HEAVY METAL BIOACCUMULATION AND DIET DIFFERENCES BETWEEN TWO PASSERINES BREEDING IN SOUTH CAROLINA

by

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(Under the Direction of James A. Martin)

ABSTRACT

Bioaccumulation of heavy metals and diet changes between seasons in wild songbird populations are basic but important information needed to make informed decisions about conservation efforts. I evaluated heavy metal concentrations in migratory Great Crested Flycatchers (*Myarchus crinitis)* and resident Northern Cardinals (*Cardinalis cardinalis)* along with trophic level changes in diet through stable isotope analysis between breeding and non-breeding seasons. Blood and feather samples were collected in South Carolina from April 2016-May 2017. Heavy metal concentrations were low compared to other related species but currently, it is unknown whether these levels have no physiological effect on individuals. Flycatchers forage at a higher trophic level than cardinals based on higher $\delta^{15}N$ values in blood and feather samples. Both cardinals' and flycatchers' trophic level unexpectedly increased between breeding and non-breeding seasons. Further research is needed to connect diet, exposure to contaminants, and fitness of migratory and resident birds.

INDEX WORDS: South Carolina, stable isotope, heavy metal, non-lethal sampling, songbirds, migratory, resident, Great Crested Flycatcher, Northern Cardinal

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DEDICATION

I dedicate this thesis to my wonderful supportive husband, Bryce who moved across the country so I could pursue this great opportunity. I also dedicate this to my parents whose endless encouragement and support helped me complete my academic career.

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CHAPTER 1

INTRODUCTION

Ecotoxicology of heavy metal contamination in wild organisms is a difficult field to study due to compounding factors that influence bioaccumulation in the environment. Heavy metals in aquatic systems have the ability to infiltrate terrestrial food webs and cause negative effects in organisms such as songbirds (Jackson et al., 2011). Bioaccumulation varies in organisms depending on specific life history traits such as seasonal movement, diet specifications, and physical characteristics (Abbasi et al., 2015). Songbirds breeding on or near the Savannah River Site (SRS) in South Carolina are exposed to many pollutants as a result of years of industrial waste deposited on the landscape. Dietary intake of such pollutants is the primary pathway of metal uptake in songbirds (Burger and Gochfeld, 2002) and provides useful information about ecosystem health after contamination events (Brait and Antoniosi- Filho, 2011; Burger and Gochfeld, 2000; Scheifler et al., 2006).

Seasonal changes in diet could result in different rates of bioaccumulation as higher trophic level species are at risk of elevated concentrations of some metals due to biomagnification (Ackerman et al., 2011; Cristol et al., 2008; Hargreaves et al., 2011). Stable isotopes, such as ^{15}N and ^{13}C, can be used to establish trophic level and diet specifications (Diggs et al., 2011; Herrera et al., 2003; Inger and Bearhop, 2008). Habitat type influences breeding territory selection for songbirds (Hogstedt, 1980; Jones et al., 2014) and prey availability (Burke and Nol, 1998; English, 1991); therefore, impacting ^{15}N and ^{13}C in their tissues. Increases in $\delta^{15}N$ generally indicate higher trophic status (Ehleringer et al., 1986) due to increased protein intake (Mizutani et al., 1992). Phenotypic attributes also alter diet (and consequently ^{15}N and

^{13}C) as nutritional requirements differ for females and males (Johnson et al., 1990; Nagy et al., 2007). The combined influences of these elements can generate wide-ranging implications.

Northern Cardinals (*Cardinalis cardinalis*) and Great Crested Flycatchers (*Myarchus crinitis*) are ideal study species as one is migratory and the other resident, both breed in similar habitats, and ostensibly consume different prey. Evaluating heavy metal concentrations and stable isotope values in these songbirds using non-lethal methods is optimal as they are protected species (Furness et al., 1986; Goede and de Bruin, 1984). Blood and feather samples provide short- and long-term metal concentration and diet variations (Bearhop et al., 2000; Burger, 1993). Creating a basic understanding of how diet changes throughout the year in both species can bring insight to potential heavy metal bioaccumulation risk. Therefore, the objectives of this thesis are to determine heavy metal concentrations in two representative songbird species breeding at or near the SRS and evaluate how physiological and habitat characteristics are associated with diet in these two species.

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HEAVY METAL BIOACCUMULATION IN TWO PASSERINES WITH DIFFERING MIGRATION STRATEGIES

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Introduction

Since the environmental revolution of the 1970s, cleanup efforts from anthropogenic environmental pollutants have been conducted nationwide, yet decades later residual repercussions to wildlife are still widespread. Heavy metal contamination results from improper disposal or release of specific chemical and nuclear byproducts. These metals can include essential (e.g., Se, Zn, and Cu) and non-essential elements (e.g., Hg, Pb, Cd, Cr, As, and Ni) with some exhibiting high toxicity at low concentrations. Biomonitoring of organisms inhabiting potentially polluted areas yields an assessment of overall ecosystem health (Burger and Gochfeld 1996). Many of the current biomonitoring efforts rely on samples taken from aquatic organisms related to possible human consumption risks. However, many terrestrial organisms also consume resources from contaminated aquatic ecosystems exposing them to such pollutants.

Higher trophic level organisms, such as piscivorous birds and raptors, have been extensively studied and monitored for heavy metals (Solonen and Lodenius 1990, Movalli 2000, Battaglia et al. 2005, Lodenius and Solonen 2013); however, heavy metal contamination from an aquatic source can bioaccumulate to levels sufficient to negatively affect organisms at a lower trophic level (Edwards et al. 2014). Jackson et al. (2011) indicated terrestrial songbird reproductive success was reduced in the terrestrial environment surrounding rivers contaminated by heavy metals. Chronic exposure to certain heavy metals can cause physiological abnormalities (Scheuhammer 1987), reducing reproductive success (Jackson et al. 2011) which could lead to local population level changes. Songbirds are low- to mid- trophic level organisms and have the ability to accumulate heavy metals through air, food, or water resources but most often through dietary intake (Burger and Gochfeld 2002). Assessing their exposure to environmental pollution can be indicative of overall ecosystem health from a ecotoxicological standpoint (Burger and

Gochfeld 2000, Scheifler et al. 2006, Brait and Antoniosi- Filho 2011, Abbasi et al. 2015) and provide information of heavy metals in the environment of which they reside in (Dauwe et al., 2002; Eens et al., 1999; Furness and Greenwood, 1993; Goede and de Bruin, 1984; Lodenius and Solonen, 2013).

Once inside the body, metals accumulate in internal tissues, are excreted in feces, or are deposited in feathers (Burger 1993). Metal concentrations in feathers can provide information on body burdens at time of molt and feather growth (Dauwe et al. 2000), especially for mercury (Bearhop et al. 2000), but relationships between feather concentrations and body burdens vary greatly among metals (Beyer and Meador 2011). Metal levels in blood provide an indication of recent short-term dietary exposure (Furness and Greenwood 1993) before being excreted or accumulated into internal tissues. Both blood and feather concentrations supply dietary information on site-specific uptake of metals such as methylmercury (Evers et al. 2005). Mercury concentrations in blood and feather tissues are generally strongly correlated (Ackerman et al. 2011). Although it has been suggested that all other metal concentrations in feathers are related to external (atmospheric deposition) contamination (Dauwe et al. 2003), this suggestion has been questioned (Burger 1993, Beyer and Meador 2011).

Feathers obtained from migratory species indicate exposure to contaminants throughout the year as these birds molt and regrow feathers between breeding and wintering grounds (Braune et al. 2002). Concentrations in resident birds are likely sourced locally and can be used to identify contaminated areas (Burger 1993). With very large home ranges, migrants are better adapted to handling toxic compounds (Rainio et al. 2012). Migratory birds have a higher basal metabolic rate (BMR) than non-migratory birds (Jetz et al. 2008) which would suggest that

heavy metals are absorbed more rapidly into the body in migrants resulting in lower blood concentrations (Cai and Calisi 2016) although this hypothesis has not been explored thoroughly. Biomonitoring studies on songbirds rarely report the sex of individuals sampled though sex-specific differences in physiology and behavior could affect heavy metal concentrations (Deng et al. 2007, Robinson et al. 2012). For example, foraging behavior of passerines often differs among sexes during the breeding season based on their space use (Holmes 1986) which, generally speaking, means that males forage at higher heights near song perches while females forage lower at nest heights. This contrast between sexes can explain the relationship of differing feeding habitats and bioaccumulation/detoxification rates (Fossi et al. 1995). Also, females have been shown to deposit trace elements into eggs and eggshells providing an alternative pathway of excretion compared to males during the breeding season (Burger 2007) leading to decreased body burden. However, female to egg metal deposition rates are not consistent among species (Robinson et al. 2012).

The objectives of this study were to 1) determine the geographic extent of heavy metal contamination in songbirds in association with potentially bioavailable contaminated (PBC) sites on the U.S. Department of Energy's Savannah River Site (SRS), 2) evaluate the congruence of two non-lethal sampling techniques, 3) compare location-specific metal concentrations between a migratory songbird, Great Crested Flycatcher (*Myiarchus crinitus*) and a resident songbird, Northern Cardinal (*Cardinalis cardinalis*), and 4) assess the differences in metal concentrations between sexes. We hypothesize that feather concentrations from Great Crested Flycatchers will be similar across sites as these feathers were grown prior to reaching the SRS while Northern Cardinals will have higher levels in their feathers at PBC locations because they reside there year round (hereafter, migratory exposure hypothesis). We also hypothesize that both species with

individuals breeding in close proximity to PBC areas will have higher concentration levels in their blood (and feathers for Northern Cardinals) compared to birds breeding in reference locations (hereafter, close proximity hypothesis).

Methods

Study species

Northern Cardinals (hereafter, cardinals) are an example of a year-round omnivorous resident species in eastern North America that generally spends its entire adult life within 2.3km of its breeding territory (Halkin and Linville 1999) which makes them an ideal species for biomonitoring of metals in a specific geographic location over a greater period of time compared to a migratory species. Great Crested Flycatchers (hereafter, flycatchers) migrate to our study area to breed from April to August and maintain a higher trophic level than cardinals as the majority (93%) of their diet consists of invertebrates (Miller and Lanyon 2014). Cardinals complete a full molt from October – November while flycatchers body molt is poorly understood but likely takes place on or near wintering grounds in Central America from March-May (Dickey and van Rossem 1938, Miller and Lanyon 2014).

Study area

The study was conducted on the U.S. Department of Energy's Savannah River Site (SRS) south of Aiken, South Carolina and the Silver Bluff Audubon Center property west of Jackson, South Carolina. The SRS is approximately 800 km^2 in size and was originally constructed in the 1950s to aid in the production of materials used for nuclear weapons. This region is characterized by a humid subtropical climate with an average annual precipitation of 1,225 mm and rarely sees ice or snowfall (Kilgo and Blake 2005). Currently, more than 50% of the area is longleaf (*Pinus palustris*) or loblolly pine (*Pinus taeda*) forest and about 20% hardwoods with a variety of other

habitat-types occupying the remaining land (Kilgo and Blake 2005). Large sections along the Savannah River are seasonally flooded wetlands and flood plains with several oak species (*Quercus* spp.*)*, sweetgum (*Liquidambar styraciflua)*, and hickories (*Carya* spp.) dominating the canopy and swamp palmetto (*Sabal minor*), winged elm (*Ulmus alata*), and hawthorns (*Crataegus* spp.*)* dominating the understory. Riparian areas along small stream bottoms contain a mix of tulip poplar (*Liriodendron tulipifera)*, sweetgum (*Liquidambar styraciflua)*, water oak (*Quercus nigra*), and American holly (*Ilex opaca*) as canopy species and switch cane (*Arundinaria tecta)*, blueberries (*Vaccinium spp.)*, red bay (*Persea borbonia*), dog hobble (*Leucothoe axillaris*) and blackhaw (*Viburnum prunifolium*) in the understory.

Study design

We sampled in four randomly selected locations in bottomland hardwood/floodplain along streams or the Savannah River in Aiken or Barnwell County. Each location was 11-28 km apart. Two locations were deemed reference locations (Silver Bluff and Upper Three Runs) due to their distance (>10 km) upstream from known or possibly contaminated areas and two locations were designated potentially bioavailable contaminated locations (PBCs: D-Area and Pen Branch) as they were in close proximity (approximately 2-4 km) to historically contaminated areas (Figure 2.1). Surface and well water along with sediment samples are collected on the SRS annually at minimum and are analyzed for many contaminants including metals (http://www.srs.gov/general/pubs/ERsum/index.html). This information has yielded some areas of higher than normal concentrations of metals. D-Area contains coal fly ash basins and drainage swamps that, in other studies, have shown heavy metal uptake by many wildlife species (Rowe et al. 1996, Hopkins et al. 1998, Hernández et al. 2016) including aquatic invertebrates (Fletcher et al. 2014). One study at D-Area found maternal transfer of trace elements from common grackles

to their young living along the basin (Bryan et al. 2003). Pen Branch, while less studied, has a history of heavy metal contamination in aquatic vertebrates and invertebrates (Loehle and Paller 1990, Bryan 2011) indicating potential for trophic transfer from the environment.

We opened mist nets 50-200m apart at each location approximately 15-30 minutes before sunrise and closed the nets by 1100 hrs or earlier if temperatures exceeded acceptable limits (i.e., ~ > 29°C). Edges between hardwoods and other habitat types were frequently used as areas to set up mist nets. We targeted flycatchers and cardinals either passively or by playback of conspecific calls and songs from April –June 2016. Once caught, each bird was sexed and given an aluminum USGS band (USGS BBL Permit Number 22002). We collected 8-12 breast feathers per individual and stored them in a marked and sealed plastic bag until analysis (Institutional Animal Care and Use Committee Permit Number A2016 02-022-Y1-A0). Blood samples (~0.10-0.30 ml) were collected from the jugular vein of each individual, placed in lithium heparin tubes, and stored on ice until analysis. We released individuals immediately upon sample collection completion.

Feather treatment and analysis

Feathers were pre-treated to remove external contamination. Before analysis, feathers were sonicated for 15 minutes in 18 MΩ water, followed by a rinse with 18 MΩ water. The feathers were then dried at 60ºC to a constant weight before determining dry weight. Whole feathers were digested in PFA vials with trace-metal grade HNO_3 at 70ºC for 24 hours following a method modified by Bond and Lavers (2011). Trace-metal grade H_2O_2 was added and the samples were heated at 70ºC for an additional 3h. After digestion, the samples were diluted with 18 MΩ water. Procedural blanks were prepared in the same manner alongside samples to monitor for potential contamination.

Blood treatment and analysis

Blood samples were pre-digested for 24h in trace-metal grade HNO_3. Samples were then heated at 90ºC for 3 hours. Trace-metal grade H_2O_2 was added and samples were heated for an additional hour at 90ºC. Samples were diluted with 18 MΩ water. Procedure blanks were prepared in the same manner alongside samples to monitor for potential contamination.

Metal analysis

Samples were analyzed at the Center for Applied Isotope Studies, University of Georgia for eight trace metals (As, Cd, Cr, Cu, Ni, Pb, Se, and Zn) by ICP-MS (VG Elemental PQ3) in solution mode. Detection limits were as follows: As 0.042 µg/mg, Cd 0.006 µg/mg, Cr 0.027 µg/mg, Cu 0.102 µg/mg, Ni 0.159 µg/mg, Pb 0.012 µg/mg, Se 0.228 µg/mg, and Zn 0.213 µg/mg. Indium was used as an internal standard. All samples were analyzed with certified QC standards and blanks. Elemental analysis was performed using the quantitative analysis method. Samples that were below detection limit were given a value of one-half the detection limit for each metal prior to statistical analysis.

Mercury analysis

Feather and blood samples were analyzed for total mercury (Hg) according to US EPA method 7473 using a Milestone DMA-80 (Savannah River Ecology Laboratory, Aiken, SC). Feather samples consisted of 2-3 whole breast feathers that had been washed and dried using the same method previously described. Quality assurance samples were run every 10-20 samples which included blanks and certified reference materials (PACS-2 and TORT-3; National Research Council Canada). Duplicate feather samples (n = 18) were run after approximately every 10 feather samples and sample averages were taken for statistical analysis. Analytes were reported in parts per million on a dry weight (dw) basis with an average detection limit of 0.123

µg/mg (ppb). Recovery of standard reference materials was 100.6% (range = 96.6-104.3%) for TORT-3 and 96.6% (range = 88.2-116.6%) for PACS-2. Blood samples were freeze-dried prior to analysis due to clotting issues to ensure the entire sample was homogenous and could be extracted. Using a wet weight (ww)/dw ratio from Newman et al. (2011), we converted the dw concentration of the blood samples to ww concentrations to facilitate comparison to other published studies.

Statistical analysis

Linear mixed-effects models were used to analyze the data with independent variables being species, sex, and site (PBC or reference locations). A random effect of site was also included because multiple samples were taken at each site. We used the lmer function from package lme4 (Bates et al. 2015) in R (version 3.2.3, R Core Team, 2015) to fit models. Models were compared using Akaike information criteria adjusted for small sample size (AIC_c) (Burnham and Anderson 2002, Arnold 2010). AIC_c values were calculated using maximum likelihood and used for model comparison and to estimate model coefficients, but r^2 values were calculated using restricted maximum likelihood (Nakagawa and Schielzeth 2013). Models were considered competing if $\Delta AIC_c < 4$ and model averaging was implemented to determine full model averaged coefficients. Biological significance was determined with 85% confidence intervals that did not cross zero (Arnold 2010). Spearman rank correlation tests were used to determine correlations between feather and blood samples among species and a few metal groups with a significance value set at $\alpha = 0.05$.

Results

Trace metals between species and location groups

Four sites were sampled across the study area (two PBC and two reference) with 70 total birds captured (37 in reference and 33 in PBC) and sampled from April-June 2016. Overall mean values in blood and feather samples for both species were relatively low for all metals analyzed (Table 2.1 and 2.2). More than 90% of the cadmium values for both tissue types were below 0.01 µg/g and were excluded from further statistical analysis.

We did not find support for our close proximity hypothesis based on blood concentrations for any metal analyzed (see Appendix A for all AICc tables). We found an unpredicted pattern of a significant species and specific site group (reference/PBC) interaction with greater Cu concentrations for cardinal feathers in the reference sites than flycatchers in the same location (β = 5.226, 85% CL = 0.939, 9.513; Fig. 2.2); however, birds overall in reference sites expressed decreased Cu concentrations (β = -5.162, 85% CL = -9.089, -1.235; Fig. 2.2) compared to PBC sites. Cardinals had less Cr in feathers than flycatchers overall (β = -1.463, 85% CL = -2.436, -0.489; Fig. 2.3) but all birds in reference sites had lower Cr feather concentrations (β = -1.361, 85% CL = -2.641, -0.081; Fig. 2.3) than PBC sites.

Zinc concentrations in feathers from reference locations, for both species combined, had an estimated 67 less µg/g than in PBC sites (β = -67.924, 85% CL = -128.705, -7.144; Fig. 2.4) which is consistent with the close proximity hypothesis but not the migratory exposure hypothesis. Similarly, cardinals had lower concentrations of Hg (β = -0.174, 85% CL = -0.261, -0.086; Fig. 2.5) and Se (β = -0.329, 85% CL = -0.500, -0.158; Fig. 2.6) in blood samples than flycatchers. Arsenic levels were negligibly lower in cardinal feathers (β = -0.061, 85% CL = -

0.116, -0.006; Fig. 2.7) than in flycatchers. Sex differences between and among species were not significant for any metal analyzed based on 85% confidence intervals after model averaging.

Metal correlations between tissues

Cardinals exhibited a significant negative feather/blood correlation for Cu (r = -0.33, p = 0.038) but significant positive correlations for Hg (r = 0.614, $p < 0.001$) and Pb (r = 0.408, p = 0.008) (Table 2.3). Correlations between the two tissues types were not observed in flycatchers for any metal.

Correlations between metals

Cardinals had significant negative feather Hg/Se (r = -0.329, p = 0.026) and positive blood Hg/Se correlations (r = 0.464, p = 0.007) but Hg/Se were not correlated in either tissue type for flycatchers (see Appendix A for figures). Feather concentrations of Pb/Zn were also positively correlated for cardinals (r = 0.34, p = 0.015) and marginally correlated for flycatchers (r = 0.43, p = 0.059); however, Pb/Zn blood concentrations were not correlated for either species.

Discussion

Overall, the lack of evidence supporting the close proximity hypothesis suggests that the bioavailability of historical heavy metal contamination to passerines on the SRS is minimal. While there may be areas of higher metal concentrations on the SRS, the spatial extent of the passerine exposure does not likely pose a risk to local population levels. This is due to the notion that only very few individuals are inhabiting areas immediately adjacent to contaminated areas which are susceptible to extremely high bioavailable concentrations. Pb and Cd are two metals of top concern in ecotoxicology however, Cd values were almost all below detection limit and no individual had higher than 0.5 µg/g in feathers or 0.15 µg/g blood for Pb. Mercury (specifically

methylmercury) is arguably, one of the most toxic compounds to humans and wildlife. The levels seen in this study however, fall below the threshold for negative reproductive consequences seen in a similar species (Jackson et al. 2011) and are therefore not concerning from a population level standpoint. Interestingly, feather concentrations of Cr, Cu, Zn, and As found in this study contradict the migratory exposure hypothesis. This suggests that individual flycatchers are apparently being exposed to differing heavy metal levels during feather growth as they travel between their breeding grounds in the US and their wintering grounds in Central and South America. Copper and chromium levels were substantially higher (as compared to cardinals) in some individuals implying some flycatchers are wintering on areas with some residual bioavailable environmental pollution. Without geolocators on said individuals however, it is impossible to determine the geographic location of their wintering grounds.

Sex-specific concentrations

Sex-specific differences were not observed for metal concentrations which was relatively surprising given that females have been known to deposit heavy metals through eggs during the breeding season (Rimmer et al. 2005), although we were unable to confirm that the females we caught were actively nesting. Even so, the deposition of heavy metals in eggs has been disputed by Burger (2007) who found that female birds in general exhibited higher levels of heavy metals. Differing foraging habits between sexes of conspecific passerines have been identified (Holmes 1986), but in these species it is likely that both sexes are maintaining approximately the same trophic level even with dissimilar diets. Sex differences in relation to mass are also not constantly observed for cardinals (Halkin and Linville 1999) or flycatchers (Miller and Lanyon 2014), but males have longer wing and tail lengths in both species. Bioaccumulation rates of heavy metals in both sexes of passerines specifically may be consistent (i.e. same uptake rate)

due to similarities in diet between the sexes. Even though diet is the primary source of metal accumulation, however, the bioaccumulation rate is very species-specific (Burger and Gochfeld 2000, Squadrone et al. 2016). While contrary to previous research in passerines (Jackson et al. 2011), subtle species specific food chain differences may affect bioaccumulation and should be explored in future research.

Species-specific concentrations

Heavy metal deposition in feathers occurs during feather formation (Goede and de Bruin 1984) and body feathers of flycatchers specifically form on or near wintering grounds before spring migration in Central or South America (Miller and Lanyon 2014), whereas cardinals molt all their feathers in the fall, post-breeding season (Halkin and Linville 1999). Due to the differences in geographic location during molting of body feathers, flycatchers were expected to have different heavy metal concentrations than cardinals regardless of treatment group effects (migratory exposure hypothesis), but this was only confirmed for certain metals: Cr, Cu, and Zn.

Species differences in blood concentration levels at both reference and PBC locations (especially for Se and Hg) suggest differences in trophic feeding levels between the two species (Deng et al. 2007, Abbasi et al. 2015). This is expected as flycatchers diet consists of >90% invertebrates (Miller and Lanyon 2014) while cardinal diet varies greatly throughout the year and are considered omnivores (De Graaf et al. 1985). Mercury (Scheuhammer et al. 2007, Newman et al. 2011, Keller et al. 2014) and possibly Se (Orr et al. 2006) biomagnify at increasing trophic levels which may indicate that flycatchers eat organisms at a higher trophic level than cardinals. Tissue correlation tests revealed that only Cu, Pb, and Hg in cardinals (not flycatchers) had any significant correlation which was expected because they are using resources from the same location year round. This trend between feather and blood concentrations was also seen in

Burger (1993) with only Hg however and was not tested in passerine species (mostly seabirds and raptors) nor was there a difference noted between migratory and resident species. This distinction is vital to address when conducting blood/feather tissue correlations as one implicates recent exposure and the other, long term, which can help distinguish when and where contaminants were accumulated. Another consideration is that if migratory birds are wintering on contaminated sites and are killed or scavenged on their breeding grounds, this could lead to trophic transfer of harmful metals to resident predators.

Certain combinations of metals can have a synergistic effect which we may have slightly observed in this study. Mercury is a primary element of concern in avian ecotoxicology; however, a selenium- enriched diet has been shown to prevent mercury toxicity (Ralston and Raymond 2010) by creating a stable complex. Cardinals had significant Se/Hg correlations in both tissue types indicating protection from possible mercury toxicity (Parizek 1978) and both metals are co-occurring at a predictable rate. Also, Dauwe et al. (2002) examined the influence of lead exposure on accumulation of Zn in feathers of zebra finches and found that it was lower when Pb intake was increased as well as a negative Pb/Zn correlation in feathers. This contradicts the findings in our study as cardinals had a positive Pb/Zn correlation (even though the correlation was weak). It should be noted however, Pb concentrations were extremely low potentially reducing the effect on higher amounts of Zn.

Site concentrations

Legacy contamination effects on terrestrial wildlife (particularly songbirds) on the SRS after initial environmental introduction and cleanup is relatively unknown. In this study, two passerine species were sampled at reference locations and locations near areas of historical contamination. Based on blood levels, a direct indication of recent dietary exposure on their

breeding territories, our results suggest no differences in local contamination for either species. Feather differences between sites did not exist for our resident species (cardinals) leading to the conclusion that PBC sites do not have bioavailable contamination. Feather differences between locations in flycatchers was explained in the previous section. In our study, sampling did not occur immediately adjacent to point sources which may have influenced metals uptake. Literature exists pertaining to contamination levels at different distances for smelters, resulting from air deposition (Dauwe et al., 2002; Eeva et al., 2008, 2006; Rogival et al., 2006), but no information was found pertaining to terrestrial bioaccumulation through water resources except those directly adjacent to the source (Bryan et al. 2003, 2012, Jackson et al. 2011). The residual contaminants may also be sufficiently contained such that bioavailability is low and bioaccumulation risk to terrestrial organisms is of minimal concern.

