# ACCUMULATION FEATURES OF PERSISTENT ORGANIC POLLUTANTS IN NEOTROPICAL MIGRATORY SONGBIRDS

A Dissertation

by

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ABSTRACT

Migratory songbirds have seen significant population declines over the last several decades. One threat to songbird populations may be due to adverse health effects of legacy contaminants such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs). Migratory songbirds spend the vast majority of their annual cycle either migrating or on their wintering grounds in Latin America; due to their annual movements they may be exposed to a wide variety of contaminants. The major aim of this dissertation was to examine accumulation features of persistent organic pollutants (POPs) in migratory songbirds. The specific objectives were to 1) examine seasonal variation of contaminant accumulation in migrant and resident songbirds, 2) determine what ecological factors, through the use of stable isotopes ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$), best explain contaminant levels and 3) determine the current state of the research on POPs levels for birds in Latin America.

In general, no significant accumulation of contaminants during migration was observed for migratory songbirds. Resident birds from Texas were significantly more contaminated with DDE and ΣPBDEs compared to resident species from wintering areas (Yucatán and Costa Rica). Residents from Costa Rica also had the lowest levels of ΣPCBs.

Stable isotopes revealed that there were only minor differences (≤ 2‰) in mean liver $\delta^{13}C$ and $\delta^{15}N$ values between migrants and residents, indicating similar diets. Results from linear regression models showed that $\delta^{15}N$ was only useful in explaining ΣPCBs concentrations in resident birds from Texas and in songbirds from Yucatán. A significant relationship was found between $\delta^{13}C$ and DDE and ΣPBDEs in residents from Texas; birds from College Station were more enriched in $^{13}C$ and had elevated levels of POPs suggesting proximity to urban environments as a source of contaminant exposure.

Significant temporal decreases in POPs levels was observed in birds from 1980 – 2018. DDE was the most frequently reported contaminant, followed by ΣPCBs. Levels of contaminants were generally below those known to cause adverse health effects. The regional distribution of the data is currently uneven with most studies coming from Mexico, followed by Brazil, and centered on coastal regions and aquatic species.

DEDICATION

This dissertation is dedicated to my family, Israel, Paula, Anabel, and Gabriel whose love and support made this endeavor possible. I love you all very much. This dissertation is also dedicated to the songbirds that were used in this research.

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## NOMENCLATURE

| | |
|---|---|
| DDE | Dichlorodiphenyldichloroethene |
| DDT | Dichlorodiphenyltrichloroethane |
| ED | Endocrine Disruptors |
| GC | Gas Chromatography |
| GERG | Geosciences Environmental Research Group |
| GCBO | Gulf Coast Bird Observatory |
| HCB | Hexachlorobenzene |
| HPLC | High Performance Liquid Chromatography |
| MDL | Method Detection Limit |
| OC | Organochlorine pesticide |
| PBDE | Polybrominated Diphenyl Ether |
| PCB | Polychlorinated Biphenyl |
| PCDD | Polychlorinated Dibenzo-*p*-Dioxin |
| PCDF | Polychlorinated Dibenzofuran |
| POP | Persistent Organic Pollutant |
| TAMU | Texas A&M University |
| TCMX | Tetrachloro-Meta-Xylene |

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# CHAPTER I

# INTRODUCTION TO PERSISTENT ORGANIC POLLUTANTS (POPS) AND THEIR ADVERSE EFFECTS IN SONGBIRDS

## Introduction

North American songbird populations, in particular Neotropical migrants, are declining at alarming rates as varied environmental threats mount. Complex and often interwoven factors such as habitat loss, urbanization, climate change and environmental pollution have contributed to these declines. However, the role of environmental pollutants on these population declines is poorly understood. This chapter provides background information on the environmental contaminants under study and the adverse health effects of these contaminants on songbird species.

## Persistent Organic Pollutants (POPs)

### *Chemical and physical characteristics of persistent organic pollutants*

Persistent organic pollutants (POPs) are “chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment” (UNEP).

As their name suggests, POPs are resistant to biological, photolytic, and chemical degradation in the environment. Their physical and chemical properties of high stability and low reactivity correlate to their prolonged persistence in the environment, which can lead to greater risk of exposure over extended periods of time. Many POPs share similar structural characteristics, which make them difficult to degrade, such as the presence of aromatic rings and halogenation (Miniero and Iamiceli 2008).

