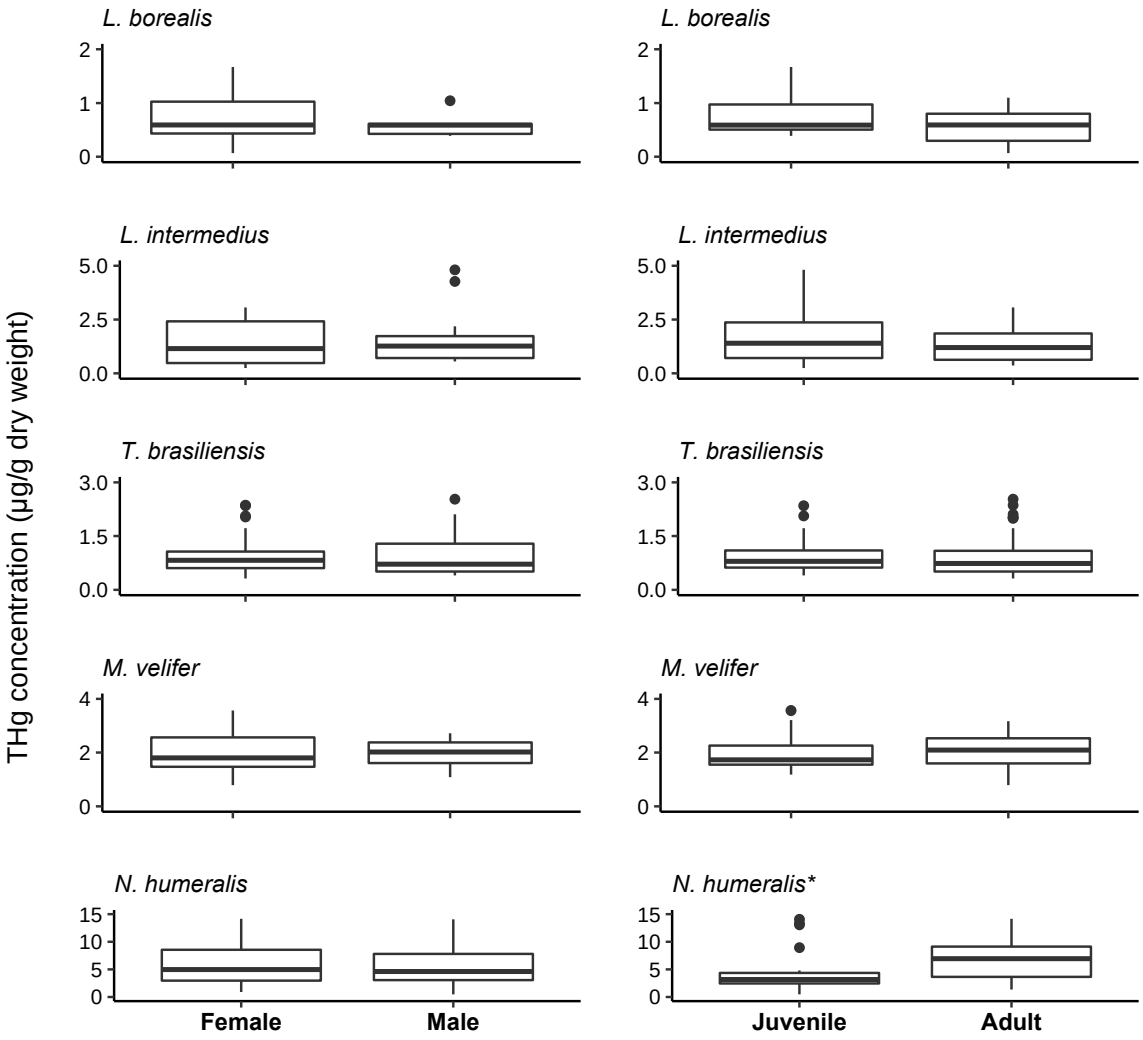

Figure 6. THg concentrations for sex and life stage among 5 bat species sampled throughout central and eastern Texas, USA. Juvenile *N. humeralis* had lower THg concentrations than adults with significance denoted by asterisk (*). Samples were pooled across sites. Total n values for each life-stage and sex are reported in Table 13.