Assessment of sampling techniques

Non-lethal sampling of organisms is ideal, especially when dealing with sensitive or protected species such as migratory birds (Goede and de Bruin 1984, Furness et al. 1986). Blood and feather samples taken from cardinals and flycatchers during their breeding season in this study provide information on heavy metal concentrations from a year-round perspective for both species. Blood levels, while not suitable for all metals, can be particularly informative about recent exposure to bioavailable concentration levels (Veerle et al. 2004, Evers et al. 2005) from their diet specifically (Furness and Greenwood 1993). Feathers yield information at time of molt which, for these species, occurs outside the breeding season and for flycatchers occur across international boundaries. Feather metal concentrations vary with the specific metal (Lodenius and Solonen 2013) and may include some atmospheric deposition, but that possibility was mitigated in this study through feather washing (Brait and Antoniosi- Filho 2011). Breast

feathers proved to be simple to collect and allowed for the entire feather to be analyzed, providing a more homogenous sample. However, contrary to other studies (Furness et al. 1986), breast feather concentration levels were not consistent when analyzed for Hg in our study. Sample duplicates (breast feathers from the same individual) had a 19% (mean, ± 16% SD) difference in concentration levels between duplicate samples. This suggests that these feathers were grown at different time periods as older feathers have lower concentrations than new feathers (Spalding et al. 2000, Ackerman et al. 2011). Even though these feathers are from the same ventral feather tract, they are not all molting simultaneously. Further exploration of molting patterns and sequence for breast feathers in both species could supply an accurate indication of why these feathers are not all grown during the same timeframe.

Conclusions

Increasing available information on heavy metal concentrations of resident and migratory passerines is necessary to determine environmental pollution changes over time and potentially predict population level effects at a local and even global scale. Passerines can provide valuable information about the bioavailability of pollutants and serve as biological sentinels for remediation. Concentration levels observed in cardinals and flycatchers at the SRS during the 2016 breeding season suggested they were not accumulating SRS-based metals and that the existing concentrations were below known levels that would indicate toxicity concern. However, if migratory birds are wintering on contaminated sites and are killed or scavenged on their breeding grounds, this could lead to trophic transfer of harmful metals to resident predators. While information on the long-term impacts of metal loads at these concentrations is relatively unknown, especially for wild populations of passerines, there could be subtle consequences rendering negative influences on the fitness of specific individuals and potentially their

offspring. Further research on breeding success and nestling fitness of passerines with similar heavy metal accumulation should be explored.

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Table 2.1: Concentrations (mean parts per million ± SD) of heavy metals in Great Crested Flycatchers (GCFL) and Northern Cardinals (NOCA) (dry weight for feathers, wet weight for blood) collected at on PBC sites April-June 2016.

	GCFL		NOCA	
Metal	Feather (*n* = 8)	Blood (*n* = 7)	Feather (*n* = 25)	Blood (*n* = 18)
As	0.175 ± 0.186	0.074 ± 0.025	0.117 ± 0.140	0.055 ± 0.044
Cd	0.011 ± 0.009	0.001 ± 0.001	0.009 ± 0.014	0.001 ± 0.001
Cr	3.026 ± 4.05	0.237 ± 0.092	1.251 ± 0.368	0.222 ± 0.086
Cu	11.504 ± 11.745	0.371 ± 0.111	10.454 ± 2.675	0.309 ± 0.105
Hg*	0.838 ± 0.603	0.397 ± 0.252	0.563 ± 0.429	0.125 ± 0.204
Ni	0.922 ± 1.194	0.004 ± 0.005	0.382 ± 0.780	0.001 ± 0.005
Pb	0.319 ± 0.253	0.002 ± 0.004	0.140 ± 0.093	0.019 ± 0.043
Se	2.012 ± 1.898	1.525 ± 0.364	1.552 ± 1.314	1.072 ± 0.368
Zn	143.90 ± 159.35	5.721 ± 1.168	104.21 ± 35.051	5.304 ± 1.314

* Sample size for GCFL feather *n* = 8 and blood *n* = 5; for NOCA feather *n* = 25 and blood *n* = 15.

Table 2.2: Concentrations (mean parts per million ± SD) of heavy metals in Great Crested Flycatchers (GCFL) and Northern Cardinals (NOCA) (dry weight for feathers, wet weight for blood) collected at on reference sites April-June 2016.

	GCFL		NOCA	
Metal	Feather (*n* = 12)	Blood (*n* = 12)	Feather (*n* = 25)	Blood (*n* = 23)
As	0.180 ± 0.083	0.043 ± 0.014	0.104 ± 0.091	0.045 ± 0.031
Cd	0.002 ± 0.006	<0.001	0.001 ± 0.003	<0.001
Cr	1.190 ± 0.678	0.187 ± 0.035	1.066 ± 0.411	0.170 ± 0.083
Cu	5.572 ± 1.574	0.347 ± 0.070	10.528 ± 2.421	0.321 ± 0.220
Hg*	0.591 ± 0.181	0.329 ± 0.155	0.452 ± 0.305	0.125 ± 0.073
Ni	0.590 ± 1.179	0.022 ± 0.054	0.205 ± 0.372	1.084 ± 5.068
Pb	0.125 ± 0.093	0.008 ± 0.006	0.222 ± 0.171	0.011 ± 0.019
Se	1.469 ± 0.352	1.320 ± 0.261	1.195 ± 0.294	1.108 ± 0.351
Zn	52.017 ± 24.233	5.309 ± 1.315	107.74 ± 42.292	5.673 ± 4.070

* Sample size for GCFL feather *n* = 4 and blood *n* = 12; for NOCA feather *n* = 21 and blood *n* = 18.

Table 2.3: Spearman rank correlation (*r*) between blood and feather tissue sample concentrations for each metal for Great Crested Flycatcher (GCFL) and Northern Cardinal (NOCA). Bold indicates significance of $P < 0.05$.

	GCFL		NOCA	
Metal	*r*	*P* value	*r*	*P* value
As	-0.341	0.153	-0.067	0.681
Cr	0.296	0.218	-0.225	0.158
Cu	-0.198	0.418	-0.325	**0.038**
Hg	0.533	0.139	0.614	**0.0004**
Ni	0.262	0.279	-0.054	0.737
Pb	-0.356	0.134	0.408	**0.008**
Se	-0.122	0.62	-0.163	0.31
Zn	-0.207	0.395	-0.197	0.216

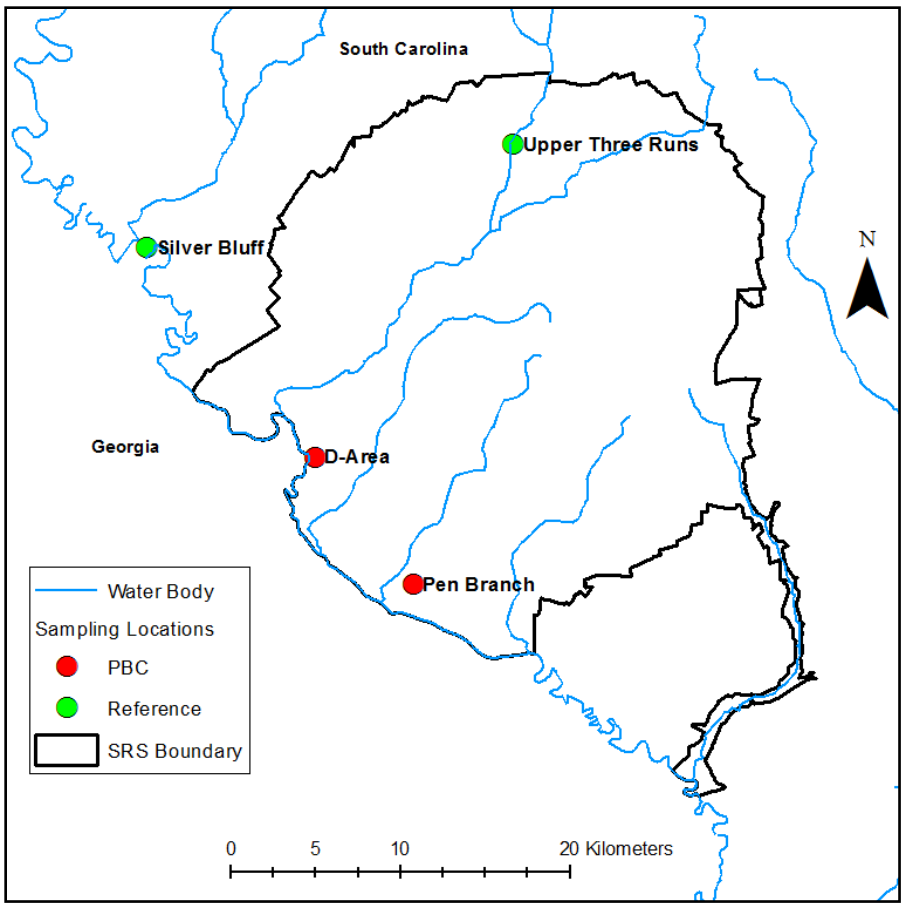

Figure 2.1: Map of the Savannah River Site (SRS) with major streams, branches, and tributaries with sampling locations denoted as potentially bioavailable contaminated (PBC) or reference.

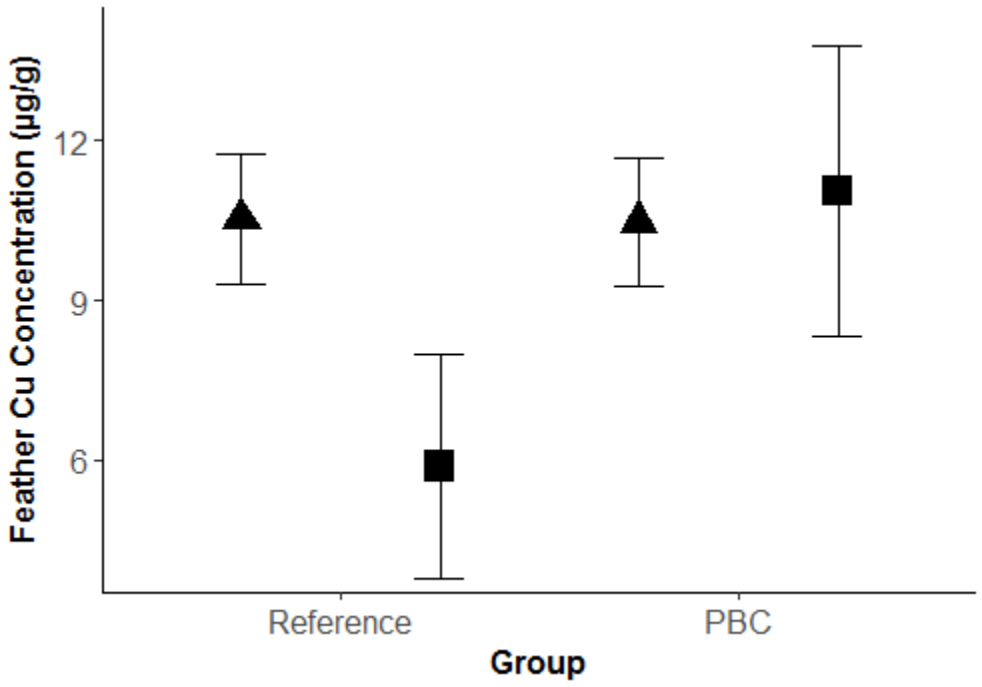

Figure 2.2: Predicted feather concentrations levels (dry weight) of copper (Cu) after model averaging (85% confidence interval) for Northern Cardinals (triangle, *n* = 50) and Great Crested Flycatchers (square, *n* = 20) collected at the Savannah River Site April-June 2016.

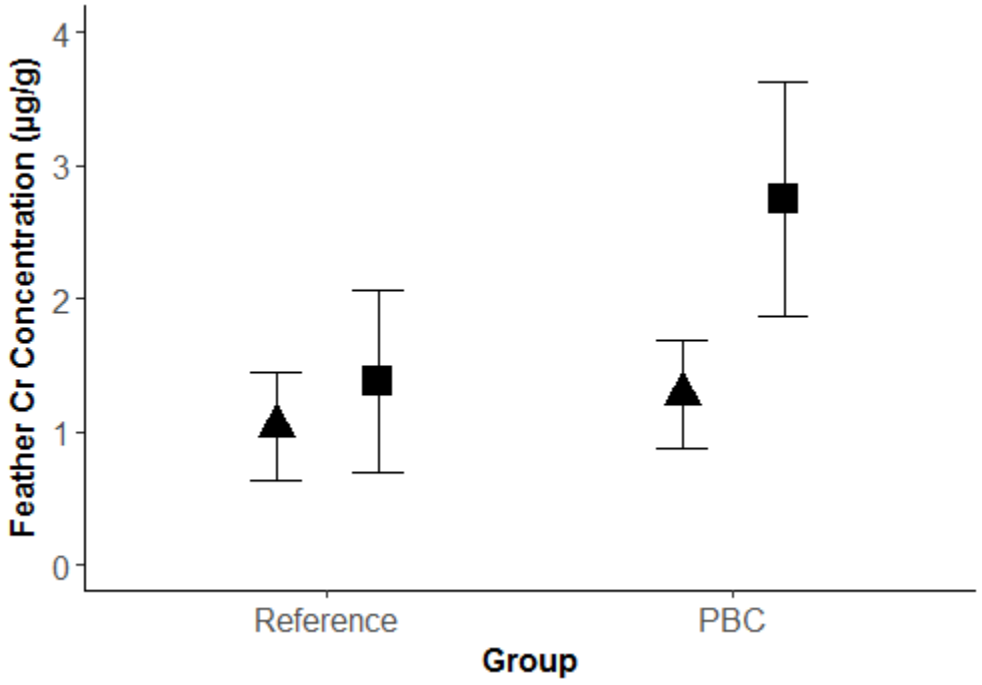

Figure 2.3: Predicted feather concentrations levels (dry weight) of chromium (Cr) after model averaging (85% confidence interval) for Northern Cardinals (triangle, *n* = 50) and Great Crested Flycatchers (square, *n* = 20) collected at the Savannah River Site April-June 2016.

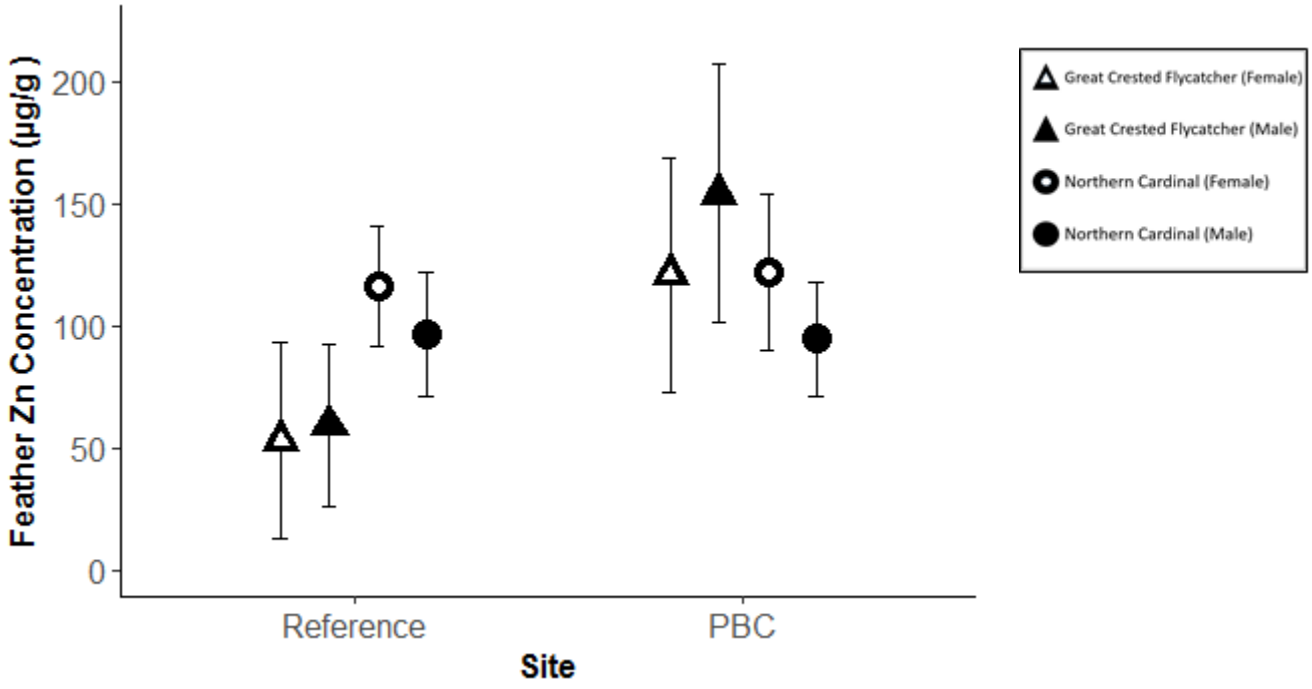

Figure 2.4: Predicted feather concentrations levels (dry weight) of zinc (Zn) after model averaging (85% confidence interval) for Northern Cardinals (circle, *n* = 50) by sex and Great Crested Flycatchers (triangle, *n* = 20) by sex that were collected at the Savannah River Site April-June 2016.

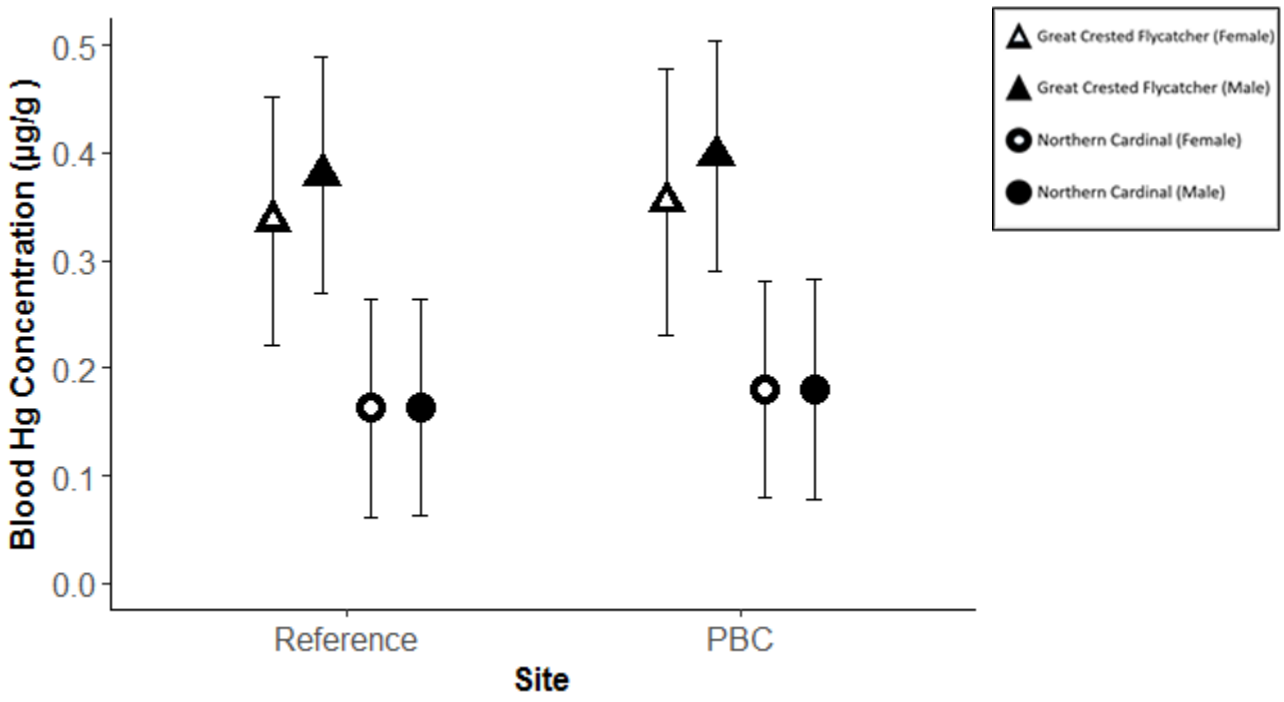

Figure 2.5: Predicted blood concentrations levels (wet weight) of mercury (Hg) after model averaging (85% confidence interval) for Northern Cardinals (circles, *n* = 33) by sex and Great Crested Flycatchers (triangles, *n* = 17) by sex that were collected at the Savannah River Site April-June 2016.

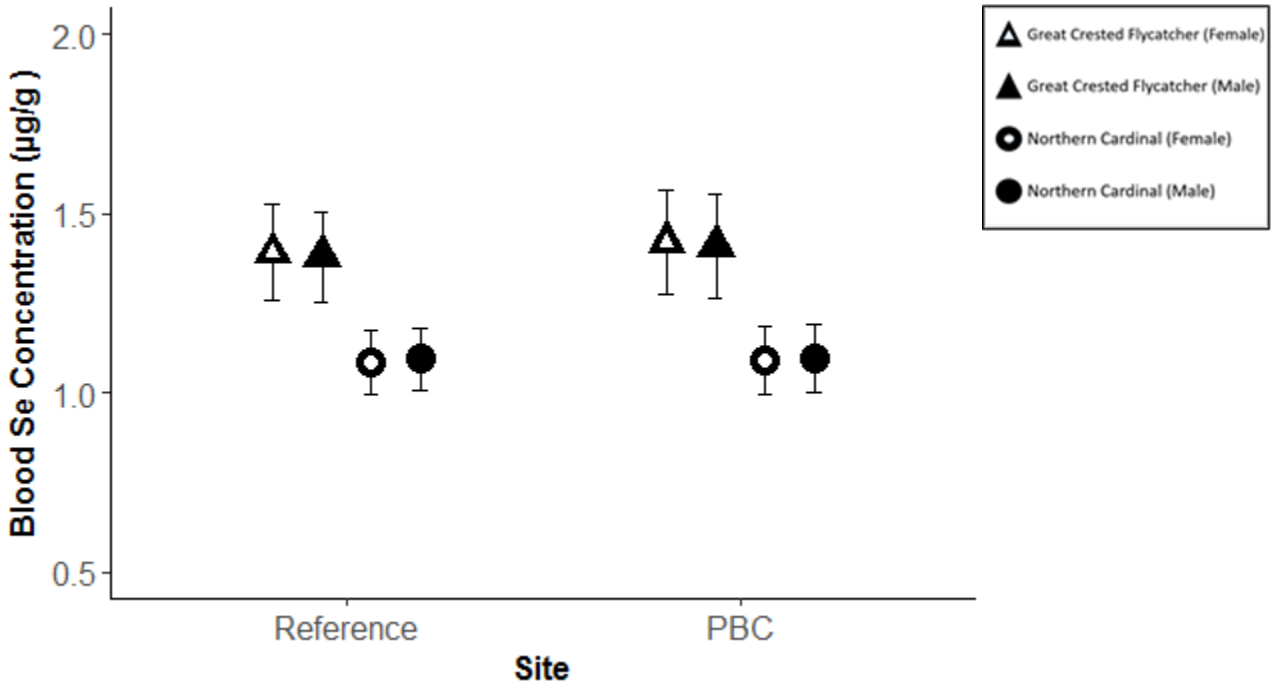

Figure 2.6: Predicted blood concentrations levels (wet weight) of selenium (Se) after model averaging (85% confidence interval) for Northern Cardinals (circles, *n* = 41) by sex and Great Crested Flycatchers (triangles, *n* = 19) by sex that were collected at the Savannah River Site April-June 2016.

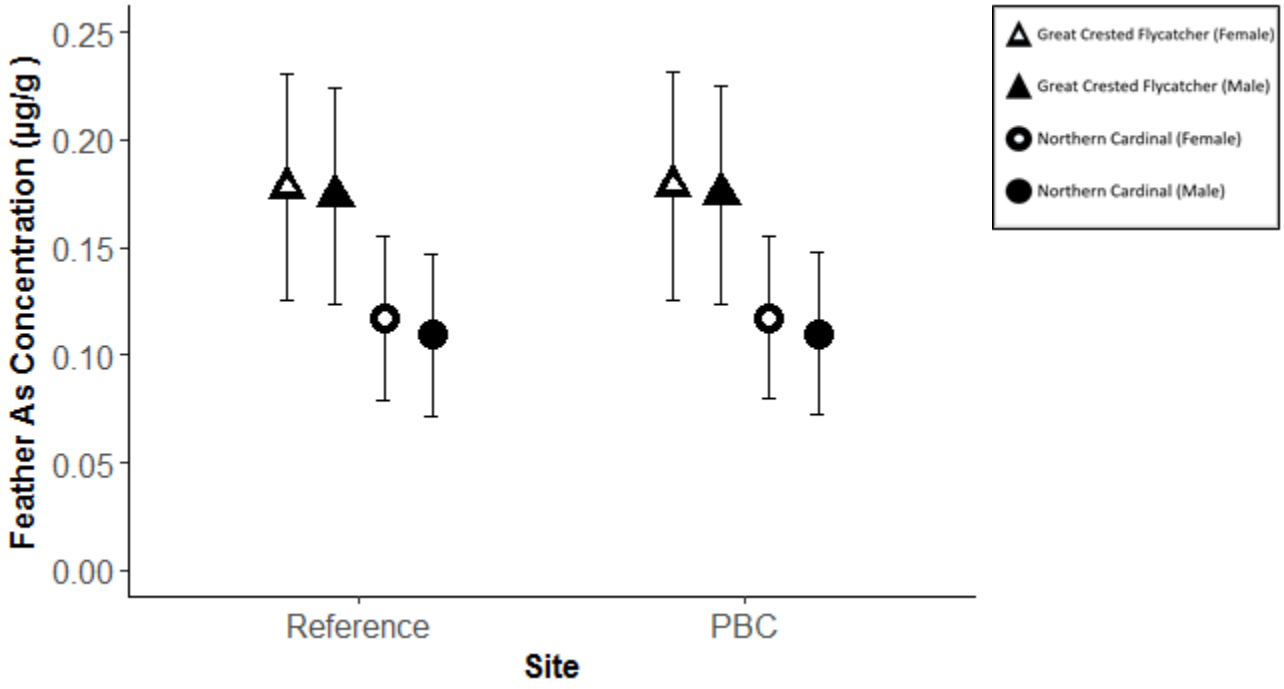

Figure 2.7: Predicted feather concentrations levels (dry weight) of arsenic (As) after model averaging (85% confidence interval) for Northern Cardinals (circle, *n* = 50) by sex and Great Crested Flycatchers (triangle, *n* = 20) by sex that were collected at the Savannah River Site April-June 2016.