POPs have also been shown to undergo long-range transport and are therefore ubiquitous in the environment. They have been found in both environmental and biotic samples in places such as the Arctic and Antarctic, far from industrial sources (Wania and Mackay 1996). Under normal conditions, they can evaporate from soils and sediment, become adsorbed onto airborne particles and transported great distances in the atmosphere before eventually settling back down to the Earth's surface. This process can repeat itself and is known as the "grasshopper effect."

Another common characteristic of POPs is the ability to bioaccumulate and biomagnify in organisms relative to their surrounding environment (Newman and Unger 2010). POPs low water solubility and lipophilic nature results in their partitioning onto sediments, organic matter, and in living organisms. Therefore, they tend to accumulate in the fatty tissues of organisms, referred to as bioaccumulation. Biomagnification, an increase in contaminant concentration from one trophic level to the next, is also a well-established phenomenon (Norstrom 2002). Organisms that feed at higher trophic scales, such as top tier predators, are at greatest risk of exposure to contaminant accumulation and subsequent adverse health effects.

In 1995, the Governing Council of the United Nations Environment Programme (UNEP) developed a global treaty with the objective of protecting human health and the environment from persistent organic pollutants. The major aims of the treaty are to eliminate, restrict, and reduce the production and use of POPs. There were initially 12 chemicals, referred to as the "dirty dozen," that were shown to cause adverse effects to humans and ecosystems. They included pesticides, industrial chemicals, and by-products (Table 1).

**Table 1.** Initial list of 12 POPs known as the "dirty dozen".

| **Chemical** | Global Historical Use and Source |
|---|---|
| Aldrin | Insecticide used on crops such as corn and cotton; also used for termite control. |
| Chlordane | Insecticide used on crops, including vegetables, small grains, potatoes, sugarcane, sugar beets, fruits nuts, citrus, and cotton. Used on home lawn and garden pests. Also used extensively to control termites. |
| Trichloroethane (DDT) | Insecticide used on agricultural crops, primarily cotton, and insects that carry disease such as malaria and typhus. |
| Dieldrin | Insecticide used on crops such as corn and cotton; also used for termite control. |
| Endrin | Insecticide used on crops such as cotton and grains; also used to control rodents. |
| Heptachlor | Insecticide used primarily against soil insects and termites. Also used against some crop pests and to combat malaria. |
| Hexachlorobenzene | Fungicide used for seed treatment. Also an industrial chemical used to make fireworks, ammunition, synthetic rubber, and other substances. |
| Mirex | Insecticide used to combat fire ants, termites, and mealybugs. Also used as a fire retardant in plastics, rubber, and electrical products. |
| Toxaphene | Insecticide used to control pests on crops and livestock, and to kill unwanted fish in lakes. |
| Polychlorinated Biphenyls (PCBs) | Used for a variety of industrial processes and purposes, including electrical transformers and capacitors, as heat exchange fluids, as paint additives, in carbonless copy paper, and in plastics. Also unintentionally produced during combustion. |
| Polychlorinated dibenzo-p-dioxins (PCDDs) | Unintentionally produced during most forms of combustion, including burning of municipal and medical wastes, backyard burning of trash, and industrial processes. |
| Polychlorinated dibenzofurans (PCDFs) | Unintentionally produced during most forms of combustion, including burning of municipal and medical wastes, backyard burning of trash, and industrial processes. |