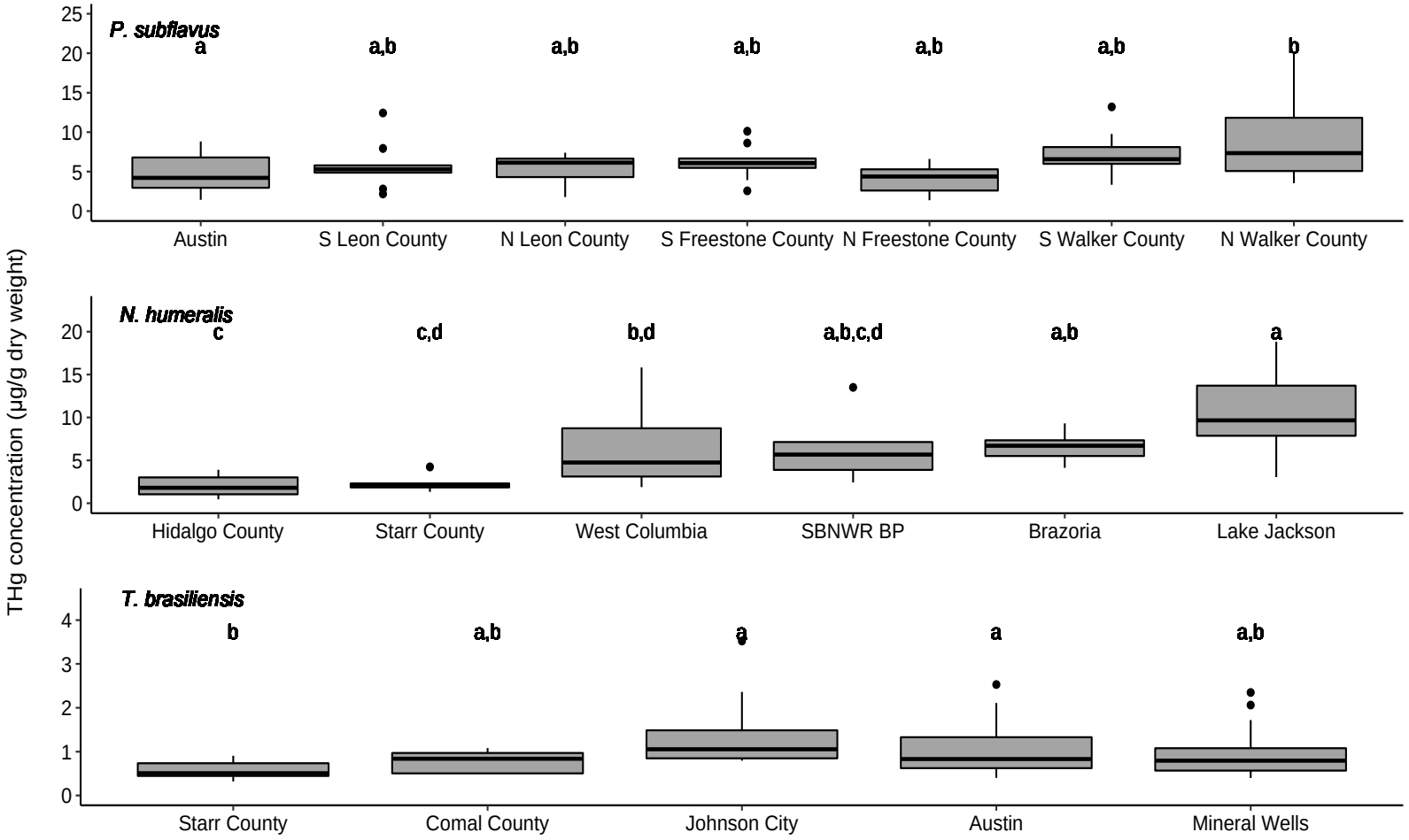

Figure 7. Inter-site variation in THg concentrations compared for *P. subflavus* (top), *N. humeralis* (middle), and *T. brasiliensis* (bottom). Letters indicate sites with similar THg concentrations based on Tukey post-hoc test for *P. subflavus* and a Dunn's pairwise comparison for *N. humeralis* and *T. brasiliensis* ($P < 0.05$). Total n values for each site are reported in Table 11 and specific site locations are reported in Figure 4.

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