INTER- AND INTRA- SPECIES VARIATION IN DIET FOR SONGBIRDS WITH DIFFERING MIGRATION STRATIGIES

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Introduction

The relationship between habitat and diet is critical to understanding the basic ecology of avian species. Describing avian diets can be done a myriad of ways such as stable isotopes and fecal analyses. In particular, stable isotope analysis in ornithological studies was introduced in the 1980s (Hobson 2011) to infer habitat use (Girard et al. 2012), food consumption (Pagani-Núñez et al. 2017), and location information (Hobson et al. 2012) without the expense of recapturing individuals or collection of fecal matter. Stable isotopes of carbon (^{13}C, ^{12}C) and nitrogen (^{15}N, ^{14}N) exist in every organism and are quantified in ratios that depend on the diet and trophic position of a species (Therrien et al. 2011). Since its inception, substantial improvements in analysis methods have led to increased use in many field studies (Rubenstein et al. 2002, Hobson 2005). Both $^{15}N/^{14}N$ ratios (hereafter $\delta^{15}N$) and $^{13}C/^{12}C$ (hereafter $\delta^{13}C$) are good predictors of trophic position or carbon source (Herrera et al. 2001, 2003, Diggs et al. 2011, Vitz and Rodewald 2012) and approximate prey composition (Inger and Bearhop 2008) as these ratios are related to protein intake (Mizutani et al. 1992). Therefore, consuming prey with higher protein concentrations could render higher $\delta^{15}N$ values (Diggs et al. 2011). Blood in songbirds produces information about recent diet intake of about 2-3 weeks (McKinnon et al. 2012) while feathers contain the isotopic make-up of diet during feather growth (Podlesak et al. 2005). Reconstructing the diet of each individual based on isotopic composition in animal tissues can be used to establish broad trophic position changes (Pearson et al. 2003) along environmental gradients such as habitat characteristics (Bearhop et al. 2006).

Vegetation structure is a common link between habitat use and diet composition for some species (Wolfe et al. 2014). Structural features of habitats may act as proximate factors for prey abundance for birds (Smith and Shugart 1987). This “structural cues” hypothesis suggests that

habitat is chosen based on the vegetation structure that generally yields the greatest arthropod abundance (i.e., resource availability). During the breeding season male songbirds establish a territory based on habitat quality (Hogstedt 1980, Jones et al. 2014) which can include prey abundance (Burke and Nol 1998, Penteriani et al. 2002) and vegetation structure for nests and cover (Germain and Arcese 2014, Romanowski and Zmihorski 2008). While the correlation between food availability and habitat use in songbirds has been researched extensively (Cody, 1985; Lack, 1968; MacArthur, 1958), some have suggested that habitat complexity itself (a diversity of mid-story and understory vegetation) drives non-breeding habitat selection (Moorman et al. 2012). In the central Savannah River area in South Carolina, there are a variety of habitats with unique vegetation structures. Insect abundance and richness is greatest in early successional habitat types than closed canopy forests in South Carolina (Horn et al. 2005, Ulyshen et al. 2005). Forest habitats associated with water bodies (streams, lakes, rivers, etc.) generally have higher insect abundance and diversity in this region (English 1991) than drier forest types such as pine stands or mixed forests. Previous research suggests early successional habitats support greater avian use during post-breeding periods (Suthers et al. 2000, Mudrzynski and Norment 2013) indicating abundant resource availability.

Resource needs are unique to each discrete phase of a bird's annual cycle with the breeding season having the most numerous requirements for protein in particular (Vitz and Rodewald 2011). Thus, diet of passerines changes based on season (McKinnon et al. 2017) and energy requirements (migration, breeding, etc.) or availability of food items (Gagnon and Hobson 2009) even when some species are considered insectivorous or frugivorous (Bairlein 1996, Parrish 2000). Songbirds typically switch from an insect-based diet during breeding season to a diet incorporating mostly fruits in the non-breeding season (Suthers et al. 2000, Mudrzynski

and Norment 2013, McKinnon et al. 2017) but seems to vary greatly among species (Gagnon and Hobson 2009). These seasonal diet changes may be extremely subtle and require molecular approaches to fully understand the changes in trophic position. Determining fine scale changes in diet differences between the breeding and non-breeding season in specific species provides details of important food items for survival (Renfrew et al. 2017). Diet is usually assessed by measuring arthropod (Moorman et al. 2012) and fruit abundance (Suthers et al. 2000) in an associated habitat but less frequently via stable isotopes (Gagnon and Hobson 2009).

Lastly, diet can vary within a species based on phenotypes. Physical attributes differ in songbirds depending on sex with males usually being slightly larger (e.g., wing chord) and having greater observed mass (Pyle 1997); also, morphometries can vary within a particular sex. During the breeding season, the nutritional diet requirements differ for females than for males as females may lose mass due to an energy deficit from feeding and incubating nestlings (Johnson et al. 1990, Nagy et al. 2007). Body condition of females also changes based on habitat use (Inger et al. 2008) with females obtaining a greater fitness in higher quality habitat. Previous studies suggest no difference in prey selection between females and males in the non-breeding season (Brown and Sherry 2006) but others disagree as McKinnon et al. (2017) found females consuming more fruit than males in the non-breeding season.

The objectives of this study were to determine diet differences in Great Crested Flycatchers (*Myarchus crinitis*) and Northern Cardinals (*Cardinalis cardinalis*) between breeding and non-breeding seasons using two types of tissue samples (blood and feather), assess the effect of breeding season habitat use on diet (i.e., $\delta^{13}C$ and $\delta^{15}N$ values) in each species, and evaluate any changes in diet based on phenotypes. Our migratory diet shift hypothesis states that diet fluctuates for migratory birds between breeding and non-breeding season due to resource

availability changes. We predict that trophic position will decrease for flycatchers between the breeding and non-breeding season because of a greater plant-based diet in the fall and winter (Podlesak et al. 2005, Mudrzynski and Norment 2013). However, cardinals will not change diet because of a similar diet for both seasons. We also hypothesize, based on the resource availability hypothesis, bird diet will change along a gradient of vegetation structure. Our prediction is that individual birds breeding in areas containing early successional vegetation structure will have higher $\delta^{15}N$ values due to higher insect abundances associated with this habitat type (English 1991, Suthers et al. 2000, Mudrzynski and Norment 2013). Finally, our individual variability hypothesis states sex and size differences among individual birds affects diet composition during the breeding season. We predict that smaller songbirds will require a diet higher in protein and females overall will also require more protein (Nagy et al. 2007) during the breeding season resulting in higher $\delta^{13}C$ and $\delta^{15}N$ values in blood samples.

Methods

Study area

The Savannah River Site (SRS) is owned by the U.S. Department of Energy and is located south of Aiken, South Carolina. At over 800 km^2, this facility has a diversity of habitats and management techniques but the majority is managed by the U.S. Forest Service for loblolly (*Pinus taeda*) and longleaf (*Pinus palustris*) pine timber with frequent prescribed fire (Kilgo and Blake 2005). Mixed pine/hardwood forests are common and include varieties of oaks (*Quercus* spp.), hickory (*Carya* spp.), red maple (*Acer rubrum*), black gum (*Nyssa sylvatica*), and pine (*Pinus* spp.) with an understory of holly (*Ilex* spp.) and fetterbush (*Lyonia lucida*) among others. Upland hardwoods also contain a variety of oaks (*Quercus* spp.), red maple (*Acer rubrum*), and sweetgum (*Liquidlambar styraciflua*) with a mid-story of dogwood (*Cornus florida*), briars

(*Smilax* spp.), blackberry (*Rubus* spp.), and sassafras (*Sassafras albidum*). Bottomland hardwoods follow tributaries, streams, and floodplains with characteristic red ash (*Fraxinus pennsylvanica*), sweetgum (*Liquidlambar styraciflua*), black gum (*Nyssa sylvatica*), water oak (*Quercus nigra*), and hackberry (*Celtis laevigata*). Sometimes dense understory species include virginia willow (*Itea virginica*) and dog hobble (*Leucothoe axillaris*). Silver Bluff Audubon Center, located west of Jackson, South Carolina, was the second location where samples were collected. This 3,250-acre preserve is located 17 km northwest of the SRS along the Savannah River with similar habitat and vegetation characteristics.

Study design

We sampled adult Great Crested Flycatchers (*Myiarchus crinitus*; hereafter, flycatchers) and Northern Cardinals (*Cardinalis cardinalis*; hereafter, cardinals) in various habitats in four randomly selected locations that were within 1 km from a body of water. Each location had a ~2.5-3.5 km radius. All locations were more than 10 km apart in the central Savannah River area with three locations on the SRS and one on the Silver Bluff Audubon property. Birds were caught using 36 mm mist nests that were opened at sunrise and closed by 1030 h or earlier if weather conditions were unacceptable (i.e., precipitation, excessive wind >25 km hr^{-1}, high temperatures >29° C). Flycatchers were targeted using conspecific playback of songs and calls on speakers while cardinals were caught either passively or with playback. Each adult individual received an aluminum USGS band (USGS BBL Permit Number 22002). Sex, mass, wing chord, and tarsus were determined and measured on each individual. Approximately 8-12 breast feathers were collected and blood samples (~0.05-0.30 ml) were drawn from the jugular vein of each individual (Institutional Animal Care and Use Committee Permit Number A2016 02-022-R1). Blood samples were stored in lithium heparin tubes on ice until taken to the lab where they

were frozen at < - 18° C. Feather samples were stored in sterile plastic bags and placed on ice. All birds caught were immediately released upon completion of sample collection.

Each individual bird's point of capture was marked with a global position system (GPS). Territories were then mapped in ArcMap 10.4.1 (Environmental Systems Research Institute, Inc., Redlands, CA) with a 87 meter radius around each point of capture as this was equal to approximate territory sizes for both species (Halkin and Linville 1999, Miller and Lanyon 2014). Cardinals are a resident species and inhabit their breeding territories year-round while flycatchers are a migratory species and only occupy their breeding territories in the spring and summer. Habitat types (e.g., pine stand, bottomland hardwood, etc.) were digitized from aerial imagery and the percent of each habitat type in each territory was calculated. Distance to the nearest permanent body of water (e.g., stream, lake, river, wetland, etc.) was also calculated from the center point of each territory.

Sample treatment and isotope analysis

Feathers were stored at ~3°C until analysis and then freeze-dried for 48hr. Approximately 1-3 feathers were removed from the storage bag and immersed into a 2:1 ratio of chloroform/methanol solution with a solvent value 3-5 times the sample volume to remove surface contaminants using methods from Bligh and Dyer (1959) modified by Logan et al. (2008). Samples were dried in a fume hood for 48hrs. Feathers were then cut to a weight between 0.10-0.50 mg and placed in 5mm x 9mm tin capsules for analysis of total % C and N as well as $\delta^{13}C$ and $\delta^{15}N$ ratios. Whole blood samples were frozen at ~-70°C and then freeze-dried for ~24h. Samples were homogenized and weighed in 5mm x 9mm tin capsules for C/N analysis using a Thermo, Delta V, isotope ratio mass spectrometer (IRMS) coupled with a Thermo CE Isolink combustion elemental analyzer. Internal reference material was used to verify

measurement precision. Stable isotope ratios were expressed as parts per thousand. All samples were analyzed at the Center for Applied Isotope Studies, University of Georgia, Athens, GA.

Statistical analysis

All statistical analyses were conducted in R (version 3.3.1, R Core Team, 2016). To determine if individual measurements (mass, tarsus, wing chord, sex) predicted $\delta^{13}C$ and $\delta^{15}N$ ratios, blood and feather were analyzed for each species using linear mixed models with independent variables being sex, tarsus length, wing chord, and mass. Site was considered a random effect because multiple individuals were captured at each location (i.e., three sites on SRS and one on Silver Bluff). Similarly, linear mixed-effect models were also utilized for the influence of habitat on $\delta^{13}C$ and $\delta^{15}N$ values with independent variables being distance to water, bottomland hardwood, upland hardwood, pine stand, mixed-forest, early successional, and any morphometric variables that were significant from the morphometric analysis. Our candidate models included high quality habitat variables (distance to water, bottomland hardwood, and early successional) tested separately and lower quality habitat variables (pine stand, upland hardwoods, and mixed-forests) were tested in a single model. Sex and morphometric variables were added to habitat model selection if they were found to be significant predictors of $\delta^{15}N$ values or $\delta^{13}C$ in each species and tissue type in the previous analysis. Site was also incorporated as a random effect. For all analyses, different sets of covariates were added to each model and tested using Akaike information criteria adjusted for small sample size (AIC_c) to choose the best model (Burnham and Anderson 2002). The lmer function from package lme4 (Bates et al. 2015) was used to fit models. Models were considered competing if $\Delta AIC < 4$. Covariates in the model were considered biologically informative if the confidence intervals (85%) did not cross zero (Arnold 2010); however, we further discerned biological relevance by considering the

change in isotope values relative to the change in the physiological and/or environmental gradient. The effects R-package (Fox 2003) was used to predict marginal means ($\bar{x}$) represented in figures and text.

Results

A total of 61 individuals (30 cardinals and 31 flycatchers) were captured and sampled from April to May 2017 in four locations in the central Savannah River area. Thirteen females, 13 males, and five unknown sex flycatchers were sampled. Of the 30 cardinals, there were 12 females and 18 males sampled. Mass ranged from 28.9 g to 38.9 g in flycatchers and 36.7 g to 45.9 g in cardinals. Mean (± sd) $\delta^{15}N$ values for cardinals were 3.64 (± 1.56) in feathers and 3.10 (± 1.32) in blood. While mean (± sd) $\delta^{13}C$ values were -25.35 (± 1.19) in feathers and -25.65 (± 0.75) in blood. Flycatchers had mean (± sd) $\delta^{15}N$ values of 7.64 (± 2.43) in feathers and 5.58 (± 1.45) in blood and mean (± sd) $\delta^{13}C$ values of -23.66 (± 0.75) in feathers and -24.87 (± 0.49) in blood (Table 3.1). Territories of both cardinals and flycatchers consisted of (in descending order of proportion) mixed forest, pine stand, upland hardwoods, early successional, and bottomland hardwoods habitat types (Table 3.2). Cardinal territories had significant correlations with upland hardwoods and mixed forests ($r = 0.61$, $P = 0.001$) and with pine stands and mixed forests ($r = -0.66$, $P < 0.001$). Flycatcher territories had a significant correlation with mixed forests and pine stands ($r = -0.65$, $P < 0.001$) but no other habitat types. Complete habitat proportion and correlation information can be found in Appendix B tables 1-3.

Migratory diet shift hypothesis

There was a significant interaction between season and species (coefficient table in Appendix B) supporting the migratory diet shift hypothesis. Cardinals had similar $\delta^{15}N$ values between breeding and non-breeding seasons ($\bar{x} = 3.19$, 85% CL = [1.52, 4.86] and $\bar{x} = 3.73$, 85%

CL = [2.05, 5.39], respectively; Fig. 3.1). However, flycatchers displayed greater $\delta^{15}N$ in the non-breeding season than the breeding season ($\bar{x}$ = 7.71, 85% CL = [6.06, 9.35] and $\bar{x}$ = 5.64, 85% CL = [4.00, 7.28], respectively, Fig. 3.1). For $\delta^{13}C$, a similar pattern was observed with flycatchers having a higher value in the non-breeding season than in the breeding season ($\bar{x}$ = -23.64, 85% CL = [-24.13, -23.15] and $\bar{x}$ = -24.85, 85% CL = [-25.34, -24.36], respectively; Fig.2). As with $\delta^{15}N$, cardinals had very similar $\delta^{13}C$ values between seasons (Fig. 3.2).

Individual morphometric analysis

Mass and tarsus were significant predictors of $\delta^{15}N$ values in blood of cardinals. Every 2.4 gram increase (1 SD) in mass decreased $\delta^{15}N$ values by 0.36 and increased by 0.45 for every 1.5 mm increase in tarsus length. This provides partial support for our hypothesis relating individual variability to diet as birds with less mass had higher nitrogen values but not size in general. Feather $\delta^{15}N$ values in cardinals increased by 0.53 for every 1.5 mm increase in tarsus length and males had 0.83 less $\delta^{15}N$ than females (β = -0.83, 85% CL = -1.51, -0.15). $\delta^{13}C$ in cardinal blood was significantly affected by sex with males having 0.52 more $\delta^{13}C$ than females (β = 0.52, 85% CL = 0.18, 0.85) and decreased by 0.33 for every 1.4 mm increase in tarsus length. $\delta^{13}C$ values in blood of flycatchers were 0.18 less per 2.5 gram increase in mass while $\delta^{13}C$ in feathers of flycatchers decreased by 0.35 with every 4.2 mm increase in wing length. $\delta^{15}N$ values in flycatcher feathers also decreased by 1.1 with every 1.2 mm increase in tarsus length. Physical attributes of individuals (sex, tarsus, mass, and wing chord) did not have any statistical significant on $\delta^{13}C$ in feathers of cardinals or $\delta^{15}N$ values in blood of flycatchers. The lack of morphometric statistical significance of $\delta^{15}N$ in flycatcher blood does not support our individual variability hypothesis. All AIC_c tables and mean $\delta^{13}C$ and $\delta^{15}N$ values are included in Appendix B.

Habitat influence on isotopic values

The resource availability habitat hypothesis was supported during the breeding season for $\delta^{15}N$ but not during the non-breeding season in cardinals. During the non-breeding season, distance to water from the territory of each cardinal was associated with $\delta^{15}N$. As distance to water increased by ~ 292 meters (1 SD) $\delta^{15}N$ decreased by 0.82 (Fig. 3.3). Male cardinals had ~0.74 less $\delta^{15}N$ than females (β = -0.74, 85% CL = -1.36, -0.12; Fig. 3.5) and conversely, every 1.5 mm increase in tarsus length increased $\delta^{15}N$ by 0.63 (Fig. 3.4). There were no significant predictor variables to explain $\delta^{13}C$ values in cardinal feathers.

The breeding season produced different results in cardinals. For every ~ 15% increase in early successional habitat, $\delta^{15}N$ increased by 0.58, for every 2.5 mm increase in tarsus length $\delta^{15}N$ increased by 0.56, and for every ~ 2.4 gram increase in weight $\delta^{15}N$ decrease by 0.35 (Figures 3.6-3.8, respectively). The four variables predicted $\delta^{13}C$ in cardinal blood. As distance to water increased by ~ 292 meters $\delta^{13}C$ increased by 0.18; every 2.5 mm increase in tarsus length decreases $\delta^{13}C$ by 0.43; males had 0.36 more $\delta^{13}C$ than females (β = 0.36, 85% CL = 0.06, 0.66) and as bottomland hardwood habitat increased by ~ 11%, $\delta^{13}C$ decreased by 0.19 (Figures 3.9-3.12, respectively).

For flycatchers breeding in the central Savannah River area, $\delta^{15}N$ increases for every ~16% increase in bottomland hardwood habitat by 0.35 (Fig. 3.13). As bottomland hardwood habitat increases by ~ 16%, $\delta^{13}C$ increases by 0.15 and every 2.6 gram increase in mass, $\delta^{13}C$ decreased by 0.18 (Figures 3.14 and 3.15, respectively). These results are not consistent with the resource availability habitat hypothesis.

Discussion

Our results suggest that different tissue types from the same individual can potentially point to seasonal changes in $\delta^{15}N$ and $\delta^{13}C$ values indicating differences in diet across a bird's annual cycle. The differences found between seasons for flycatchers support our belief that diet composition changes from breeding to time of molt even though the changes are not drastic. $\delta^{15}N$ and $\delta^{13}C$ values found in cardinals during the breeding season agree with our resource availability hypothesis but flycatcher results do not.

Cardinals partially supported the individual variability hypothesis as smaller individuals (less mass) had higher $\delta^{15}N$ values during the breeding season even though having a larger tarsus increased levels. A similar effect was seen in breeding flycatchers as $\delta^{15}N$ values decreased with mass. On the contrary, Diggs et al. (2011) found an opposite pattern with an increase in mass leading to higher $\delta^{15}N$ and $\delta^{13}C$. Although, both cardinals and flycatchers with small tarsus length had higher $\delta^{15}N$ values, which disagreed with our small size prediction, was also observed in other studies (Moller and Hobson 2004). Because sex was not a statistically significant factor influencing $\delta^{15}N$ in the breeding season, this supports with the notion that while males and females may forage at different vegetation heights (Holmes 1986), both sexes may consume similar prey (Busby and Sealy 1979). Females during the breeding season lose more mass than males (Nagy et al. 2007) which we would have expected to observe a change associated with their diet. However, the quantitative amount of prey items may have increased or changed in either sex but this information is not measured with stable isotope analysis. We cannot be certain that individual morphometric measurements are associated with $\delta^{13}C$ and $\delta^{15}N$ in feathers of our two species because these measurements (especially mass) could have changed since their molt in the fall. Overall, it is difficult to discern whether subtle phenotypic differences have a

biological influence on $\delta^{15}N$ and $\delta^{13}C$ values in these two species or if other unknown factors alter these values.

Flycatchers, as a migratory species, showed the greatest shift in $\delta^{13}C$ and $\delta^{15}N$ values between tissue types -- and therefore, seasons -- this conformed to our predictions. The increased $\delta^{13}C$ and $\delta^{15}N$ in the non-breeding season could be an indication of migratory and molt requirements that necessitate more protein for increase in muscle mass (Schwilch et al. 2002). Higher $\delta^{15}N$ values are associated with insect laden diet [specifically larvae and spiders; (Fair et al. 2013, Pagani-Núñez et al. 2017)] while lower values are indicative of lower trophic level insects and fruits (McKinnon et al. 2017). Arthropod prey species availability may have also shifted between seasons as higher trophic level predatory arthropods emerge later in year (Tibbets et al. 2008) and could have led to the observed $\delta^{15}N$ increase. Although, the increase may not be significantly related to diet but instead could be a result of a difference in location (Kelly 2000). However, others have disputed that nitrogen in prey is unrelated to $\delta^{15}N$ values but instead to the physiological effect in $\delta^{15}N$ enrichment of tissues (Hobson et al. 1993). While many species of songbirds switch from an insect-based diet during the breeding season to a frugivorous one during migration or the non-breeding season (Gagnon and Hobson 2009, McKinnon et al. 2017), this may not be the case of flycatchers in this study. Insect availability is prolonged in the southeast and therefore may be chosen over fruits (Long and Stouffer 2003) as protein and nitrogen are essential for muscle mass (Blem 1990) and may have influenced $\delta^{15}N$ values. As predicted, cardinal $\delta^{13}C$ and $\delta^{15}N$ values did not differ significantly between seasons. Cardinals, as a resident species in the southeastern US, would not be expected to have a major diet shift in between seasons as many of the same resources are found in both seasons. Our results also support the notion that using blood and tissue can indicate diet changes in songbirds.

Resource availability seemed to be associated with cardinal $\delta^{15}N$ values in the breeding season of cardinals indicating early successional habitat may yield higher insect prey use that possibly let to greater $\delta^{15}N$ values. Similarly, the distance to water association with $\delta^{15}N$ during the non-breeding season points to a possible increase in insect prey abundance closer to bodies of water in the fall. The slight increase in $\delta^{15}N$ in bottomland hardwood habitats for flycatchers is insufficient to conclude there is an association of such habitat on $\delta^{15}N$ values for this species. $\delta^{13}C$ changes based on habitat in both species were statistically significant but likely do not render any biological significance as the differences were minimal. If the $\delta^{13}C$ value changes were much greater (e.g. ~ > 10 ‰), there would be an indication of a transfer from a C3 to a C4 plant-based food webs (Ehleringer et al. 1986, Hobson 2011) and we would not expect such a drastic change in this system.

Our assumption that $\delta^{15}N$ is directly associated with protein levels in food items may be incorrect as noted in previous research (Hobson et al. 1993) which would alter the interpretation of our results. Measuring $\delta^{13}C$ and $\delta^{15}N$ values in a migratory species may be problematic as the changes could be attributed to the physical change in location (Hobson 2005); however, recent research supports our interpretation as higher $\delta^{15}N$ especially were correlated higher trophic level prey such as spiders (Pagani-Núñez et al. 2017). Also, body molt timing and location in Great Crested Flycatchers is currently unknown and could have occurred on the breeding grounds, but we assumed it was during the fall on or near their wintering territories (Miller and Lanyon 2014). Further research into how exactly prey protein translates into $\delta^{15}N$ in these species would give better dietary information. Additional research into small-scale movement of both resident and migratory adult songbirds between breeding and non-breeding seasons would improve habitat requirement information. Probable prey item sample may also improve details into the specific

choice of prey for each species aiding in knowledge of habitat requirements for the birds that offer such prey. Habitat type influences on diet may have less to do with resource availability and more with vegetation structure requirements during contrasting seasons.

In conclusion, traditional classifications of songbirds into foraging guilds (frugivorous, insectivorous, etc.) may be too broad, as it appears the diet of some birds can change between seasons. Using $\delta^{13}C$ and $\delta^{15}N$, we observed seasonal changes in diet between our two species; however, we cannot say specifically how their diet changed (e.g. more insects, less fruit, etc.). Habitat and phenotypic characteristics appear to be associated with $\delta^{13}C$ and $\delta^{15}N$ values in these two species although these associations may not be biologically significant.