*Classes of Persistent Organic Pollutants (POPs)*

**Organochlorine pesticides (OCPs)**

Organochlorine pesticides are a diverse group of chlorinated hydrocarbons that exhibit similar biological and environmental effects. They were used extensively from the 1940's through the 1960's, mostly in agriculture and vector control applications. Representative compounds include dichlorodiphenyltrichloroethane (DDT) and its metabolites, aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene, methoxychlor, mirex, and, toxaphene (Figure 1). The parent compound of *p,p'*–DDT is converted to the highly persistent metabolite *p,p'*–DDE via loss of a chlorine atom (Figure 2). As early as the 1950's and 60's evidence of the accumulation of dichlorodiphenyldichloroethene (DDE) in organisms was observed. In the 1960's several species of piscivorous bids across North America and Europe including bald eagles (*Haliaeetus leucocephalus*), peregrine falcon (*Falco peregrinus*), brown pelican (*Pelecanus occidentalis*), American kestrel (*F. sparvrius*), Aplomado falcon (*F. femoralis*) and golden eagle (*Aquila chrysaetos*) experienced severe population declines (Ratcliffe 1967). Through field studies and later experiments, it was discovered that DDE was biomagnifying through food webs and causing eggshell thinning by inhibiting Ca-dependent ATPase in the shell gland of birds (Cade et al. 1971, Findholt and Trost 1985, Lincer 1975, Peterle 1991). Thin eggshells resulted in low reproductive success due to crushed eggs, reduced hatching success, and smaller clutch sizes, ultimately resulting in population declines.

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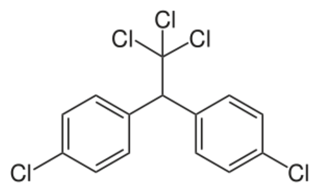
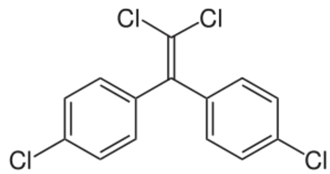
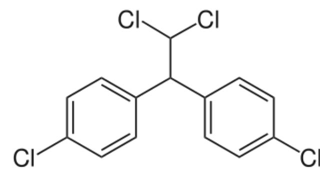
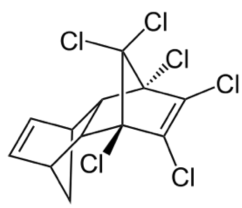
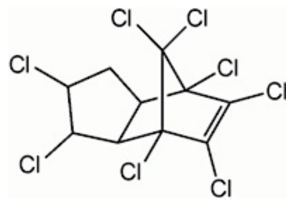
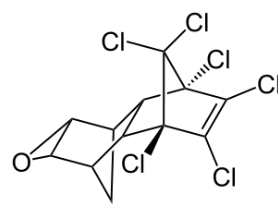
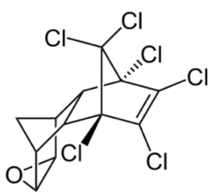
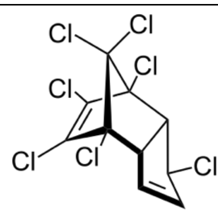
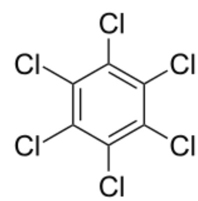
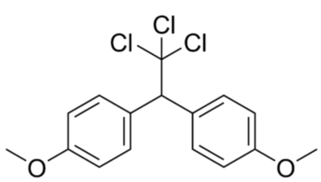
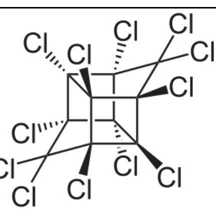
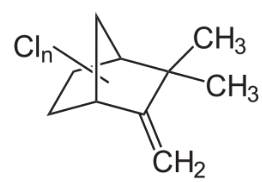
|  |  |  |
| DDT | DDE | DDD |
|  |  |  |
| Aldrin | Chlordane | Dieldrin |
|  |  |  |
| Endrin | Heptachlor | Hexachlorobenzene |
|  |  |  |
| Methoxychlor | Mirex | Toxaphene |