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Table 3.1: Average isotope values (± SD) based on tissue type for Northern Cardinals (*Cardinalis cardinalis*; n = 26) and Great Crested Flycatchers (*Myarchus crinitis*; n = 29) captured in the central Savannah River area in South Carolina from April to May 2017.

	Northern Cardinal				Great Crested Flycatcher			
	Feather		Blood		Feather		Blood	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$\delta^{15}N$	3.64	1.56	3.10	1.32	7.64	2.43	5.58	1.45
$\delta^{13}C$	-25.35	1.19	-25.65	0.75	-23.66	0.75	-24.87	0.49

Table 3.2: Average habitat type proportions in the breeding territories of Northern Cardinals (*Cardinalis cardinalis*; *n* = 26) and Great Crested Flycatchers (*Myarchus crinitis*; *n* = 29) captured in the central Savannah River area in South Carolina from April to May 2017.

	Northern Cardinal		Great Crested Flycatcher	
Habitat type	Mean	SD	Mean	SD
Early Successional	0.10	0.15	0.04	0.08
Pine Stand	0.26	0.28	0.27	0.35
Mixed Forest	0.42	0.32	0.39	0.34
Bottomland Hardwood	0.06	0.11	0.08	0.16
Upland Hardwood	0.10	0.15	0.10	0.15

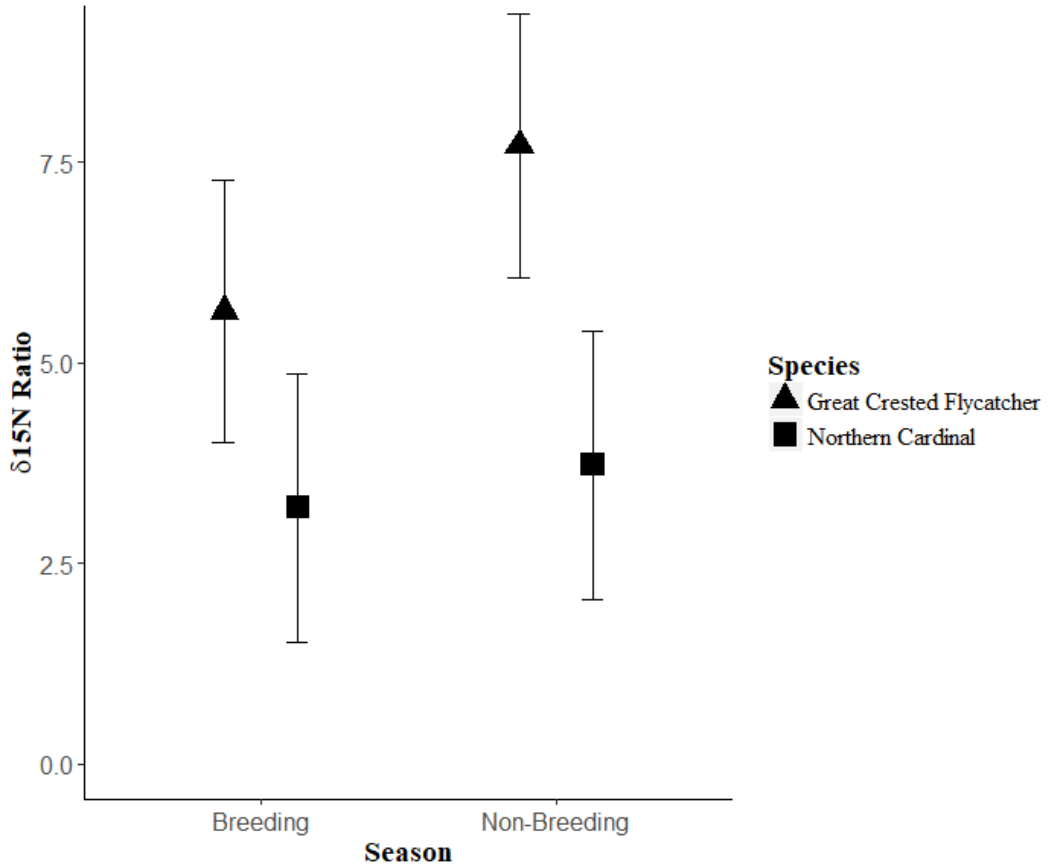

Figure 3.8: Predicted means from linear-mixed model using effects R-Package. $\delta^{15}N$ values for Great Crested Flycatchers (*Myarchus crinitis*; *n* = 29) and Northern Cardinals (*Cardinalis cardinalis*; *n* = 26) captured in the central Savannah River area of South Carolina from April to May 2017. Blood samples were analyzed for breeding season values and feathers were analyzed for non-breeding season values.

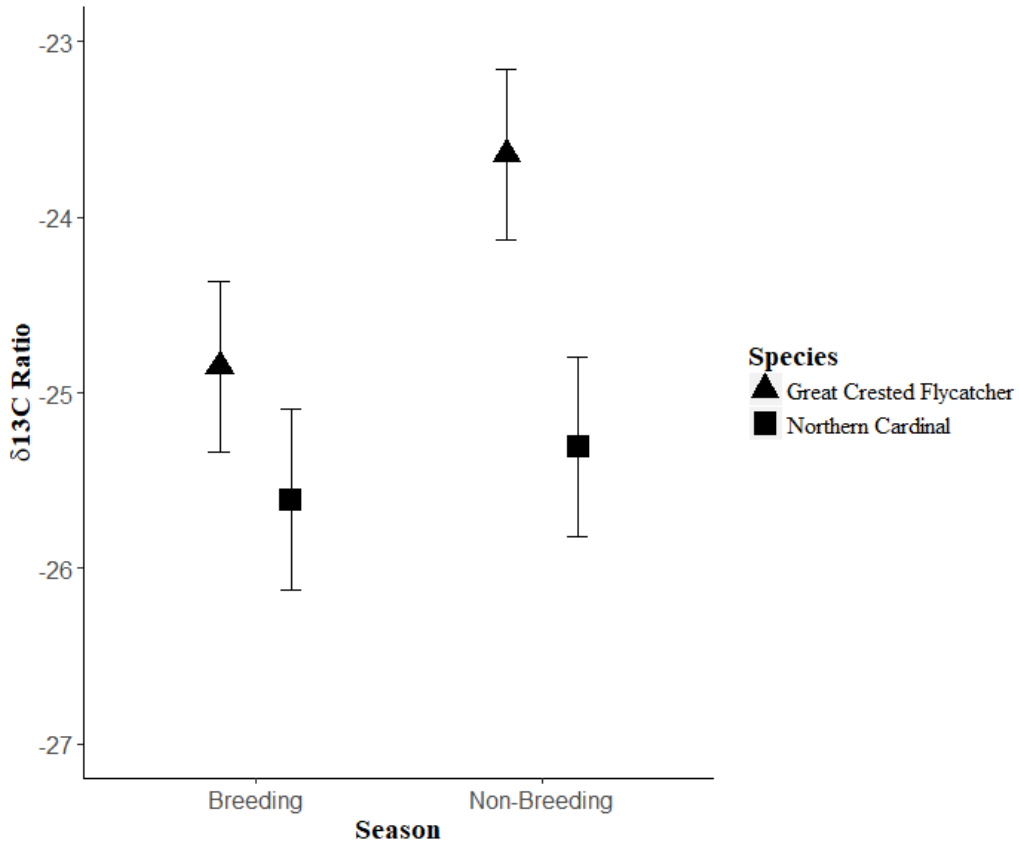

Figure 3.9: Predicted means from linear-mixed model using effects R-Package. $\delta^{13}C$ values for Great Crested Flycatchers (*Myarchus crinitis*; *n* = 29) and Northern Cardinals (*Cardinalis cardinalis*; *n* = 26) captured in the central Savannah River area of South Carolina from April to May 2017. Blood samples were analyzed for breeding season values and feathers were analyzed for non-breeding season values.

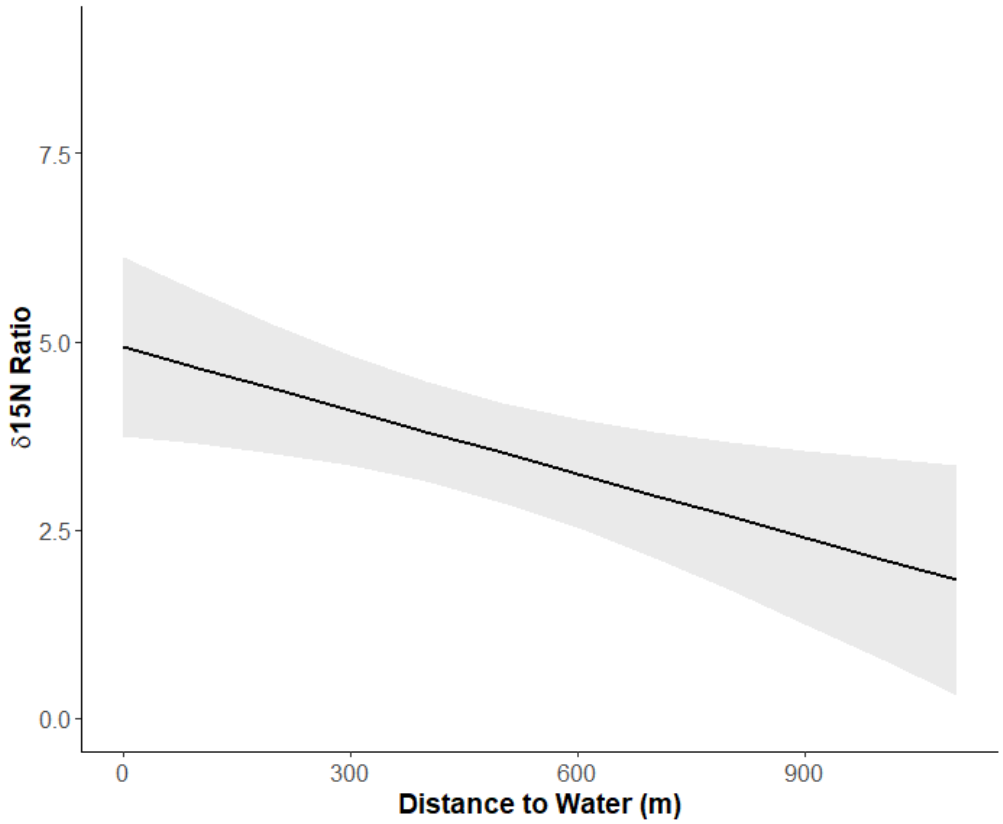

Figure 3.10: Changes in $\delta^{15}N$ values in feathers based on distance to nearest body of water (85% CI) during the non-breeding season in Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

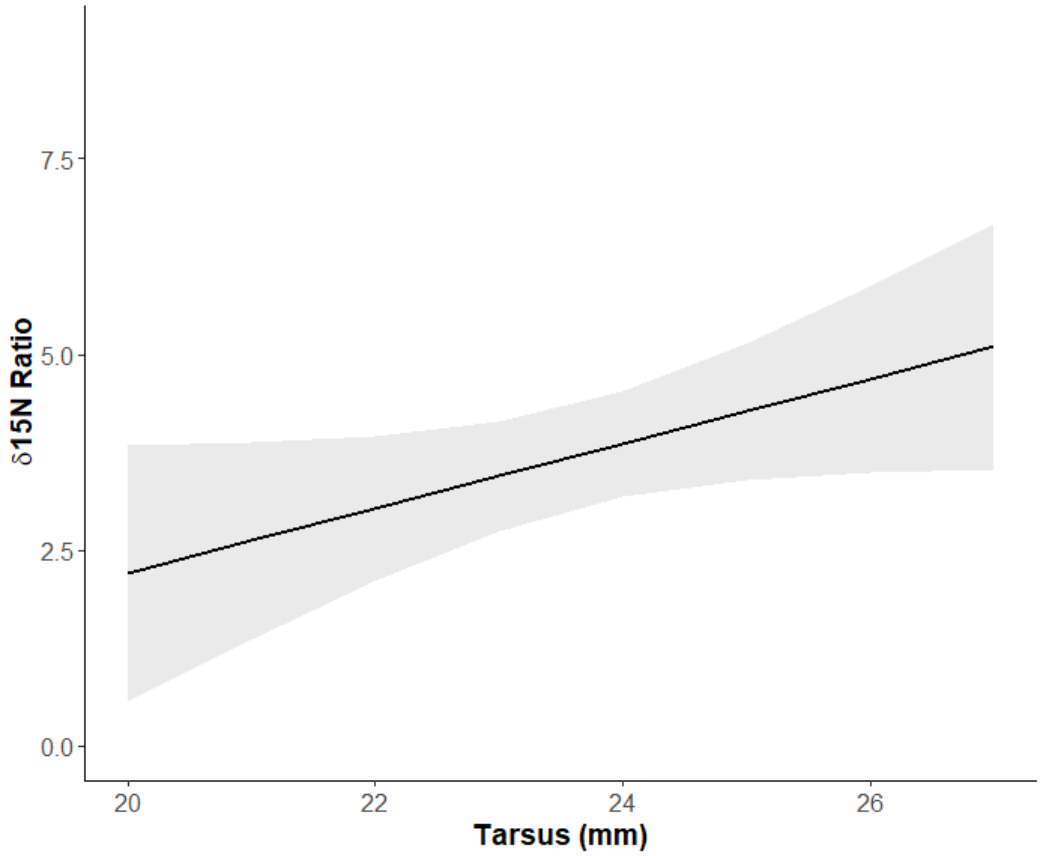

Figure 3.11: Changes in $\delta^{15}N$ values in feathers based on tarsus length (85% CI) in Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

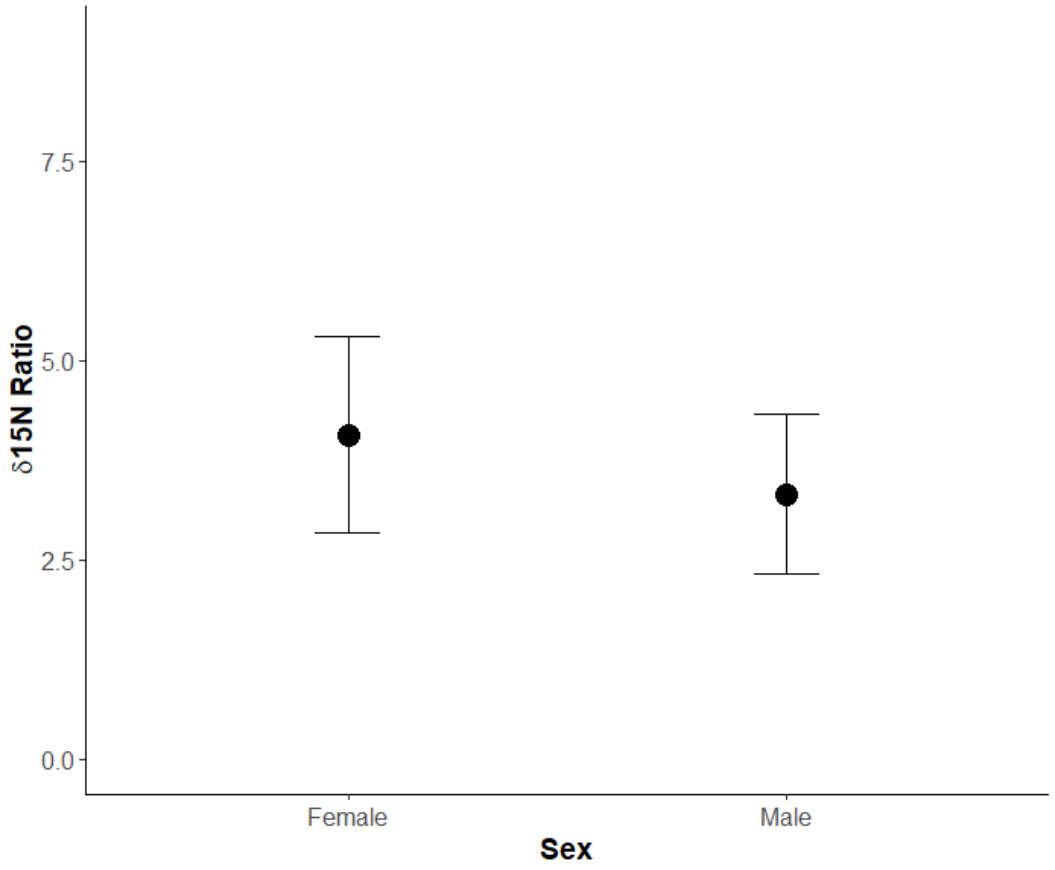

Figure 3.12: Changes in $\delta^{15}N$ values in feathers based on sex (85% CI) in Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

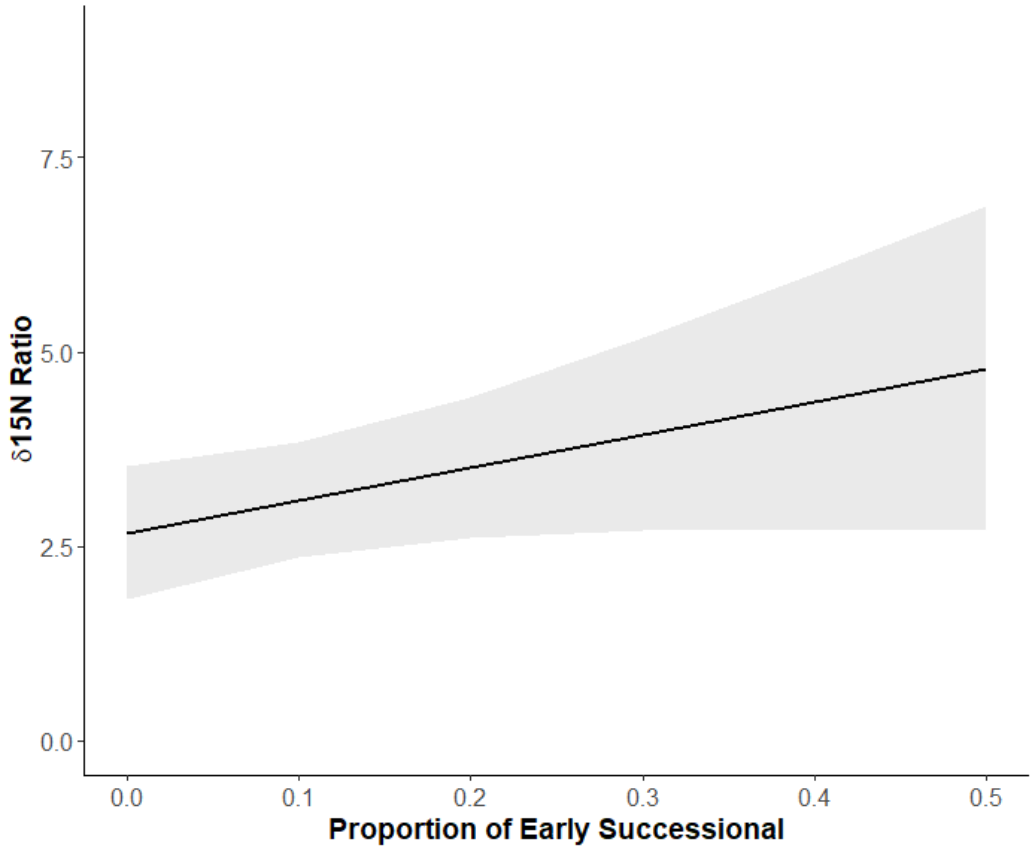

Figure 3.13: Changes in $\delta^{15}N$ values in blood based on proportion of early successional habitat (85% CI) in the territories of Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

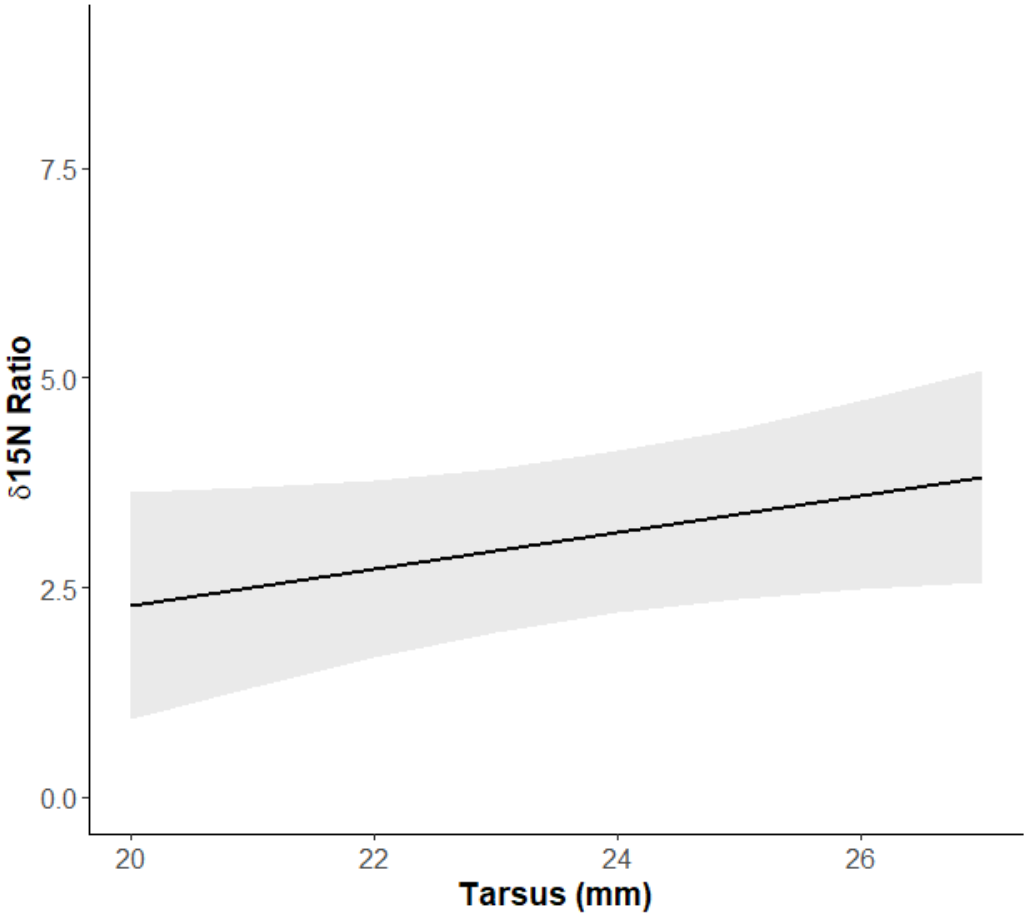

Figure 3.14: Changes in $\delta^{15}N$ values in blood based on tarsus length (85% CI) of Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

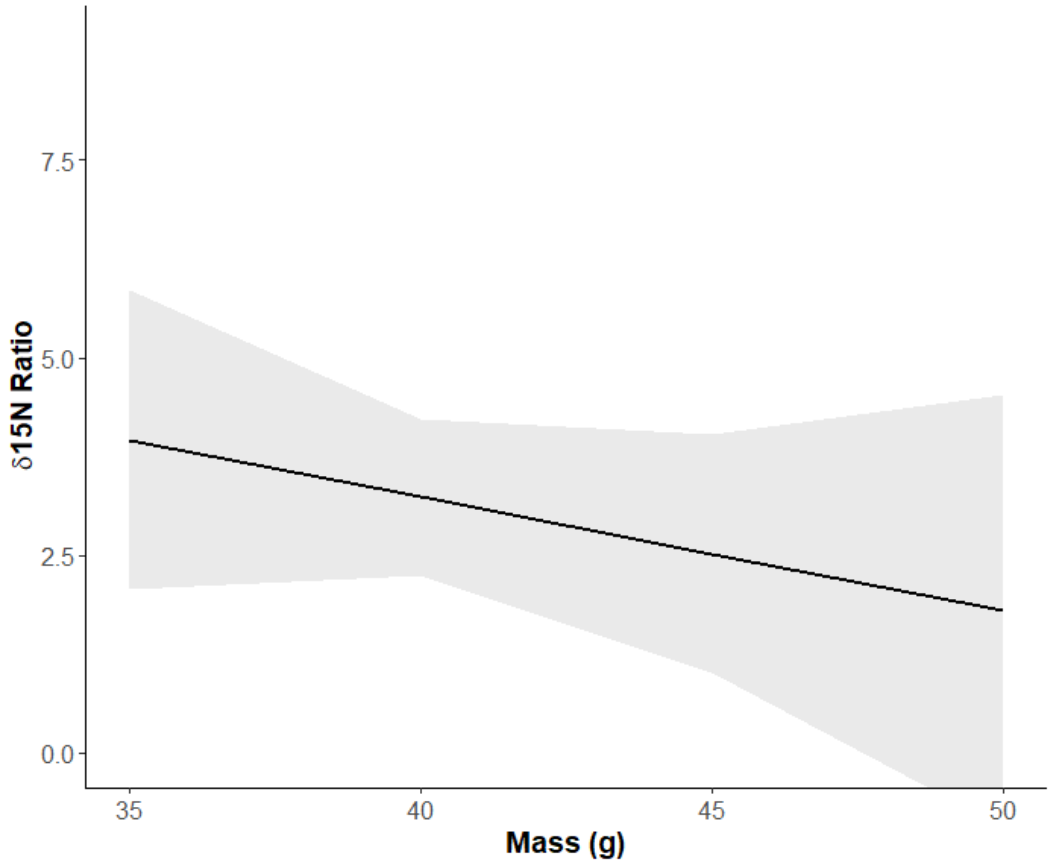

Figure 3.15: Changes in $\delta^{15}N$ values in blood based on mass (85% CI) of Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

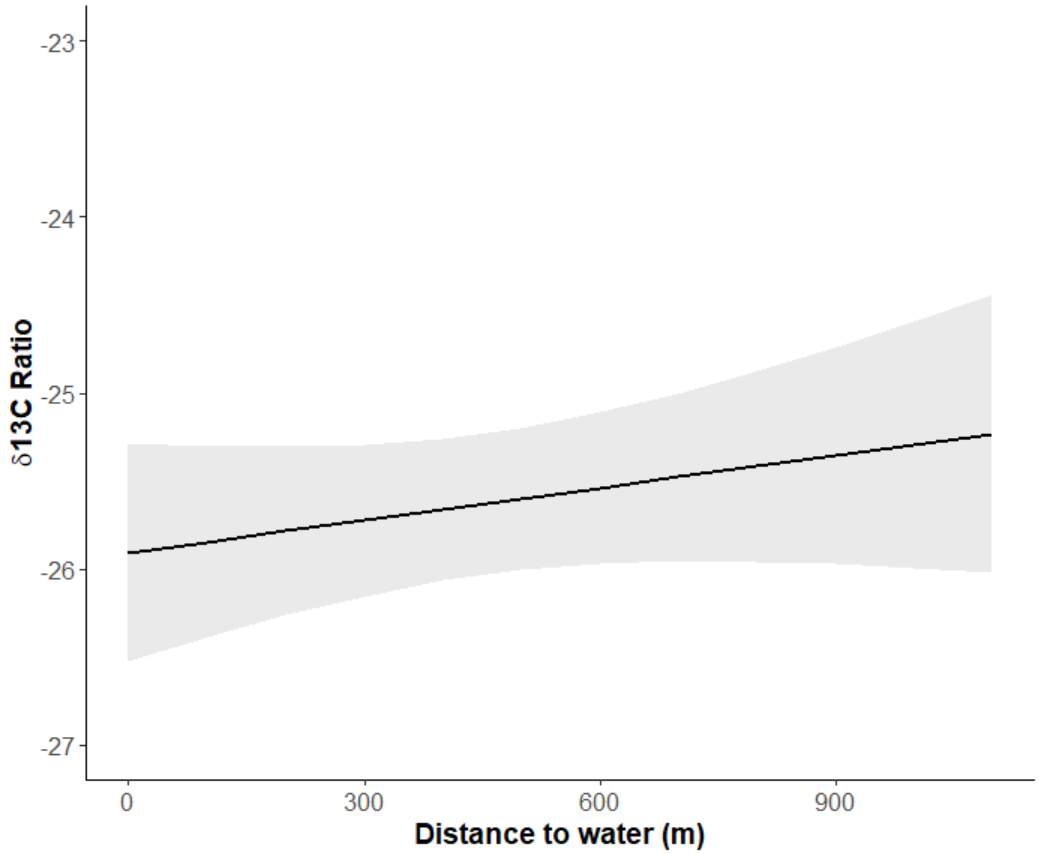

Figure 3.16: Changes in $\delta^{13}C$ values in blood based on distance to water (85% CI) of Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

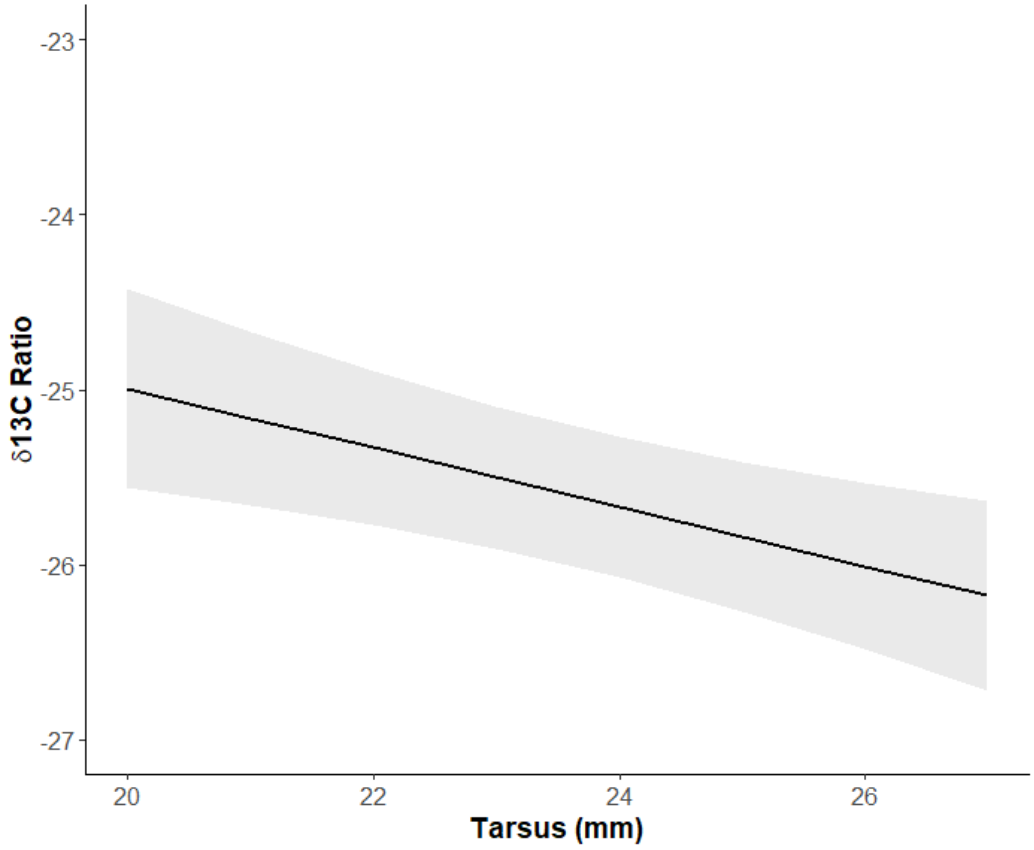

Figure 3.17: Changes in δ13C values in blood based on tarsus length (85% CI) in Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

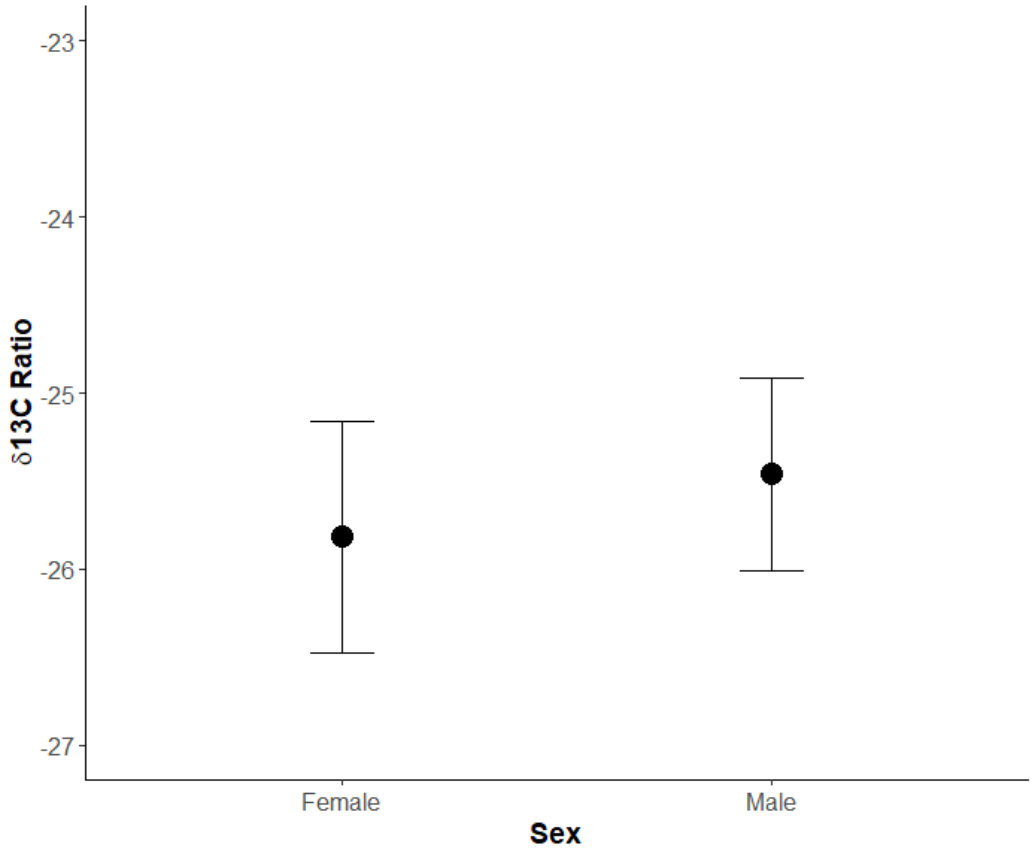

Figure 3.18: Changes in δ13C values in blood based on sex (85% CI) in Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

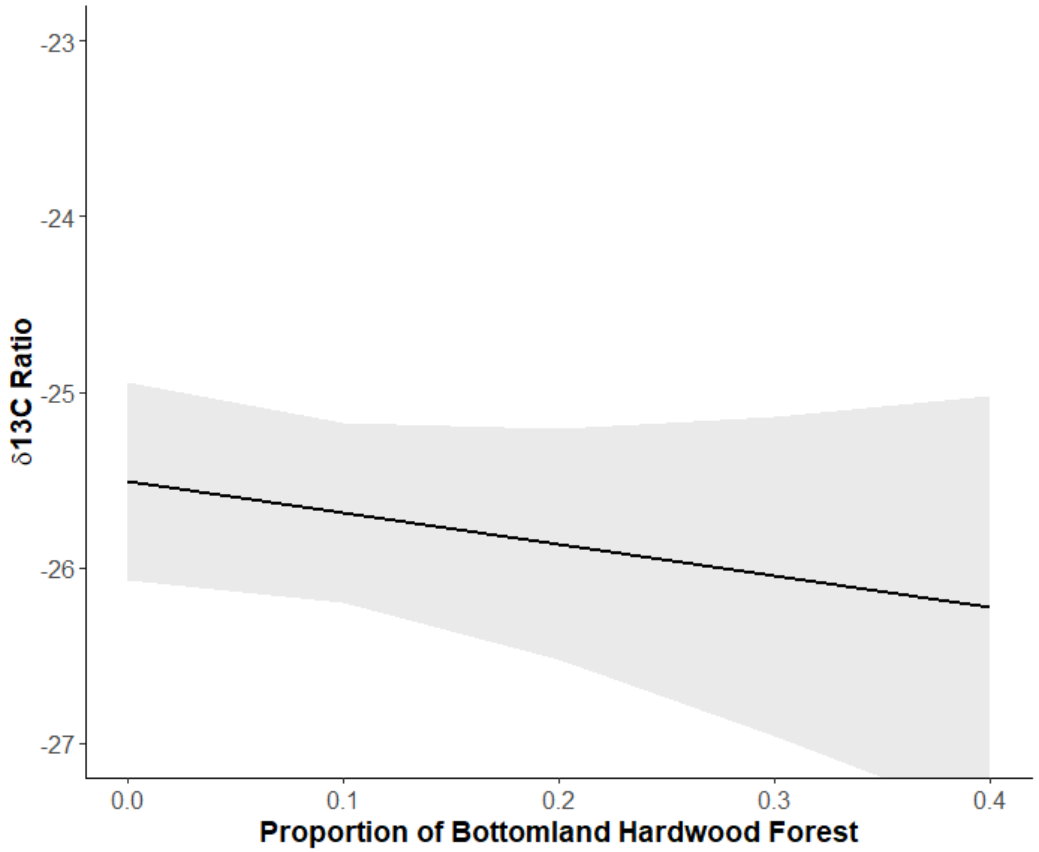

Figure 3.19: Changes in δ13C values in blood based on proportion of bottomland hardwood forest (85% CI) in the territories of Northern Cardinals (*Cardinalis cardinalis*; *n* = 27) captured in the central Savannah River area in South Carolina from April to May 2017.

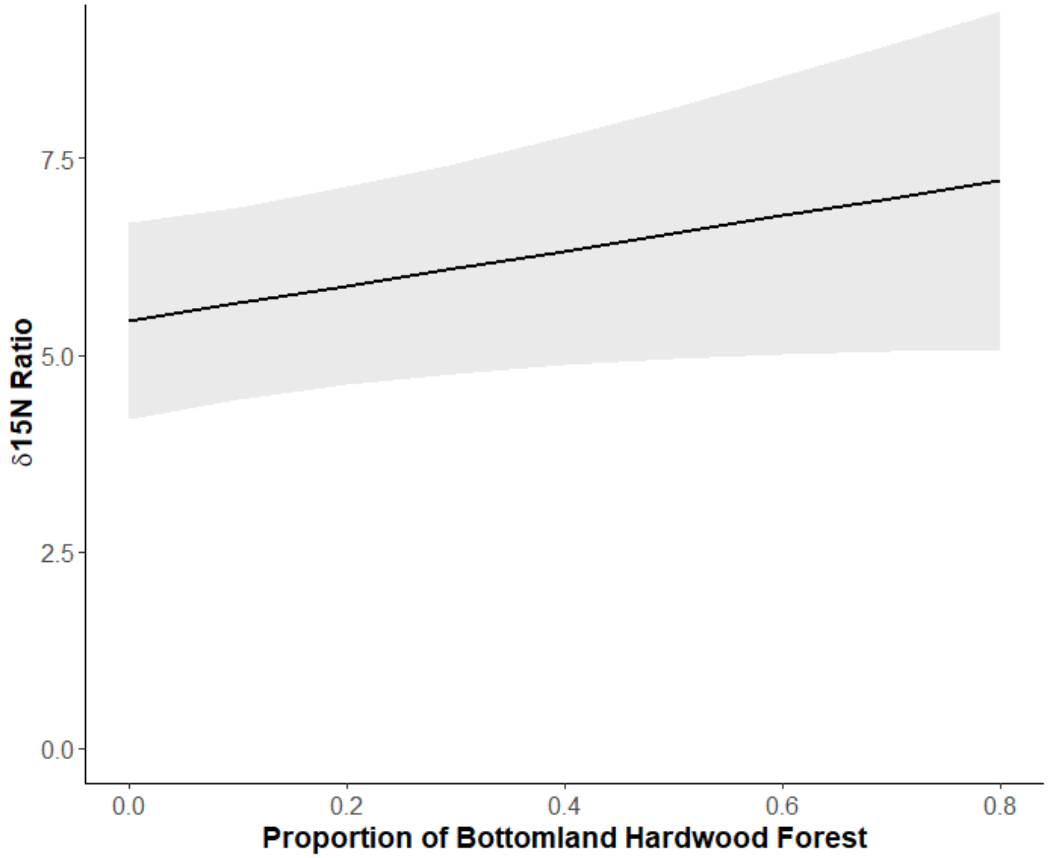

Figure 3.20: Changes in $\delta^{15}N$ values in blood based on proportion of bottomland hardwood forest (85% CL) in territories of Great Crested Flycatchers (*Myarchus crinitis*; *n* = 29) captured in the central Savannah River area in South Carolina from April to May 2017.

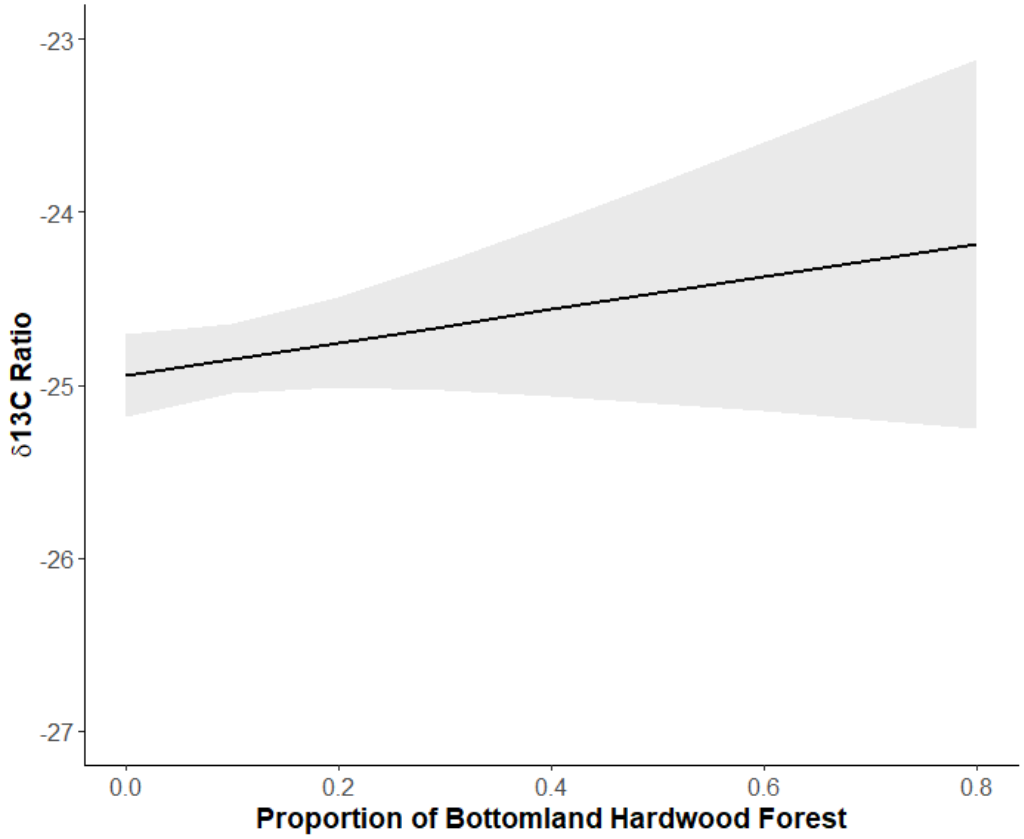

Figure 3.21: Changes in $\delta^{13}C$ values in blood based on proportion of bottomland hardwood forest (85% CL) in territories of Great Crested Flycatchers (*Myarchus crinitis*; *n* = 29) captured in the central Savannah River area in South Carolina from April to May 2017.

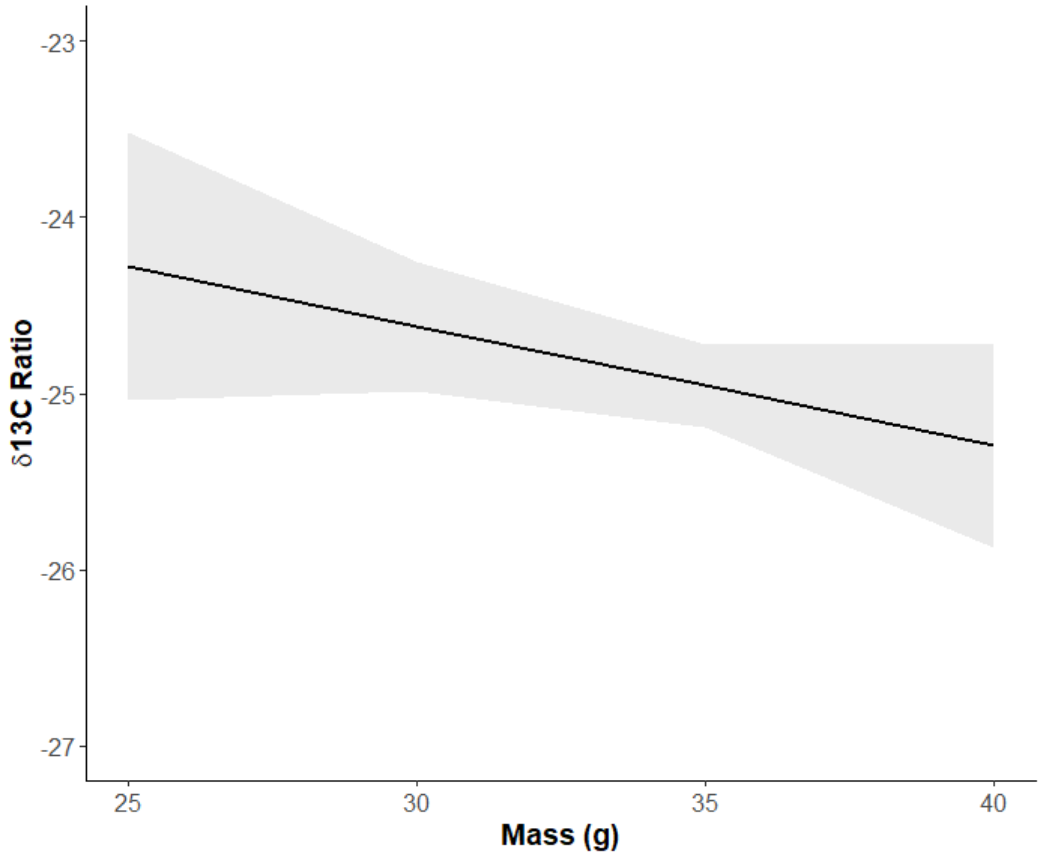

Figure 3.22: Changes in $\delta^{13}C$ values in blood based on mass (85% CL) in Great Crested Flycatchers (*Myarchus crinitis*; *n* = 29) captured in the central Savannah River area in South Carolina from April to May 2017.

CHAPTER 4

CONCLUSION

Bioaccumulation of heavy metal obtained from food resources is common among birds around the globe (Burger, 1993; Burger and Gochfeld, 2002; Hofer et al., 2010). Two common species that breed in South Carolina, Northern Cardinals (*Cardinalis cardinalis*) and Great Crested Flycatchers (*Myarchus crinitis*), exhibited differences in diet and heavy metal accumulation between seasons based on blood and feather tissue analysis. As two distinct species, these differences were expected but provide useful information pertaining to diet changes in a neotropical migrant (flycatchers) and changes in heavy metal bioaccumulation between breeding and molting grounds. The observed metal concentrations and stable isotope values in flycatchers seem to provide fairly reliable local contamination bioaccumulation (in blood samples) and concentrations outside of the breeding range where feathers are grown. The unexpected increase in $\delta^{13}C$ and $\delta^{15}N$ from breeding to non-breeding seasons in flycatchers suggests their diet may not switch to fruits in the non-breeding season. Cardinals are a common and abundant resident species and may act as a proxy for similar species in determining terrestrial bioavailability of heavy metals and seasonal food resource changes in one geographic area.

Non-lethal tissue sampling (such as blood and feathers) proves to be potentially effective at generating diet change information and heavy metal concentrations in these two species over an annual cycle. Results from the 2016 data suggested flycatchers had higher selenium and mercury than cardinals breeding in the same locations. Stable isotope information yielded information pertaining to the differences in trophic position with flycatchers foraging at a slightly higher trophic status than cardinals. Due to the higher trophic position and higher

mercury and selenium concentrations in flycatchers compared to cardinals, there is supporting evidence that biomagnification of selenium and mercury is occurring in this species. Many other studies have supported the notion that mercury biomagnifies as trophic level increases (Cristol et al., 2008; Keller et al., 2014; Newman et al., 2011; Scheuhammer et al., 2007; Varian-Ramos et al., 2014) but the biomagnification of selenium has been less observed (Orr et al., 2006). Concentration information gathered from both species has capacity to be an indication of overall terrestrial ecosystem health in the areas they occupy (Furness and Greenwood, 1993). While we cannot be certain if the observed concentrations are harmful to either species, we have no reason to believe there are harmful effects at these levels at a population scale.

No significant differences in heavy metal concentrations between sexes possibly indicate that when overall metal levels are low, females do not deposit metals in their eggs during the breeding season (Burger, 2007) contrary to other research (Rimmer et al., 2005). Flycatcher stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) were not significantly associated with sex and the differences seen in cardinals were negligible. Both results suggest sex is not a primary factor in bioaccumulation of metals or in diet differentiation. Male and female songbirds may have very similar nutritional requirements and physiological processes in dealing with heavy metals or have alternative pathways of excreting them.

Several morphometric variables impacted $\delta^{13}C$ and $\delta^{15}N$ values in different tissues of the two species. However, the impacts were minimal in terms of noticeable diet changes and biological relevance to possible prey selection due to said variables. Overall, observed diet changes regarded in $\delta^{13}C$ and $\delta^{15}N$ were only subtly associated with habitat. The magnitude of changes in $\delta^{13}C$ and $\delta^{15}N$ as habitat and/or size changed was not enough to constitute entire trophic level differences (Herrera et al., 2003).

This study provided novel heavy metal concentration information for wild populations of cardinals and flycatchers in South Carolina. Stable isotope analysis on different tissue types in these two species to determine seasonal diet changes is controversial (Hobson, 2005; Hobson et al., 1993) although our results indicate differences can be observed using this method similar to previous research (Maldonado et al., 2012; Pagani-Núñez et al., 2017; Vitz and Rodewald, 2012). Grouping songbirds by feeding guild may be too broad as it is clear we may not fully understand the details of diet composition using stable isotopes. However, comparing $\delta^{13}C$ and $\delta^{15}N$ between species allowed us to determine that there are measurable differences between the diets of flycatchers and cardinals with flycatchers foraging at a higher trophic level and acquiring higher levels of mercury and selenium which could indicate biomagnification. Therefore, flycatchers may be more susceptible to the physiological consequences of biomagnification than lower trophic level cardinals.