**Figure 1.** Chemical structures of representative organochlorine pesticides.

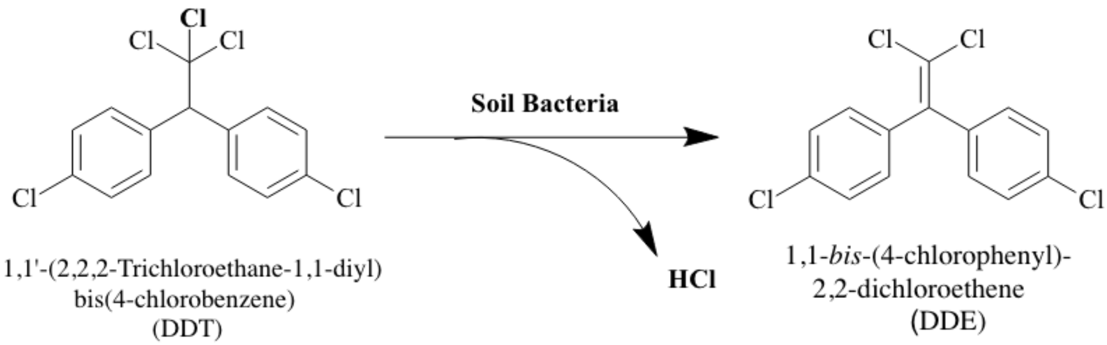


**Figure 2.** Metabolization of parent compound DDT to DDE.

**Polychlorinated biphenyls (PCBs)**

Polychlorinated biphenyls (PCBs) are a type of industrial contaminant first introduced in the 1930's. PCBs were commercially produced by the chlorination of biphenyl, which resulted in chemical mixtures of a variety of individual chlorinated biphenyl congeners. In the United States, PCB mixtures were produced under the trade name "Aroclor". There are potentially 209 different congeners of PCBs with varying degrees of chlorination; the general structure for PCB is shown in Figure 3. They have a wide range of industrial applications due to their low reactivity and high chemical stability. These chemical properties are what have led to their persistence and accumulation in the environment and cause for concern. They were widely used as pesticide extenders, heat transfer fluids, hydraulic lubricants, adhesives, flame retardants, cutting oils, components of plasticizers in paints, inks, and toners (Grube et al. 2011, Hutzinger, Safe, and Zitko 1974). They were banned in 1979 by the EPA under the Toxic Substances Control Act due to the high risk they posed to humans and wildlife (Peterle 1991). PCBs are highly lipophilic and can bioaccumulate and biomagnify through food webs. They have also been linked to a variety of health problems in birds and other wildlife. The number and position of chlorine atoms on the biphenyl rings are important determinants of the biological and physical properties, as well as toxicity, of PCBs. PCB congeners with chlorines positioned in the non- and mono-*ortho* positions have a coplanar configuration and exhibit dioxin-like activity, making them among the more toxic of the congeners. PCB congeners that are considered to be dioxin-like compounds and are most environmentally significant are PCBs 77, 126, 169, 105, 114, 118, 123, 156, 157, 167, and 189 (EPA 2010). Like dioxins, non- and mono-*ortho* substituted PCB congeners can activate the aryl hydrocarbon receptor (AhR), which is responsible for the regulation of metabolic cytochrome P450 enzymes (Harris and Elliott 2011). Anthropogenic activities are the only source of PCBs in the environment and hence, sites that are more highly contaminated are in industrialized areas.

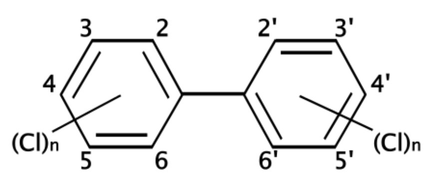



**Figure 3.** General structure of polychlorinated biphenyls (PCBs).

**Polybrominated diphenyl ethers (PBDEs)**

Polybrominated diphenyl ethers (PBDEs) are a group of flame retardants widely used to reduce fire hazards by interfering with combustion reactions (Rahman et al. 2001).

PBDEs are most commonly used in polymer materials with applications in electronics, building materials, and upholstery (de Wit 2002). PBDEs are a type of additive flame retardant where they are generally blended with polymers (Alaee 2003, de Wit 2002). Since they are not chemically bound, this leads to a higher likelihood of PBDEs leaching into the environment. PBDEs are similar in structure to PCBs with bromine atoms attached to different positions on phenyl rings linked by an ether bond (Figure 4). The most common commercial mixture of PBDEs are decabromodiphenyl ehter (DBDE), octabromodiphenyl ether (OBDE), and pentabromodiphenyl (pentaBDE) (Darnerud 2008). The major PBDE product in all markets is DBDE and accounts for approximately 80% of the total production worldwide (Darnerud et al. 2001). Like PCBs, PBDEs are chemically stable, making them resistant to environmental degradation. They have also been shown to bioaccumulate and biomagnify through food webs (Burreau et al. 2006). Unlike PCBs however, which are considered legacy contaminants, PBDEs are considered a new emergent contaminant of concern. Samples from sediments, biota and humans show an increasing trend over time (Park et al. 2009, Shaw and Kannan 2009). PBDEs have also been linked to a variety of human and wildlife health problems (Darnerud et al. 2001). Polybrominated diphenyl ethers are of particular concern due to their effects on the thyroid hormone endocrine system. Previous studies have indicated that exposure to PBDEs can decrease thyroid hormone levels, in particular thyroxine ($T_4$), in birds, fish, and rodents (Jugan, Levi, and Blondeau 2010). In addition, researchers have demonstrated that hydroxylated metabolites of PBDEs have competitive binding affinity relative to $T_4$ with transthyretin (TTR) indicating that metabolites may pose a greater risk than parent compounds (Cao et al. 2010). TTR is an essential plasma binding protein that transports thyroid hormones to target tissues.