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APPENDIX A

Supplemental Material Part 1

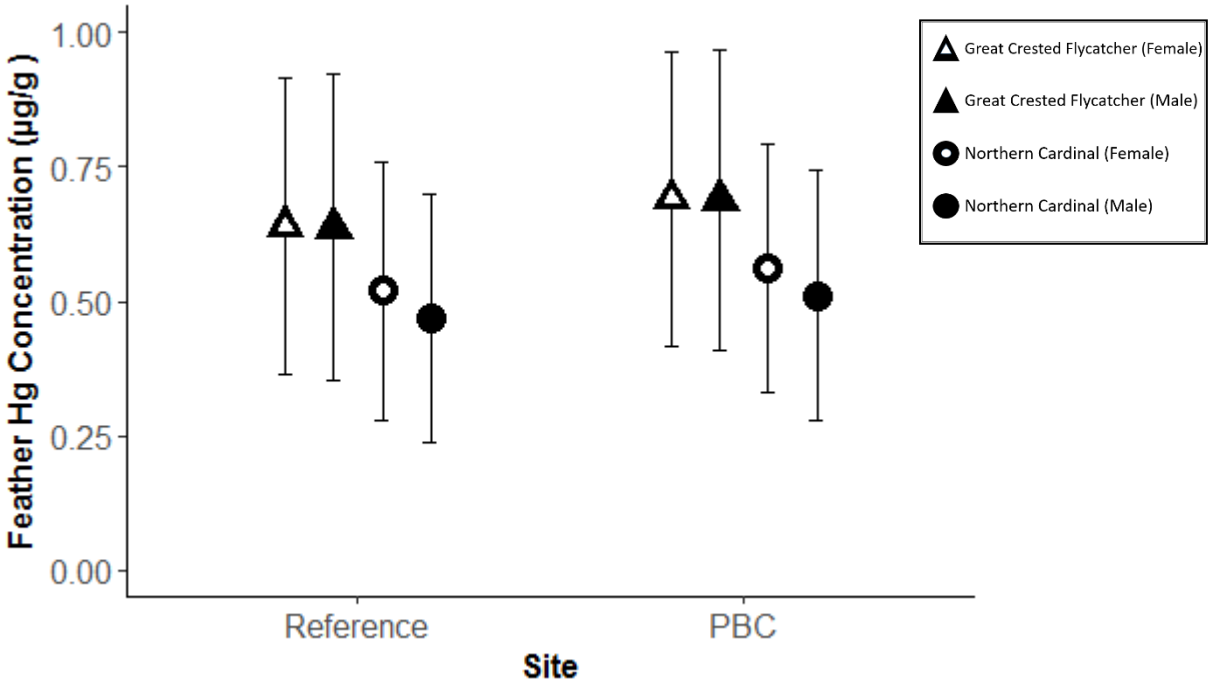

Figure A.23: Predicted feather concentrations levels (dry weight) of mercury (Hg) after model averaging (85% confidence interval) for Northern Cardinals (triangles, *n* = 46) by sex and Great Crested Flycatchers (squares, *n* = 12) by sex collected at the Savannah River Site April-June 2016.

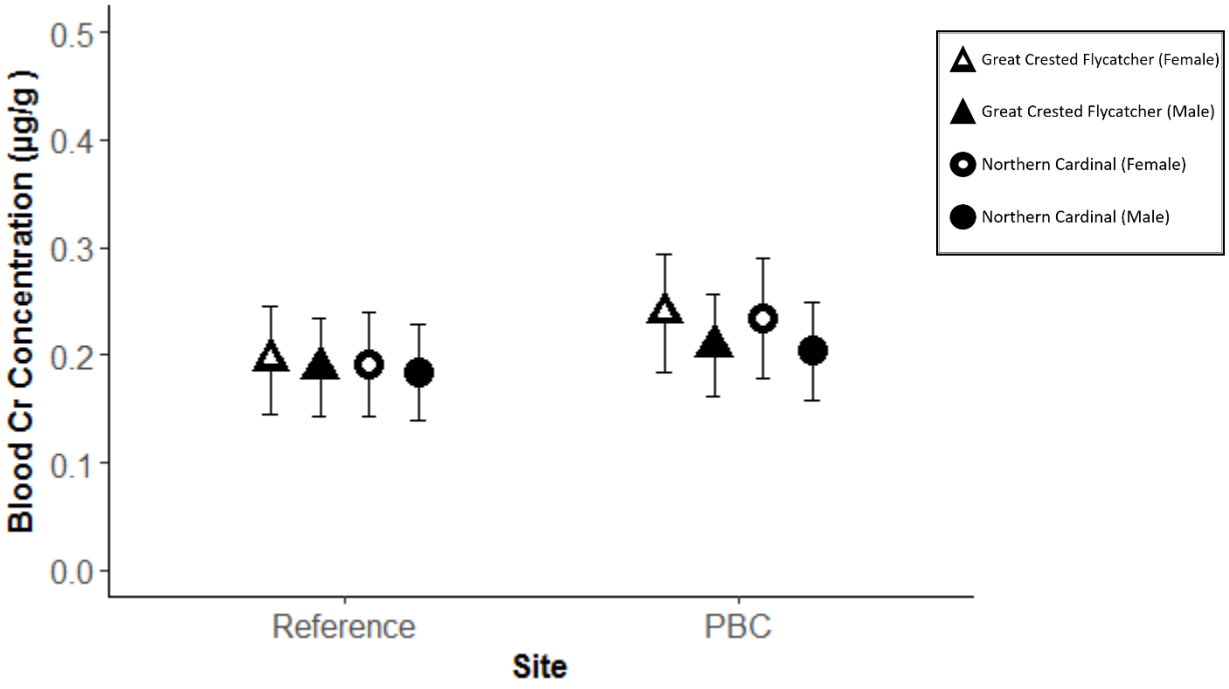

Figure A.24: Predicted blood concentrations levels (wet weight) of chromium (Cr) after model averaging (85% confidence interval) for Northern Cardinals (triangles, *n* = 41) by sex and Great Crested Flycatchers by sex (squares, *n* = 19) collected at the Savannah River Site April-June 2016.

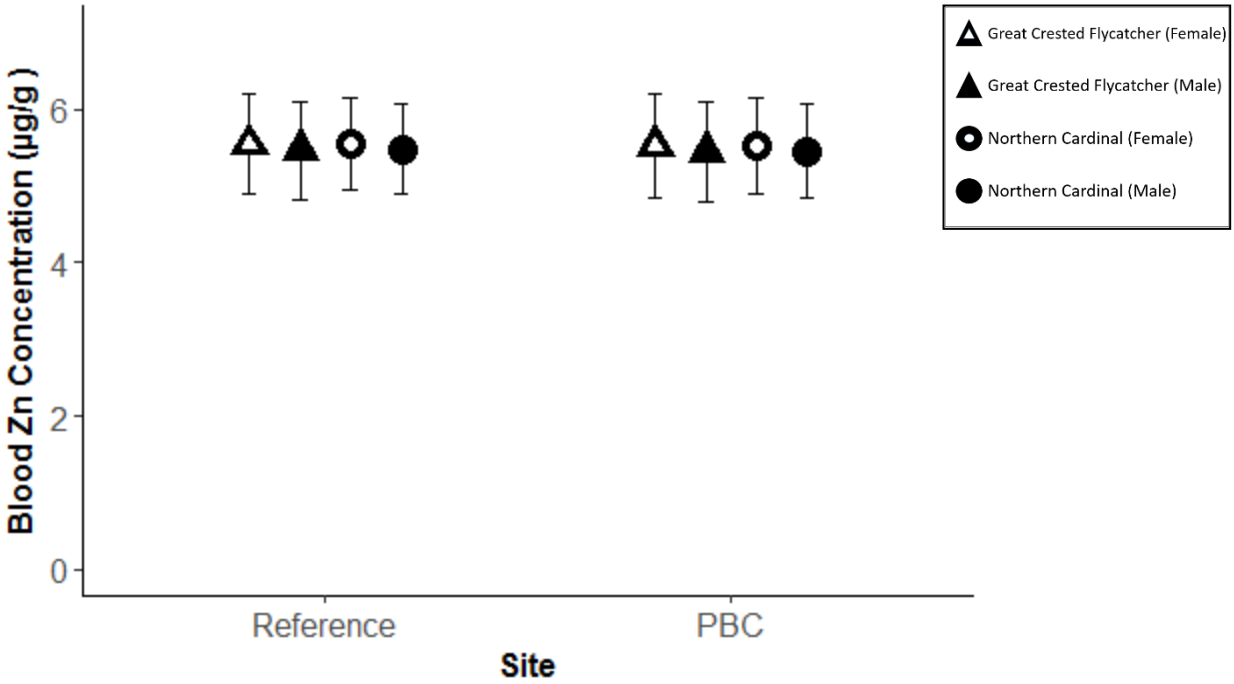

Figure A.25: Predicted blood concentrations levels (wet weight) of zinc (Zn) after model averaging (85% confidence interval) for Northern Cardinals (triangles, *n* = 41) by sex and Great Crested Flycatchers (squares, *n* = 19) by sex collected at the Savannah River Site April-June 2016.

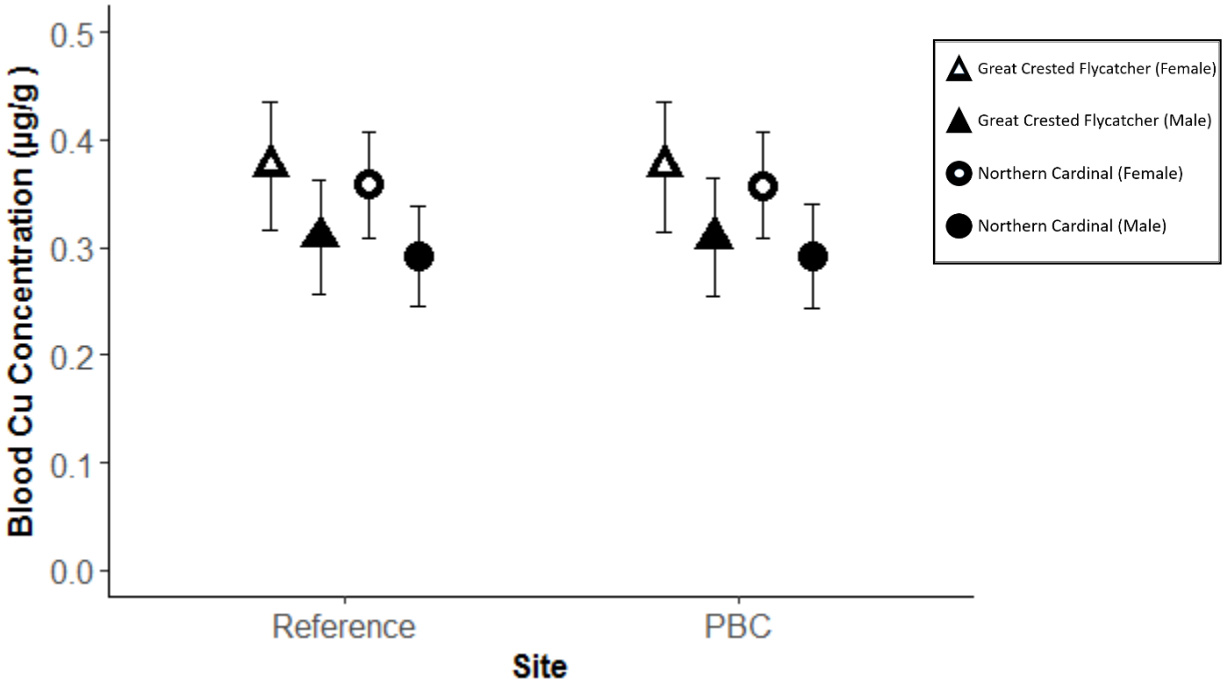

Figure A.26: Predicted blood concentrations levels (wet weight) of copper (Cu) after model averaging (85% confidence interval) for Northern Cardinals (triangles, *n* = 41) by sex and Great Crested Flycatchers (squares, *n* = 19) by sex collected at the Savannah River Site April-June 2016.

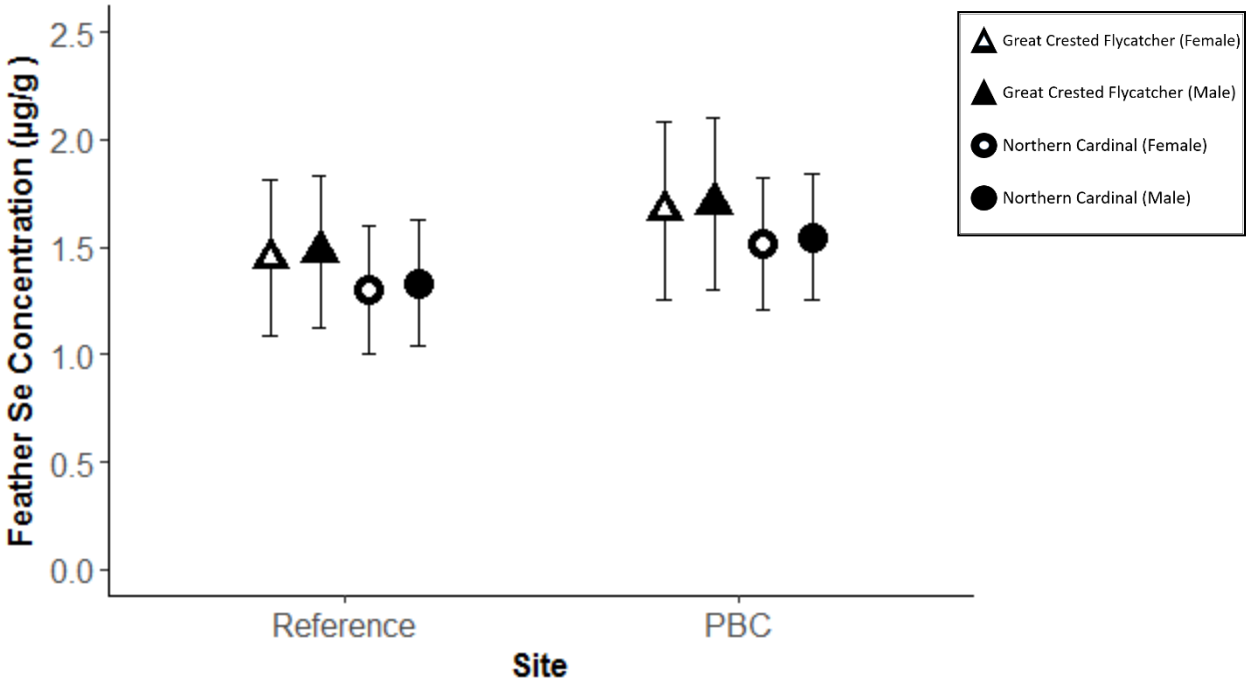

Figure A.27: Predicted feather concentrations levels (dry weight) of selenium (Se) after model averaging (85% confidence interval) for Northern Cardinals (triangles, *n* = 50) by sex and Great Crested Flycatchers (squares, *n* = 20) by sex collected at the Savannah River Site April-June 2016.

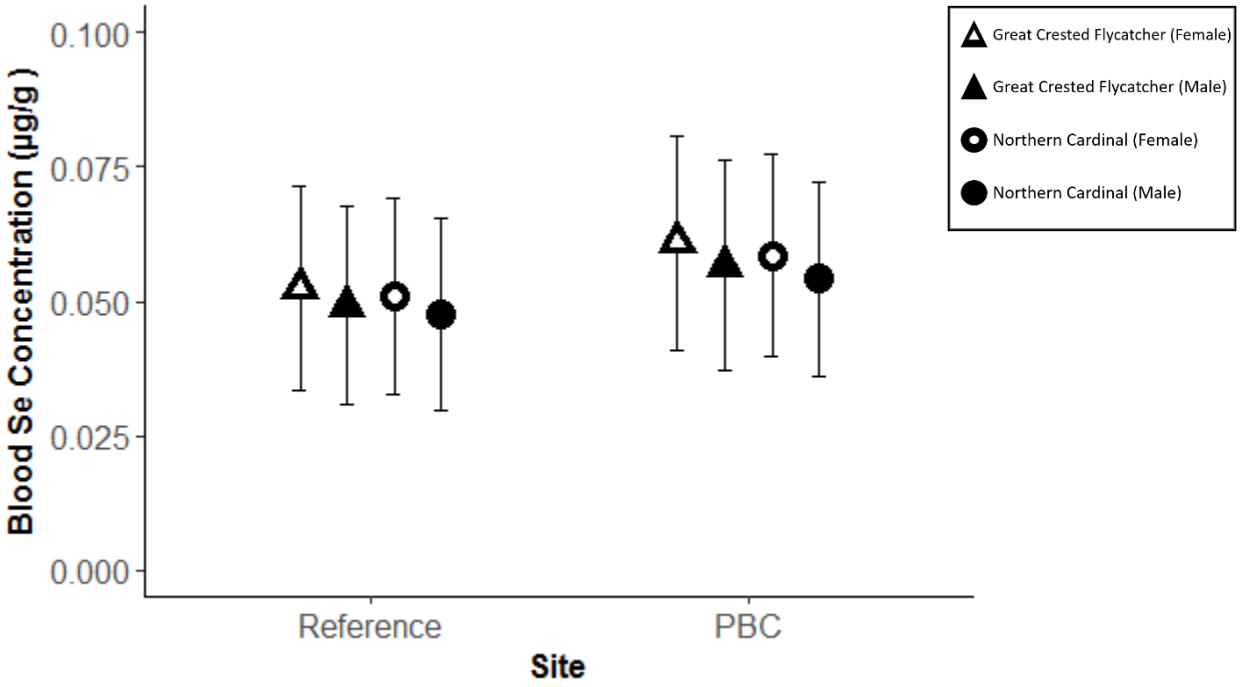

Figure A.6: Predicted blood concentrations levels (wet weight) of arsenic (As) after model averaging (85% confidence interval) for Northern Cardinals (circles, *n* = 41) by sex and Great Crested Flycatchers (triangles, *n* = 19) by sex collected at the Savannah River Site April-June 2016.

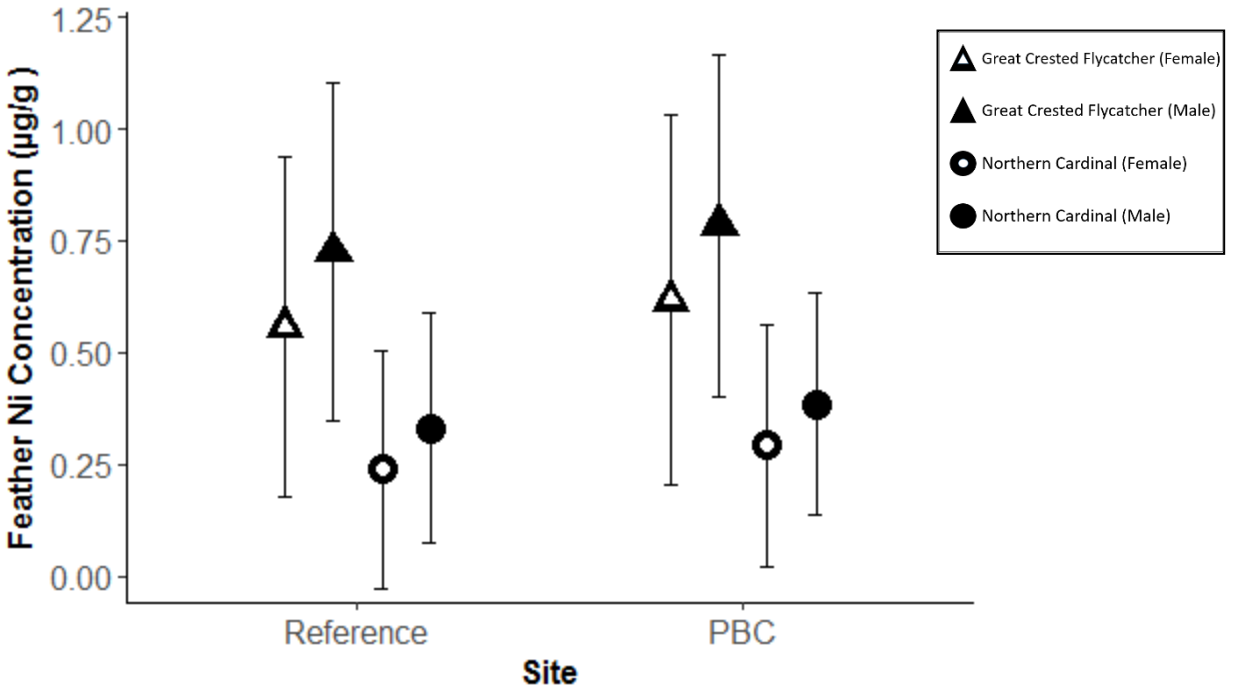

Figure A.7: Predicted feather concentrations levels (dry weight) of nickel (Ni) after model averaging (85% confidence interval) for Northern Cardinals (triangles, *n* = 50) by sex and Great Crested Flycatchers (squares, *n* = 20) by sex collected at the Savannah River Site April-June 2016.

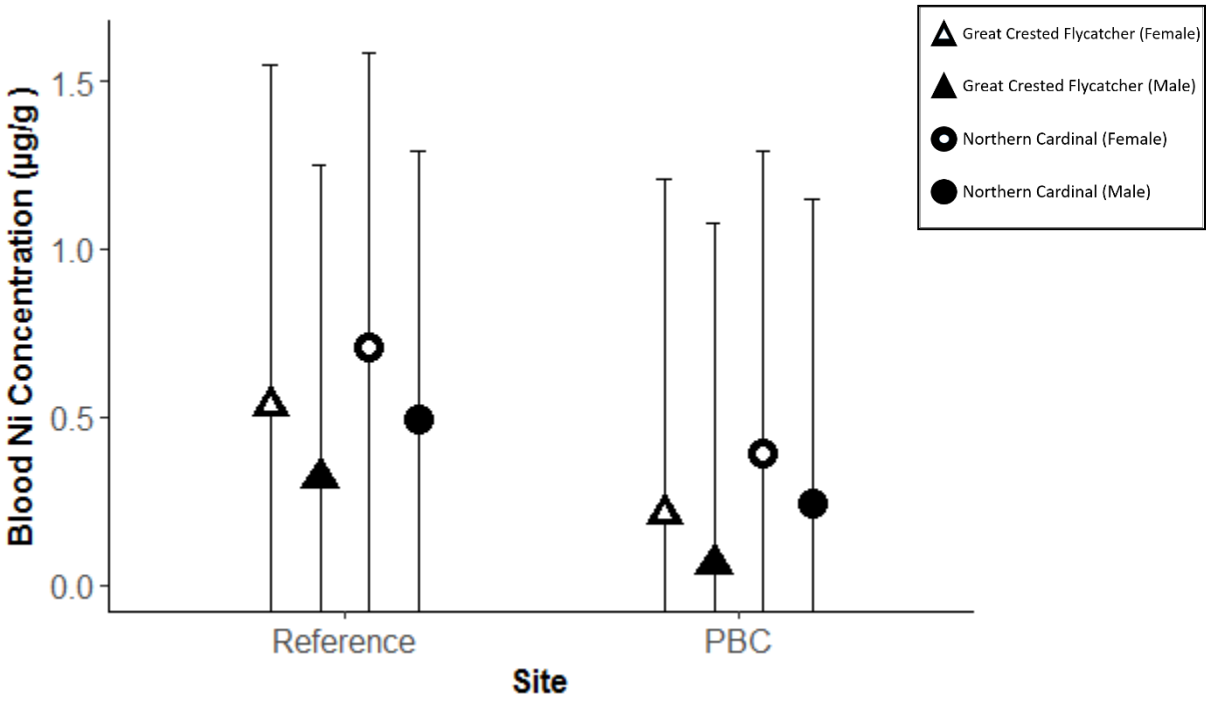

Figure A.8: Predicted blood concentrations levels (wet weight) of nickel (Ni) after model averaging (85% confidence interval) for Northern Cardinals (triangles, *n* = 41) by sex and Great Crested Flycatchers (squares, *n* = 19) by sex collected at the Savannah River Site April-June 2016.

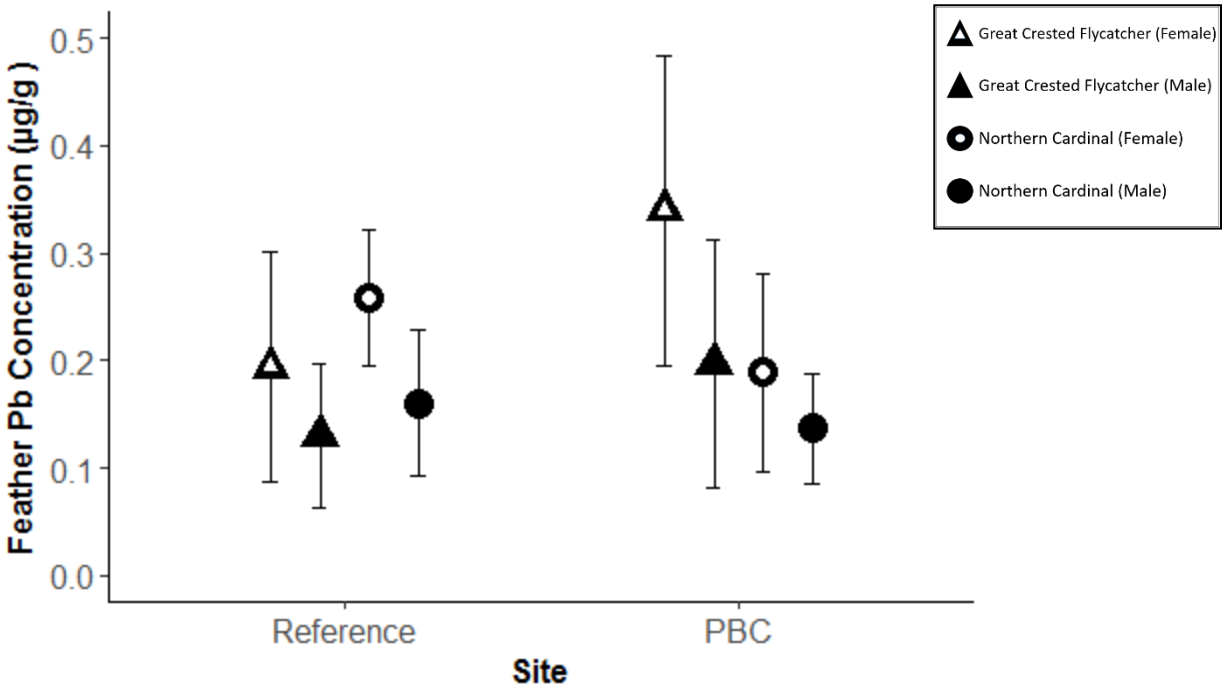

Figure A.9: Predicted feather concentrations levels (dry weight) of lead (Pb) after model averaging (85% confidence interval) for Northern Cardinals (triangles, *n* = 50) by sex and Great Crested Flycatchers (squares, *n* = 20) by sex collected at the Savannah River Site April-June 2016.

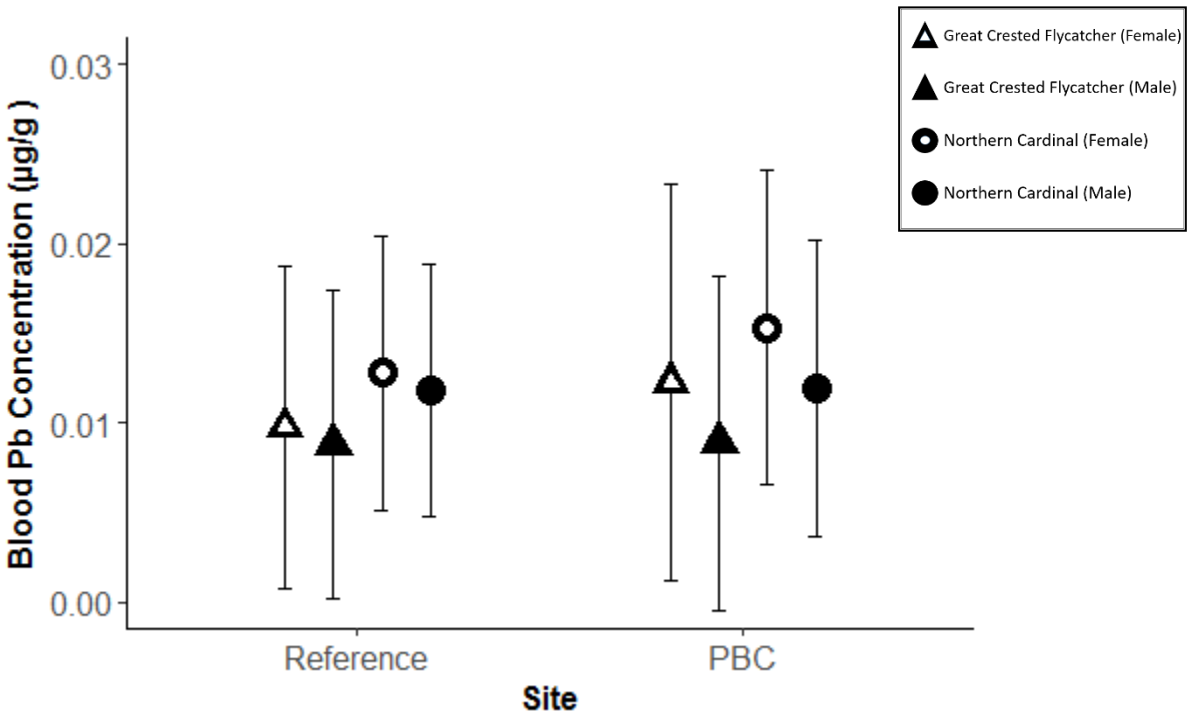

Figure A.10: Predicted blood concentrations levels (wet weight) of lead (Pb) after model averaging (85% confidence interval) for Northern Cardinals (triangles, *n* = 41) by sex and Great Crested Flycatchers (squares, *n* = 19) by sex collected at the Savannah River Site April-June 2016.

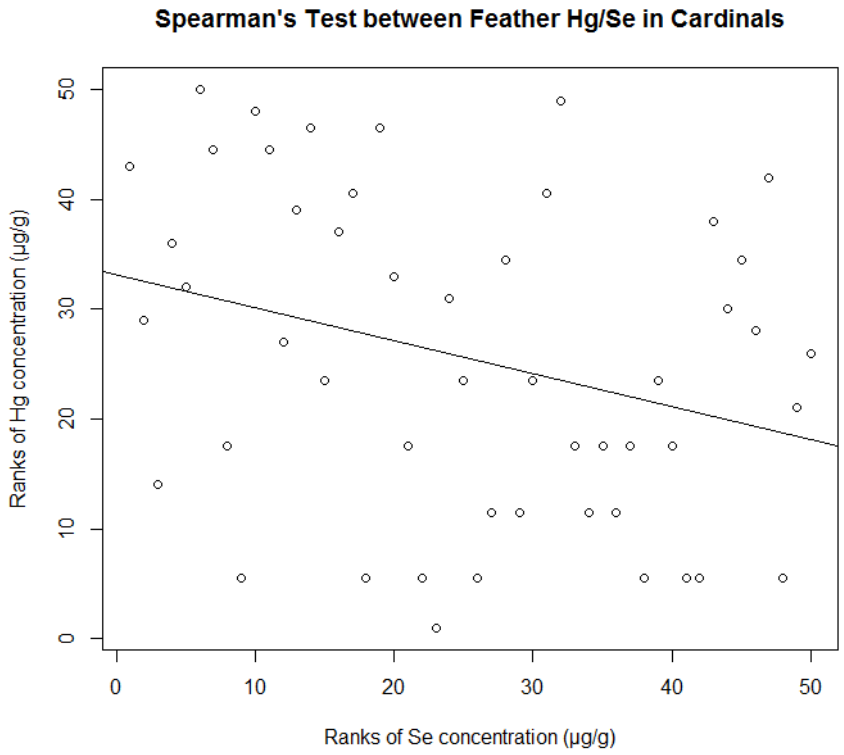

Figure A.11: Spearman's rank correlation test between feather Hg and feather Se concentrations in Northern Cardinals ((*r* = -0.329, *p* = 0.026).

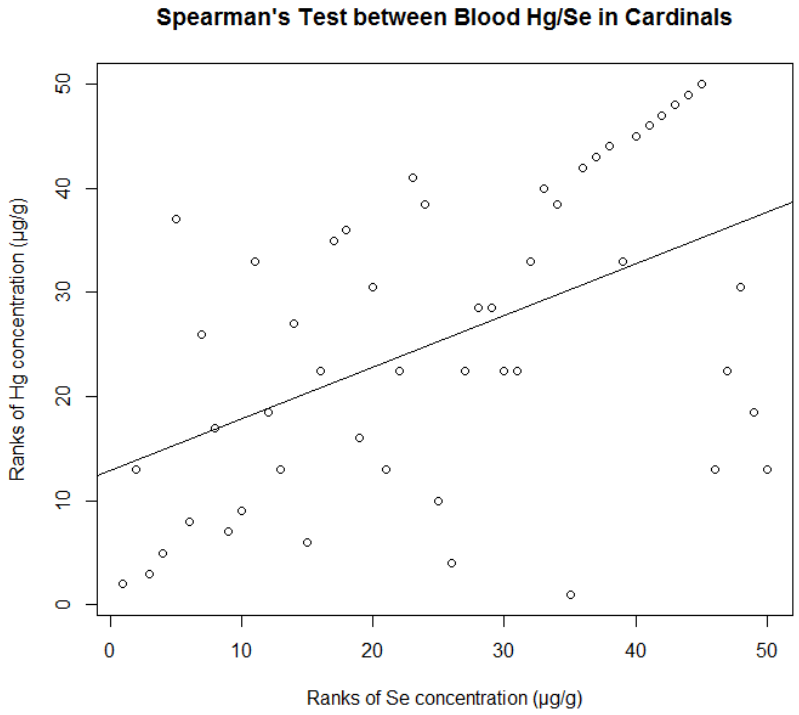

Figure A.12: Spearman's rank correlation test between blood Hg and blood Se concentrations in Northern Cardinals (r = 0.464, p = 0.007).

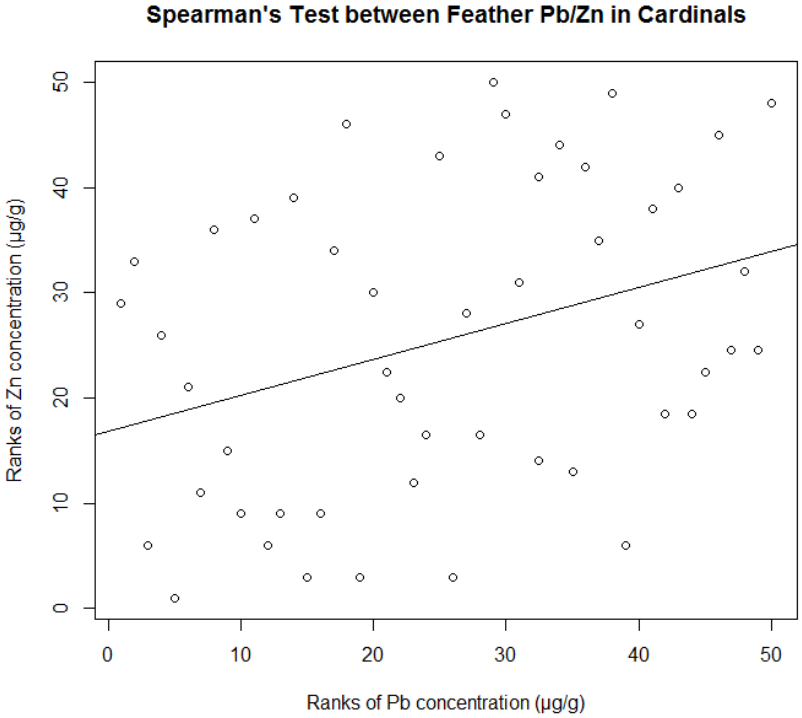

Figure A.13: Spearman's rank correlation test between feather Pb and feather Zn concentrations in Northern Cardinals (r = 0.34, p = 0.015).

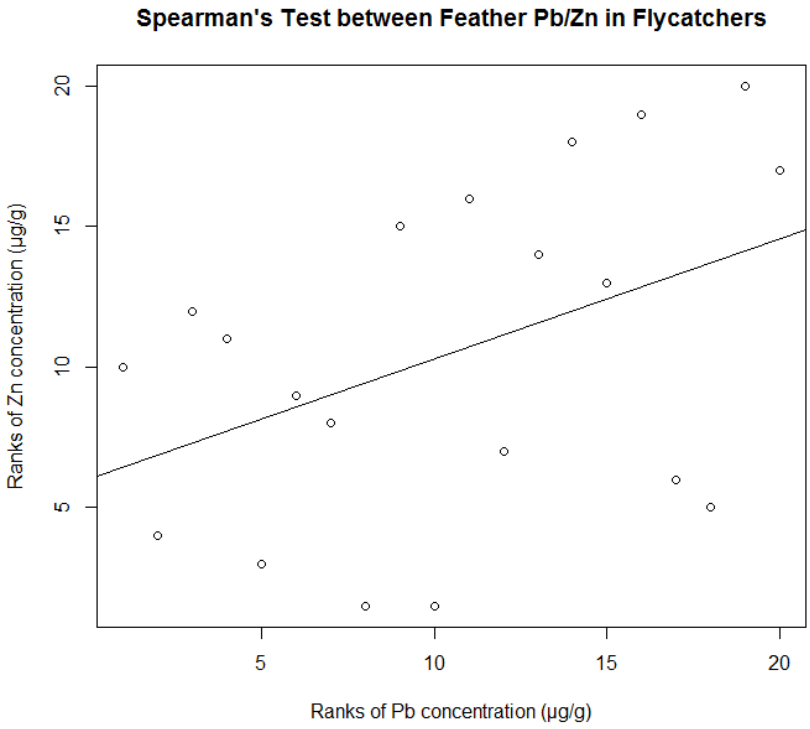

Figure A.14: Spearman's rank correlation test between feather Pb and feather Zn concentrations in Great Crested Flycatchers (r = 0.43, p = 0.059).

AICc Tables

[a] Akaike's Information Criterion corrected for small sample size using maximum likelihood.

[b] The r^2 values were calculated using restricted maximum likelihood (Nakagawa and Schielzeth, 2013).

[c] The number of parameters in the model including the intercept.

[d] The difference in the AIC_c values of the current model and the model with the lowest AIC_c.

[e] The weight of evidence that the model with the lowest AIC_c value is better than the current model.

Table A.1: Summary of model selection results for linear mixed effects models describing the variation in arsenic (As) concentrations in feathers (dry weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers

(GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA *n* = 50 and for GCFL *n* = 20.

Feather As

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Species + (Site)	50.5	-92.3	0.07	0.16	2	0.00	0.382
Species + Sex + (Site)	50.8	-90.7	0.07	0.16	3	1.65	0.168
Species + Group + (Site)	50.5	-90.0	0.06	0.22	3	2.31	0.120
(Site)	47.9	-89.5	0.00	0.08	1	2.87	0.091
Species*Sex + (Site)	51.1	-88.8	0.08	0.16	4	3.54	0.065
Species + Sex + Group + (Site)	50.8	-88.3	0.07	0.21	4	4.02	0.051
Species*Group + (Site)	50.5	-87.8	0.06	0.21	4	4.58	0.039
Sex + (Site)	48.2	-87.7	0.01	0.08	2	4.62	0.038
Group + (Site)	47.9	-87.2	0.00	0.14	2	5.12	0.029
Sex + Group + (Site)	48.2	-85.4	0.01	0.14	3	6.94	0.012
Sex*Group+ (Site)	48.3	-83.3	0.01	0.14	4	9.02	0.004
Sex*Species*Group +(Site)	51.5	-79.2	0.08	0.21	9	13.10	0.001

Table A.2: Summary of model selection results for linear mixed effects models describing the variation in arsenic (As) concentrations in blood (wet weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA *n* = 41 and for GCFL *n* = 20.

Blood As

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
(Site)	124.6	-242.8	0.00	0.38	1	0.00	0.249
Sex + (Site)	125.4	-242.0	0.02	0.39	2	0.80	0.167
Group + (Site)	125.1	-241.5	0.06	0.46	2	1.32	0.129
Species + (Site)	124.9	-241.1	0.01	0.38	2	1.75	0.104
Sex + Group + (Site)	125.9	-240.6	0.07	0.47	3	2.24	0.082
Species + Sex + (Site)	125.7	-240.3	0.02	0.40	3	2.50	0.072
Species + Group + (Site)	125.4	-239.7	0.06	0.46	3	3.13	0.052
Sex*Group+ (Site)	126.6	-239.7	0.08	0.49	4	3.15	0.051
Species*Group + (Site)	126.3	-239.1	0.08	0.48	4	3.75	0.038
Species + Sex + Group + (Site)	126.2	-238.9	0.08	0.48	4	3.99	0.034
Species*Sex + (Site)	125.8	-237.9	0.02	0.39	4	4.92	0.021
Sex*Species*Group +(Site)	127.9	-231.3	0.10	0.50	9	11.57	0.001

Table A.3: Summary of model selection results for linear mixed effects models describing the variation in copper (Cu) concentrations in feathers (dry weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA $n = 50$ and for GCFL $n = 20$.

Feather Cu

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Species*Group + (Site)	-201.72	416.78	0.16	0.16	4	0.00	0.605

Species + (Site)	-205.98	420.58	0.06	0.06	2	3.80	0.090
Species + Group + (Site)	-204.93	420.79	0.08	0.08	3	4.01	0.081
Species + Sex + (Site)	-205.52	421.98	0.07	0.07	3	5.20	0.045
Group + (Site)	-206.85	422.31	0.04	0.04	2	5.53	0.038
Species + Sex + Group + (Site)	-204.54	422.42	0.09	0.09	4	5.64	0.036
(Site)	-208.12	422.60	0.00	0.00	1	5.82	0.033
Sex*Species*Group +(Site)	-200.01	423.75	0.19	0.19	9	6.97	0.019
Sex + Group + (Site)	-206.55	424.04	0.04	0.04	3	7.27	0.016
Sex + (Site)	-207.75	424.11	0.01	0.01	2	7.33	0.015
Species*Sex + (Site)	-205.42	424.17	0.07	0.07	4	7.39	0.015
Sex*Group+ (Site)	-206.17	425.67	0.05	0.05	4	8.90	0.007

Table A.4: Summary of model selection results for linear mixed effects models describing the variation in copper (Cu) concentrations in blood (wet weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA $n = 41$ and for GCFL $n = 20$.

Blood Cu

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Sex + (Site)	29.61	-50.48	0.07	0.08	2	0.00	0.33
Species + Sex + (Site)	30.23	-49.36	0.09	0.10	3	1.12	0.19
(Site)	27.43	-48.42	0.00	0.02	1	2.06	0.12
Sex + Group + (Site)	29.62	-48.12	0.06	0.11	3	2.36	0.10

Species + (Site)	27.88	-47.04	0.02	0.04	2	3.45	0.06
Species + Sex + Group + (Site)	30.24	-46.89	0.08	0.13	4	3.59	0.05
Species*Sex + (Site)	30.23	-46.89	0.08	0.10	4	3.60	0.05
Group + (Site)	27.43	-46.13	0.00	0.06	2	4.35	0.04
Sex*Group+ (Site)	29.69	-45.79	0.07	0.11	4	4.69	0.03
Species + Group + (Site)	27.88	-44.65	0.02	0.07	3	5.83	0.02
Species*Group + (Site)	27.97	-42.35	0.02	0.07	4	8.14	0.01
Sex*Species*Group +(Site)	30.38	-36.27	0.08	0.14	9	14.22	0.00

Table A.5: Summary of model selection results for linear mixed effects models describing the variation in chromium (Cr) concentrations in feathers (dry weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA $n = 50$ and for GCFL $n = 20$.

Feather Cr

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Species*Group + (Site)	-120.24	253.81	0.16	0.20	4	0.00	0.53
Species + Group + (Site)	-122.75	256.43	0.10	0.14	3	2.62	0.14
Species + (Site)	-124.18	256.97	0.06	0.13	2	3.15	0.11
Species + Sex + Group + (Site)	-122.74	258.82	0.10	0.14	4	5.01	0.04
Group + (Site)	-125.16	258.94	0.04	0.08	2	5.12	0.04
(Site)	-126.31	258.98	0.00	0.06	1	5.16	0.04
Species + Sex + (Site)	-124.17	259.28	0.06	0.13	3	5.47	0.03

Sex*Species*Group +(Site)	-118.33	260.40	0.19	0.23	9	6.58	0.02
Sex + (Site)	-126.29	261.20	0.00	0.06	2	7.39	0.01
Sex + Group + (Site)	-125.15	261.23	0.04	0.08	3	7.42	0.01
Species*Sex + (Site)	-124.08	261.49	0.06	0.14	4	7.67	0.01
Sex*Group+ (Site)	-124.89	263.12	0.04	0.08	4	9.30	0.01

Table A.6: Summary of model selection results for linear mixed effects models describing the variation in chromium (Cr) concentrations in blood (wet weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA $n = 41$ and for GCFL $n = 20$.

Blood Cr

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Sex*Group+ (Site)	76.98	-140.37	0.15	0.53	4	0.00	0.29
(Site)	72.66	-138.88	0.00	0.39	1	1.49	0.14
Sex + (Site)	73.79	-138.85	0.02	0.41	2	1.52	0.13
Group + (Site)	73.39	-138.06	0.09	0.46	2	2.31	0.09
Sex + Group + (Site)	74.51	-137.92	0.11	0.48	3	2.46	0.08
Species + Sex + (Site)	74.35	-137.59	0.03	0.42	3	2.78	0.07
Species + (Site)	73.10	-137.48	0.01	0.40	2	2.89	0.07
Species + Group + (Site)	73.86	-136.61	0.10	0.46	3	3.76	0.04
Species + Sex + Group + (Site)	75.10	-136.61	0.12	0.48	4	3.77	0.04
Species*Sex + (Site)	74.67	-135.75	0.04	0.42	4	4.63	0.03
Species*Group + (Site)	73.86	-134.14	0.10	0.46	4	6.23	0.01

Sex*Species*Group +(Site)	78.18	-131.86	0.16	0.54	9	8.51	0.00

Table A.7: Summary of model selection results for linear mixed effects models describing the variation in mercury (Hg) concentrations in feathers (dry weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA *n* = 46 and for GCFL *n* = 12.

Feather Hg

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Species + (Site)	-17.92	44.60	0.04	0.51	2	0.00	0.25
Species*Sex + (Site)	-16.00	45.64	0.06	0.56	4	1.04	0.15
Species + Sex + (Site)	-17.29	45.74	0.04	0.53	3	1.14	0.14
(Site)	-19.94	46.32	0.00	0.49	1	1.72	0.11
Species + Group + (Site)	-17.70	46.55	0.07	0.60	3	1.94	0.10
Sex + (Site)	-19.16	47.08	0.01	0.52	2	2.48	0.07
Species + Sex + Group + (Site)	-17.05	47.75	0.08	0.61	4	3.15	0.05
Group + (Site)	-19.67	48.10	0.04	0.58	2	3.50	0.04
Species*Group + (Site)	-17.32	48.29	0.07	0.60	4	3.68	0.04
Sex + Group + (Site)	-18.88	48.92	0.05	0.60	3	4.32	0.03
Sex*Group+ (Site)	-18.83	51.30	0.05	0.60	4	6.70	0.01
Sex*Species*Group +(Site)	-14.95	54.58	0.10	0.65	9	9.98	0.00

Table A.8: Summary of model selection results for linear mixed effects models describing the variation in mercury (Hg) concentrations in blood (wet weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers

(GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA *n* = 33 and for GCFL *n* = 17.

Blood Hg

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Species + (Site)	29.23	-49.56	0.22	0.65	2	0.00	0.35
Species*Sex + (Site)	31.52	-49.08	0.24	0.68	4	0.48	0.28
Species + Sex + (Site)	29.56	-47.75	0.22	0.64	3	1.82	0.14
Species + Group + (Site)	29.53	-47.69	0.20	0.69	3	1.87	0.14
Species + Sex + Group + (Site)	29.84	-45.72	0.21	0.69	4	3.84	0.05
Species*Group + (Site)	29.54	-45.13	0.20	0.69	4	4.44	0.04
Sex*Species*Group +(Site)	33.27	-40.91	0.23	0.73	9	8.65	0.00
(Site)	17.99	-29.45	0.00	0.40	1	20.11	0.00
Sex + (Site)	18.94	-28.99	0.02	0.41	2	20.57	0.00
Group + (Site)	18.11	-27.33	0.02	0.51	2	22.23	0.00
Sex + Group + (Site)	19.04	-26.71	0.04	0.52	3	22.85	0.00
Sex*Group+ (Site)	19.06	-24.17	0.04	0.51	4	25.39	0.00

Table A.9: Summary of model selection results for linear mixed effects models describing the variation in nickel (Ni) concentrations in feathers (dry weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA *n* = 50 and for GCFL *n* = 20.

Feather Ni

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Species + (Site)	-82.7	174.1	0.06	0.13	2	0.00	0.246
Species + Sex + (Site)	-81.9	174.7	0.08	0.15	3	0.64	0.179
Species*Sex + (Site)	-81.0	175.3	0.10	0.16	4	1.26	0.131
Species + Group + (Site)	-82.3	175.6	0.07	0.16	3	1.53	0.115
(Site)	-85.0	176.4	0.00	0.05	1	2.28	0.079
Species + Sex + Group + (Site)	-81.6	176.5	0.09	0.18	4	2.40	0.074
Sex + (Site)	-84.1	176.9	0.02	0.08	2	2.79	0.061
Species*Group + (Site)	-82.3	177.9	0.07	0.16	4	3.80	0.037
Group + (Site)	-84.7	178.0	0.01	0.08	2	3.92	0.035
Sex + Group + (Site)	-83.9	178.7	0.03	0.12	3	4.64	0.024
Sex*Group+ (Site)	-83.3	179.9	0.05	0.14	4	5.84	0.013
Sex*Species*Group +(Site)	-79.0	181.7	0.14	0.22	9	7.62	0.005

Table A.10: Summary of model selection results for linear mixed effects models describing the variation in nickel (Ni) concentrations in blood (wet weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA *n* = 41 and for GCFL *n* = 20.

Blood Ni

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
(Site)	-153.3	313.0	0.00	0.00	1	0.00	0.291

Sex + (Site)	-152.7	314.2	0.02	0.02	2	1.17	0.162
Group + (Site)	-152.9	314.5	0.01	0.01	2	1.52	0.136
Species + (Site)	-153.0	314.8	0.01	0.01	2	1.83	0.117
Sex + Group + (Site)	-152.3	315.7	0.03	0.03	3	2.71	0.075
Species + Sex + (Site)	-152.5	316.1	0.02	0.03	3	3.16	0.060
Species + Group + (Site)	-152.6	316.3	0.02	0.02	3	3.33	0.055
Sex*Group+ (Site)	-151.9	317.3	0.05	0.05	4	4.32	0.034
Species + Sex + Group + (Site)	-152.1	317.7	0.04	0.04	4	4.70	0.028
Species*Sex + (Site)	-152.3	318.1	0.04	0.04	4	5.13	0.022
Species*Group + (Site)	-152.4	318.4	0.03	0.03	4	5.44	0.019
Sex*Species*Group +(Site)	-151.2	326.9	0.08	0.08	9	13.88	0.000

Table A.11: Summary of model selection results for linear mixed effects models describing the variation in lead (Pb) concentrations in feathers (dry weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA $n = 50$ and for GCFL $n = 20$.

Feather Pb

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Sex*Species*Group +(Site)	43.08	-62.43	0.25	0.37	9	0.00	0.37
Sex + (Site)	35.00	-61.38	0.11	0.16	2	1.05	0.22
Species*Group + (Site)	36.95	-60.57	0.14	0.27	4	1.86	0.15

Species + Sex + (Site)	35.26	-59.58	0.11	0.17	3	2.85	0.09
Sex + Group + (Site)	35.00	-59.06	0.10	0.2	3	3.37	0.07
Species*Sex + (Site)	35.63	-57.93	0.12	0.19	4	4.50	0.04
Species + Sex + Group + (Site)	35.26	-57.19	0.11	0.21	4	5.24	0.03
Sex*Group+ (Site)	35.04	-56.75	0.10	0.2	4	5.68	0.02
(Site)	30.67	-54.97	0.00	0.07	1	7.46	0.01
Species + (Site)	30.83	-53.05	0.00	0.08	2	9.38	0.00
Group + (Site)	30.69	-52.76	0.00	0.13	2	9.67	0.00
Species + Group + (Site)	30.84	-50.75	0.01	0.13	3	11.68	0.00

Table A.12: Summary of model selection results for linear mixed effects models describing the variation in lead (Pb) concentrations in blood (wet weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA $n = 41$ and for GCFL $n = 20$.