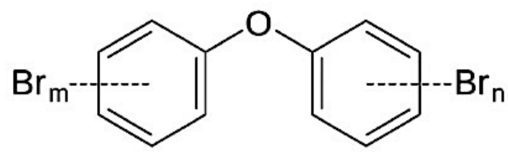


**Figure 4.** Chemical structure of polybrominated diphenyl ether (PBDE).

## Effects of POPs observed in songbirds

Birds were among the first indicators of the adverse effects posed by persistent organic pollutants. Many POPs, are known or suspected endocrine disruptors (EDs). They can interfere with natural hormone system function both directly, by binding to hormone, receptors or indirectly, such as interfering with synthesis, metabolic breakdown or excretion of hormones. DeLeon et al. (2013) looked at the effects of PCBs on song in two species of passerines. For black-capped chickadees (*Poecile atricapillus*), the species-specific identity signal varied significantly. Neigh et al. (2007) looked at reproductive success in two species of passerines, Eastern bluebirds (*Sialia sialis*) and house wrens (*Troglodytes aedon*), exposed to PCBs from a contaminated superfund site in the Kalamazoo River. Hatching success and clutch size in house wrens were significantly lower at the contaminated site compared to a reference site, however, it is likely that other factors such as habitat quality and prey abundance were contributing factors to observed differences. McCarty and Secord (1999a) measured nest building behavior and quality of tree swallows (*Tachycineta bicolor*) breeding along the Hudson River in New York in PCB contaminated areas. Tree swallows from PCB contaminated sites built smaller nests of lower quality when compared to birds from a reference site. Nest quality is an important factor in reproductive success and the researchers results indicate that PCBs may be acting as EDs in regards to nest building behavior, ultimately affecting reproduction. Flahr et al. (2015) exposed juvenile European starlings (*Sturnus vulgaris*) to environmentally relevant concentrations of PCB mixture Aroclor 1254. Juvenile starlings in the high dose treatment group (1.05 μg/g-body weight) failed to orient properly during simulated peak migration time. Songbirds exposed to high levels of PCBs also had incomplete molts. Improper timing and orientation of migratory activity in addition to incomplete molt could result in negative implications at the individual level and ultimately to populations. Eng et al. (2014) measured ΣPBDEs in European starlings from an agricultural area in British Columbia; ΣPBDE concentrations in eggs were low (range: 2–307 ng/g ww). No correlation was observed for PBDE concentrations in eggs and reproductive success, maternal condition, or nestling condition (Eng et al. 2014), indicating passerines might not be as sensitive to PBDEs at low levels. However, a recent study by Eng et al. (2018) found that *in ovo* exposure to high environmental concentrations of PBDEs (≥ 1,000 ng/g) could affect avian brain structure that is responsible for song-control in passerines.