Blood Pb

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
(Site)	133.6	-260.8	0.00	0.04	1	0.00	0.258
Species + (Site)	134.4	-260.1	0.03	0.06	2	0.74	0.178
Sex + (Site)	134.0	-259.2	0.01	0.04	2	1.56	0.118
Group + (Site)	133.8	-258.9	0.00	0.09	2	1.94	0.098
Sex*Group+ (Site)	136.1	-258.6	0.07	0.14	4	2.23	0.085
Species + Sex + (Site)	134.7	-258.3	0.03	0.07	3	2.49	0.074
Species + Group + (Site)	134.5	-257.9	0.03	0.11	3	2.85	0.062

Sex + Group + (Site)	134.2	-257.2	0.01	0.09	3	3.60	0.043
Species*Group + (Site)	134.9	-256.2	0.04	0.12	4	4.56	0.026
Species*Sex + (Site)	134.9	-256.2	0.04	0.07	4	4.60	0.026
Species + Sex + Group + (Site)	134.8	-256.1	0.03	0.11	4	4.70	0.025
Sex*Species*Group +(Site)	139.1	-253.8	0.14	0.19	9	7.00	0.008

Table A.13: Summary of model selection results for linear mixed effects models describing the variation in selenium (Se) concentrations in feathers (dry weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA $n = 50$ and for GCFL $n = 20$.

Feather Se

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Group + (Site)	-100.3	209.2	0.03	0.03	2	0.00	0.204
(Site)	-101.5	209.4	0.00	0.02	1	0.14	0.190
Species + Group + (Site)	-99.4	209.8	0.06	0.06	3	0.58	0.153
Species + (Site)	-100.8	210.2	0.02	0.05	2	1.01	0.123
Sex + (Site)	-101.3	211.3	0.00	0.03	2	2.06	0.073
Sex + Group + (Site)	-100.2	211.3	0.04	0.04	3	2.09	0.072
Species + Sex + Group + (Site)	-99.3	212.0	0.06	0.06	4	2.79	0.051
Species*Group + (Site)	-99.4	212.1	0.06	0.06	4	2.85	0.049
Species + Sex + (Site)	-100.7	212.3	0.02	0.05	3	3.03	0.045
Sex*Group+ (Site)	-100.0	213.4	0.04	0.04	4	4.17	0.025

Species*Sex + (Site)	-100.6	214.5	0.03	0.06	4	5.27	0.015
Sex*Species*Group +(Site)	-98.4	220.5	0.08	0.08	9	11.29	0.001

Table A.14: Summary of model selection results for linear mixed effects models describing the variation in selenium (Se) concentrations in blood (wet weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*).Sample size for NOCA n = 41 and for GCFL n = 20.

Blood Se

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Species + (Site)	-19.7	48.1	0.15	0.15	2	0.00	0.453
Species + Group + (Site)	-19.6	50.3	0.15	0.15	3	2.20	0.151
Species + Sex + (Site)	-19.7	50.5	0.15	0.15	3	2.38	0.138
Species*Group + (Site)	-18.8	51.1	0.17	0.18	4	3.06	0.098
Species*Sex + (Site)	-19.1	51.7	0.16	0.16	4	3.66	0.073
Species + Sex + Group + (Site)	-19.6	52.8	0.15	0.15	4	4.67	0.044
Sex*Species*Group +(Site)	-15.0	54.4	0.25	0.27	9	6.35	0.019
(Site)	-24.6	55.6	0.00	0.00	1	7.46	0.011
Sex*Group+ (Site)	-21.5	56.6	0.10	0.10	4	8.48	0.007
Sex + (Site)	-24.5	57.8	0.00	0.00	2	9.70	0.004
Group + (Site)	-24.5	57.8	0.00	0.00	2	9.73	0.003
Sex + Group + (Site)	-24.5	60.1	0.00	0.00	3	12.05	0.001

Table A.15: Summary of model selection results for linear mixed effects models describing the variation in zinc (Zn) concentrations in feathers (dry weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with (*). Sample size for NOCA *n* = 50 and for GCFL *n* = 20.

Feather Zn

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Species*Group + (Site)	-386.6	786.5	0.14	0.17	4	0.00	0.336
Sex*Species*Group +(Site)	-381.4	786.5	0.25	0.29	9	0.05	0.328
Species*Sex + (Site)	-388.3	789.9	0.11	0.18	4	3.43	0.060
Sex + Group + (Site)	-389.6	790.2	0.07	0.08	3	3.70	0.053
Sex + (Site)	-391.0	790.6	0.03	0.07	2	4.14	0.042
Group + (Site)	-391.0	790.6	0.03	0.04	2	4.17	0.042
(Site)	-392.2	790.8	0.00	0.03	1	4.31	0.039
Species + Sex + Group + (Site)	-389.3	791.9	0.08	0.09	4	5.42	0.022
Species + Sex + (Site)	-390.5	792.0	0.05	0.08	3	5.57	0.021
Species + (Site)	-391.7	792.0	0.01	0.04	2	5.58	0.021
Species + Group + (Site)	-390.6	792.2	0.04	0.05	3	5.73	0.019
Sex*Group+ (Site)	-389.6	792.5	0.07	0.08	4	6.06	0.016

Table A.16: Summary of model selection results for linear mixed effects models describing the variation in zinc (Zn) concentrations in blood (wet weight) of both Northern Cardinals (NOCA) and Great Crested Flycatchers (GCFL) from four sampling locations near the Savannah River Site. Models containing an interaction effect are denoted with(*). Sample size for NOCA *n* = 41 and for GCFL *n* = 20.

Blood Zn

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
(Site)	-143.74	293.91	0.00	0.00	1	0.00	0.39
Sex + (Site)	-143.58	295.88	0.01	0.01	2	1.97	0.15
Group + (Site)	-143.73	296.18	0.00	0.00	2	2.27	0.13
Species + (Site)	-143.74	296.21	0.00	0.00	2	2.29	0.12
Sex + Group + (Site)	-143.56	298.23	0.01	0.01	3	4.31	0.05
Species + Sex + (Site)	-143.58	298.27	0.01	0.01	3	4.35	0.04
Species + Group + (Site)	-143.72	298.56	0.00	0.00	3	4.64	0.04
Species*Group + (Site)	-142.75	299.09	0.00	0.00	4	5.18	0.03
Species*Sex + (Site)	-142.99	299.56	0.02	0.02	4	5.65	0.02
Species + Sex + Group + (Site)	-143.56	300.70	0.01	0.01	4	6.79	0.01
Sex*Group+ (Site)	-143.59	300.76	0.03	0.03	4	6.85	0.01
Sex*Species*Group +(Site)	-141.05	306.59	0.08	0.08	9	12.68	0.00

APPENDIX B

Supplemental Material Part 2

Table B.1: Pearson's correlation for different habitat types in Northern Cardinal (*Cardinalis cardinalis*; *n* = 29) territories in the central Savannah River area in South Carolina.

	Agriculture	Bottomland Hardwood	Early Successional	Mixed Forest	Pine Stand	Road	Upland Hardwood	Wetland
Agriculture	1.000	0.060	-0.136	0.216	-0.195	-0.122	-0.137	1.000
Bottomland Hardwood	0.060	1.000	0.155	-0.248	-0.117	-0.227	-0.074	0.060
Early Successional	-0.136	0.155	1.000	-0.253	-0.375	0.316	0.073	-0.136
Mixed Forest	0.216	-0.248	-0.253	1.000	-0.662	0.305	-0.607	0.216
Pine Stand	-0.195	-0.117	-0.375	-0.662	1.000	-0.465	0.182	-0.195
Road	-0.122	-0.227	0.316	0.305	-0.465	1.000	-0.163	-0.122
Upland Hardwood	-0.137	-0.074	0.073	-0.607	0.182	-0.163	1.000	-0.137
Wetland	1.000	0.060	-0.136	0.216	-0.195	-0.122	-0.137	1.000

Table B.2: Pearson's correlation for different habitat types in Great Crested Flycatcher (*Myarchus crinitis*; $n = 30$) breeding territories in the central Savannah River area in South Carolina.

	Agriculture	Bottomland Hardwood	Early Successional	Industrial	Mixed Forest	Pine Stand	Road	Upland Hardwood	Water
Agriculture	1.000	0.094	-0.102	-0.036	0.013	-0.151	-0.074	-0.125	-0.051
Bottomland Hardwood	0.094	1.000	-0.054	-0.096	-0.353	-0.093	-0.306	-0.022	0.092
Early Successional	-0.102	-0.054	1.000	0.249	-0.059	-0.201	0.324	0.276	-0.146
Industrial	-0.036	-0.096	0.249	1.000	0.113	-0.078	0.281	-0.125	-0.051
Mixed Forest	0.013	-0.353	-0.059	0.113	1.000	-0.655	0.297	-0.155	-0.101
Pine Stand	-0.151	-0.093	-0.201	-0.078	-0.655	1.000	-0.460	-0.226	0.141
Road	-0.074	-0.306	0.324	0.281	0.297	-0.460	1.000	0.008	-0.461
Upland Hardwood	-0.125	-0.022	0.276	-0.125	-0.155	-0.226	0.008	1.000	0.019
Water	-0.051	0.092	-0.146	-0.051	-0.101	0.141	-0.461	0.019	1.000

Table B.4: Summary of fixed effects output from a linear mixed model with $\delta^{15}N$ values as the response variable, tissue type (blood or feather) and species (Great Crested Flycatcher or Northern Cardinal) as the fixed, independent variables and site being a random effect. R^2 values were calculated using restricted maximum likelihood and confidence limits (CL) are 85%.

$\delta^{15}N$

	Estimate	Upper CL	Lower CL	Pr(>|t|)
(Intercept)	5.64	6.43	4.85	<0.001
Feather	2.07	2.63	1.51	<0.001
Cardinal	-2.45	-1.87	-3.03	<0.001
Feather:Cardinal	-1.53	-0.72	-2.35	0.008

$r^{2m} = 0.48$, $r^{2c} = 0.66$

Table B.5: Summary of fixed effects output from a linear mixed model with $\delta^{13}C$ values as the response variable, tissue type (blood or feather) and species (Great Crested Flycatcher or Northern Cardinal) as the fixed, independent variables and site being a random effect. R^2 values were calculated using restricted maximum likelihood and confidence limits (CL) are 85%.

$\delta^{13}C$

	Estimate	Upper CL	Lower CL	Pr(>|t|)
(Intercept)	-24.85	-24.60	-25.10	<0.001
Feather	1.21	1.51	0.91	<0.001
Cardinal	-0.76	-0.45	-1.07	0.001
Feather:Cardinal	-0.91	-0.48	-1.34	0.003

$r^2m = 0.46$, $r^2c = 0.50$

AICc Tables

[a] Akaike's Information Criterion corrected for small sample size using maximum likelihood.

[b] The r^2 values were calculated using restricted maximum likelihood (Nakagawa and Schielzeth, 2013).

[c] The number of parameters in the model including the intercept.

[d] The difference in the AIC_c values of the current model and the model with the lowest AIC_c.

[e] The weight of evidence that the model with the lowest AIC_c value is better than the current model.

Table B.1: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{15}N$ values in feathers of Northern Cardinals (n = 29) based on significant morphometric and sex differences from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Tarsus + (Site)	-48.07	105.88	0.11	0.31	3	0.00	0.28
Sex + Tarsus + (Site)	-46.65	106.04	0.16	0.42	4	0.16	0.26
Sex + (Site)	-48.82	107.38	0.07	0.38	3	1.50	0.13
Mass + Tarsus + (Site)	-47.88	108.49	0.11	0.34	4	2.61	0.08
Mass + (Site)	-49.43	108.59	0.04	0.34	3	2.72	0.07
Tarsus + Wing + Mass + (Site)	-46.53	109.05	0.19	0.32	5	3.18	0.06
Wing + (Site)	-49.72	109.17	0.02	0.23	3	3.29	0.05
Sex + Mass + (Site)	-48.47	109.67	0.09	0.41	4	3.79	0.04
Sex + Wing + (Site)	-48.82	110.36	0.07	0.40	4	4.48	0.03

Sex + Tarsus + Wing + Mass + (Site)	-46.31	112.22	0.18	0.36	6	6.34	0.01

Table B.2: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{13}C$ values in feathers of Northern Cardinals (n = 29) based on morphometric and sex differences from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Wing + (Site)	-42.14	94.02	0.01	0.34	3	0.00	0.22
Tarsus + (Site)	-42.26	94.25	0.00	0.32	3	0.23	0.20
Mass + (Site)	-42.26	94.26	0.00	0.31	3	0.23	0.20
Sex + (Site)	-42.26	94.26	0.00	0.31	3	0.23	0.20
Sex + Wing + (Site)	-42.08	96.90	0.01	0.37	4	2.87	0.05
Sex + Tarsus + (Site)	-42.26	97.24	0.00	0.31	4	3.22	0.04
Mass + Tarsus + (Site)	-42.26	97.24	0.00	0.31	4	3.22	0.04
Sex + Mass + (Site)	-42.26	97.24	0.00	0.30	4	3.22	0.04
Tarsus + Wing + Mass + (Site)	-42.11	100.23	0.01	0.34	5	6.20	0.01
Sex + Tarsus + Wing + Mass + (Site)	-42.01	103.62	0.02	0.38	6	9.60	0.00

Table B.3: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{15}N$ values in feathers of Great Crested Flycatchers (n = 30) based on morphometric and sex differences from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Tarsus + (Site)	-64.02	137.64	0.20	0.34	3	0.00	0.50
Mass + Tarsus + (Site)	-62.91	138.32	0.24	0.36	4	0.68	0.36
Tarsus + Wing + Mass + (Site)	-62.91	141.48	0.24	0.35	5	3.84	0.07
Sex + Tarsus + (Site)	-63.76	143.17	0.20	0.31	4	5.53	0.03
Mass + (Site)	-67.41	144.41	0.02	0.15	3	6.77	0.02
Wing + (Site)	-67.83	145.26	0.00	0.14	3	7.62	0.01
Sex + (Site)	-67.83	148.17	0.00	0.13	3	10.53	0.00
Sex + Tarsus + Wing + Mass + (Site)	-62.86	148.58	0.23	0.33	6	10.94	0.00
Sex + Mass + (Site)	-67.30	150.25	0.03	0.15	4	12.61	0.00
Sex + Wing + (Site)	-67.83	151.31	0.00	0.13	4	13.67	0.00

Table B.4: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{13}C$ values in feathers of Great Crested Flycatchers (n = 30) based on morphometric and sex differences from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Wing + (Site)	-31.58	72.76	0.20	0.28	3	0.00	0.67
Tarsus + Wing + Mass + (Site)	-30.44	76.53	0.23	0.27	5	3.77	0.10

Sex + (Site)	-32.60	77.70	0.13	0.13	3	4.94	0.06
Tarsus + (Site)	-34.12	77.83	0.04	0.05	3	5.08	0.05
Sex + Wing + (Site)	-31.30	78.25	0.20	0.26	4	5.49	0.04
Mass + (Site)	-34.83	79.25	0.00	0.00	3	6.50	0.03
Sex + Tarsus + (Site)	-32.20	80.05	0.15	0.15	4	7.29	0.02
Sex + Mass + (Site)	-32.40	80.45	0.14	0.14	4	7.70	0.01
Mass + Tarsus + (Site)	-34.11	80.72	0.04	0.05	4	7.96	0.01
Sex + Tarsus + Wing + Mass + (Site)	-30.19	83.24	0.23	0.26	6	10.48	0.00

Table B.5: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{15}N$ values in blood of Great Crested Flycatchers (n = 30) based on morphometric and sex differences from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Mass + (Site)	-43.98	97.57	0.02	0.66	3	0.00	0.23
Wing + (Site)	-44.08	97.77	0.01	0.64	3	0.20	0.21
Sex + (Site)	-42.68	97.87	0.05	0.69	3	0.30	0.20
Tarsus + (Site)	-44.64	98.87	0.00	0.62	3	1.30	0.12
Sex + Wing + (Site)	-42.15	99.95	0.06	0.70	4	2.38	0.07
Sex + Mass + (Site)	-42.25	100.14	0.06	0.70	4	2.57	0.06
Mass + Tarsus + (Site)	-43.97	100.45	0.02	0.65	4	2.88	0.05
Sex + Tarsus + (Site)	-42.62	100.89	0.05	0.68	4	3.32	0.04

Tarsus + Wing + Mass + (Site)	-43.71	103.08	0.02	0.65	5	5.51	0.02
Sex + Tarsus + Wing + Mass + (Site)	-41.75	106.35	0.07	0.70	6	8.78	0.00

Table B.6: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{13}C$ values in blood of Great Crested Flycatchers (n = 30) based on morphometric and sex differences from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Mass + (Site)	-17.95	45.49	0.13	0.14	3	0.00	0.38
Mass + Tarsus + (Site)	-17.50	47.50	0.15	0.15	4	2.01	0.14
Sex + Mass + (Site)	-15.93	47.51	0.22	0.22	4	2.02	0.14
Sex + (Site)	-17.83	48.16	0.13	0.13	3	2.67	0.10
Tarsus + (Site)	-19.38	48.36	0.04	0.09	3	2.86	0.09
Wing + (Site)	-19.81	49.21	0.01	0.08	3	3.72	0.06
Sex + Wing + (Site)	-17.33	50.31	0.15	0.15	4	4.82	0.03
Sex + Tarsus + (Site)	-17.34	50.34	0.15	0.15	4	4.85	0.03
Tarsus + Wing + Mass + (Site)	-17.41	50.48	0.15	0.15	5	4.99	0.03
Sex + Tarsus + Wing + Mass + (Site)	-15.46	53.78	0.23	0.23	6	8.28	0.01

Table B.7: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{15}N$ values in blood of Northern Cardinals (n = 29) based on morphometric and sex differences from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Tarsus + (Site)	-40.90	91.62	0.23	0.36	3	0.00	0.33
Mass + Tarsus + (Site)	-39.39	91.65	0.30	0.44	4	0.03	0.33
Sex + Tarsus + Wing + Mass + (Site)	-36.76	93.41	0.35	0.59	6	1.79	0.14
Sex + Tarsus + (Site)	-40.52	93.89	0.24	0.37	4	2.27	0.11
Tarsus + Wing + Mass + (Site)	-39.39	94.98	0.29	0.43	5	3.36	0.06
Mass + (Site)	-44.50	98.83	0.02	0.10	3	7.21	0.01
Sex + (Site)	-44.65	99.12	0.01	0.11	3	7.51	0.01
Wing + (Site)	-44.65	99.13	0.02	0.17	3	7.51	0.01
Sex + Wing + (Site)	-43.80	100.45	0.09	0.34	4	8.83	0.00
Sex + Mass + (Site)	-44.05	100.95	0.05	0.12	4	9.34	0.00

Table B.8: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{13}C$ values in blood of Northern Cardinals (n = 29) based on morphometric and sex differences from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]

Sex + Tarsus + (Site)	-24.60	62.05	0.29	0.39	4	0.00	0.41
Tarsus + (Site)	-26.90	63.62	0.11	0.12	3	1.57	0.19
Sex + Wing + (Site)	-25.74	64.34	0.23	0.23	4	2.28	0.13
Sex + (Site)	-27.86	65.53	0.11	0.12	3	3.48	0.07
Mass + Tarsus + (Site)	-26.48	65.82	0.19	0.28	4	3.76	0.06
Mass + (Site)	-28.45	66.73	0.07	0.07	3	4.67	0.04
Sex + Tarsus + Wing + Mass + (Site)	-23.51	66.91	0.32	0.39	6	4.85	0.04
Sex + Mass + (Site)	-27.36	67.57	0.14	0.14	4	5.52	0.03
Wing + (Site)	-29.30	68.43	0.01	0.03	3	6.37	0.02
Tarsus + Wing + Mass + (Site)	-26.48	69.16	0.19	0.27	5	7.10	0.01

Table B.9: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{15}N$ values in feathers of Northern Cardinals (n = 26) in different habitat types from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Distance to water + Tarsus + (Site)	-40.26	93.52	0.42	0.42	5	0.00	0.49
Distance to water + Sex + Tarsus + (Site)	-38.86	94.13	0.42	0.50	5	0.61	0.36
Distance to water + (Site)	-43.63	97.16	0.24	0.29	3	3.64	0.08
Early Successional + (Site)	-45.37	100.65	0.14	0.25	3	7.13	0.01

Bottomland Hardwood + (Site)	-45.51	100.92	0.12	0.28	3	7.40	0.01
Bottomland Hardwood + Sex + Tarsus + (Site)	-42.40	101.22	0.24	0.48	5	7.70	0.01
Early Successional + Sex + Tarsus + (Site)	-42.43	101.27	0.26	0.51	5	7.75	0.01
(Site)	-47.38	101.85	0.00	0.27	2	8.33	0.01
Mixed Forest + Upland Hardwood + Pine Stand + (Site)	-42.89	102.20	0.32	0.42	5	8.68	0.01
Mixed Forest + Upland Hardwood + Pine Stand + Sex + Tarsus + (Site)	-40.38	105.23	0.39	0.55	7	11.71	0.00

Table B.10: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{13}C$ values in feathers of Northern Cardinals (n = 26) in different habitat types from four sampling locations near the Savannah River Site from April – May 2017. Note: no individual covariates were significant predictors of $\delta^{13}C$ in cardinal feather.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
(Site)	-39.06	85.21	0.00	0.37	2	0.00	0.44
Bottomland Hardwood + (Site)	-38.22	86.34	0.05	0.54	3	1.13	0.25
Distance to water + (Site)	-38.65	87.20	0.03	0.47	3	1.99	0.16
Early Successional + (Site)	-38.72	87.34	0.02	0.33	3	2.13	0.15

Mixed Forest + Upland Hardwood + Pine Stand + (Site)	-38.57	93.55	0.02	0.46	5	8.34	0.01

Table B.11: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{15}N$ values in blood of Northern Cardinals (n = 26) in different habitat types from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Early Successional + (Site)	-40.59	91.08	0.26	0.39	3	0.00	0.41
Early Successional + Mass + Tarsus + (Site)	-37.76	91.94	0.36	0.49	5	0.85	0.27
(Site)	-43.67	94.43	0.00	0.11	2	3.35	0.08
Bottomland Hardwood + (Site)	-42.77	95.44	0.06	0.15	3	4.36	0.05
Bottomland Hardwood + Mass + Tarsus + (Site)	-39.55	95.52	0.24	0.33	5	4.43	0.04
Distance to water + (Site)	-42.93	95.77	0.05	0.05	3	4.69	0.04
Distance to water + Tarsus + Mass + (Site)	-39.70	95.83	0.24	0.33	5	4.74	0.04
Distance to water + Tarsus + (Site)	-41.46	95.92	0.15	0.15	4	4.84	0.04

Mixed Forest + Upland Hardwood + Pine Stand + (Site)	-39.77	95.97	0.33	0.52	5	4.88	0.04
Mixed Forest + Upland Hardwood + Pine Stand + Mass + Tarsus + (Site)	-36.81	98.09	0.41	0.59	7	7.01	0.01

Table B.12: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{13}C$ values in blood of Northern Cardinals (n = 26) in different habitat types from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Distance to water + Tarsus + (Site)	-22.19	57.39	0.37	0.45	4	0.00	0.34
Bottomland Hardwood + Sex + Tarsus + (Site)	-20.78	57.98	0.41	0.53	5	0.59	0.26
Distance to water + Tarsus + Sex + (Site)	-20.85	58.13	0.42	0.48	5	0.74	0.24
Early Successional + Sex + Tarsus + (Site)	-21.80	60.02	0.38	0.44	5	2.63	0.09
Mixed Forest + Upland Hardwood + Pine Stand + Sex + Tarsus + (Site)	-18.41	61.30	0.46	0.77	7	3.91	0.05
(Site)	-28.89	64.86	0.00	0.03	2	7.48	0.01

Bottomland Hardwood + (Site)	-27.63	65.16	0.14	0.33	3	7.77	0.01
Distance to water + (Site)	-28.10	66.11	0.07	0.13	3	8.72	0.00
Early Successional + (Site)	-28.88	67.66	0.01	0.11	3	10.28	0.00
Mixed Forest + Upland Hardwood + Pine Stand + (Site)	-27.64	71.70	0.24	0.59	5	14.31	0.00

Table B.13: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{15}N$ values in blood of Great Crested Flycatchers (n = 29) in different habitat types from four sampling locations near the Savannah River Site from April – May 2017. Note: no individual covariates were significant predictors of $\delta^{15}N$ in flycatcher blood.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Bottomland Hardwood + (Site)	-41.35	92.36	0.05	0.66	3	0.00	0.43
(Site)	-43.03	93.02	0.00	0.66	2	0.66	0.31
Distance to water + (Site)	-42.40	94.46	0.02	0.73	3	2.10	0.15
Early Successional + (Site)	-42.78	95.23	0.01	0.66	3	2.87	0.10
Mixed Forest + Upland Hardwood + Pine Stand + (Site)	-42.88	101.57	0.01	0.69	5	9.21	0.00

Table B.14: Summary of model selection results for linear mixed effects models describing the variation in $\delta^{13}C$ values in blood of Great Crested Flycatchers (n = 29) in different habitat types from four sampling locations near the Savannah River Site from April – May 2017.

Model	logLik	AIC_C[a]	r^2m[b]	r^2c[b]	K[c]	ΔAIC[d]	AIC weight[e]
Bottomland Hardwood + Mass + (Site)	-16.07	44.75	0.21	0.21	4	0.00	0.37
Bottomland Hardwood + (Site)	-18.28	46.22	0.09	0.12	3	1.47	0.18
(Site)	-19.71	46.39	0.00	0.09	2	1.64	0.17
Early Successional + Mass + (Site)	-17.44	47.49	0.14	0.15	4	2.74	0.10
Distance to water + Mass + (Site)	-17.73	48.07	0.11	0.17	4	3.32	0.07
Distance to water + (Site)	-19.48	48.63	0.03	0.17	3	3.88	0.05
Early Successional + (Site)	-19.60	48.87	0.00	0.08	3	4.12	0.05
Mixed Forest + Upland Hardwood + Pine Stand + Mass + (Site)	-16.50	52.34	0.18	0.18	6	7.59	0.01
Mixed Forest + Upland Hardwood + Pine Stand + (Site)	-18.59	52.99	0.06	0.08	5	8.24	0.01