CHAPTER II

# SEASONAL DIFFERENCES IN CONTAMINANT ACCUMULATION IN NEOTROPICAL MIGRANT AND RESIDENT SONGBIRDS*

**Summary**

For many years, it has been hypothesized that Neotropical migrants that breed in the United States and Canada accumulate organochlorine pesticides (OCPs) while on their wintering grounds in Latin America. We investigated the seasonal accumulation of persistent organic pollutant (POPs) in migrant and resident passerines in Texas, Yucatán, and Costa Rica collected during the fall, winter and spring from 2011 to 2013. A total of 153 birds were collected and all contained detectable levels of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and OCPs, dichlorodiphenyldichloroethene (DDE) being the most predominant pesticide. OCPs and PCBs were the predominant contaminants, accounting for ≥ 80% of the total POPs burden, while PBDEs accounted for ≤ 16%. Only spring migrants from Texas had significantly higher DDE concentrations (64.6 ng/g dw) than migrants collected in Costa Rica (23.2 ng/g dw). Resident birds in Texas had significantly greater levels of DDE (121 ng/g dw) and ΣPBDEs (34.8 ng/g dw) compared to residents in Yucatán and Costa Rica. For ΣPCBs, resident birds from Costa Rica had significantly lower concentrations (9.60 ng/g dw) compared to their migrant counterparts (43.7 ng/g dw) and residents from Texas (48.3

ng/g dw) and the Yucatán (32.1 ng/g dw). Migrant and resident passerines had similar congener profiles for PCBs and PBDEs suggesting similar exposure and retention of these contaminants. No significant accumulation of DDE was observed in migrants while on their wintering grounds. Relatively high concentrations of PBDEs in resident birds from Costa Rica warrant future studies of PBDE contamination in Latin America.

## Introduction

Neotropical migratory passerines are those songbird species that breed in North America (United States and Canada) during the summer months and migrate south in the fall to their wintering grounds (Caribbean, Mexico, Central and South America). Due to their annual movements, these long-distance migrants may be exposed to a wide range of pollutants over large geographic areas. Some migratory songbirds have suffered significant population declines over the past several decades; explanations for these have included habitat loss, parasitism, climate change and environmental pollutants (Robbins et al. 1989, Robinson and Wilcove 1994, DeGraaf and Rappole 1995, Both et al. 2006). However, the relationship between environmental contaminants and population declines of Neotropical migratory songbirds is poorly understood (Finch and Martin 1995, Gard, Hooper, and Bennett 1993). In contrast, population declines for larger avian species and dichlorodiphenyltrichloroethane (DDT) are well documented in the literature. The primary breakdown product of DDT, dichlorodiphenyldichloroethene (DDE), was shown to cause eggshell thinning, thus critically impacting reproduction and hatching success (Peakall 1993, Fry 1995, King et al. 2003). Since most Neotropical migrants spend the vast majority of their annual cycle either migrating or on their wintering grounds (Newton 2010) it is critical to understand how contaminants in these areas may impact individuals and populations during migration. Migratory behavior can play an important role in the accumulation of contaminants and their distribution within the body (Van Velzen, Stiles, and Stickel 1972, Henny et al. 1982). Several studies have shown that there are seasonal differences in body burdens for migratory birds; there are also observed differences in the levels and specific contaminant profiles between migrants and residents (Tanabe et al. 1998, Kunisue et al. 2003, Yogui and Sericano 2009, Seegar et al. 2015). Migration is energetically costly and birds rely primarily on fat stores to fuel their flights. Lipophilic contaminants especially can thus become mobilized and redistributed to different organs, posing additional risk to migrating birds that may already be under physiological stress (Scollon et al. 2012). Additionally, contaminant exposure in songbirds has been shown to alter migratory behavior by disrupting orientation capabilities and impairing molt completion, both critical for successful migration (Flahr et al. 2015).

The use of many persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were banned in North America in the early 1970s, which lead to significant declines of these contaminants in passerines and other avian species (Johnston 1974, Olsson et al. 2000, Henny, Yates, and Seegar 2009, Mora et al. 2016). However, due to their persistence in the environment, detectable levels are still observed in many passerine species and have the potential to cause adverse health effects (McCarty and Secord 1999a, Neigh et al. 2007, DeLeon et al. 2013). In addition, polybrominated diphenyl ethers (PBDEs), a class of flame retardants, are relatively new emergent contaminants of concern with increasing temporal trends seen in environmental media and biota (Shaw and Kannan 2009). Encouragingly, restrictions and bans on the use and production of certain PBDE formulations in developing countries have led to observable declines in the levels of flame retardants in wildlife and the environment (Law et al. 2014). However, the persistence of these contaminants and the ability to undergo long-range transport, has led to their ubiquity in all environmental media, humans, and wildlife (Jones and De Voogt 1999).

A long held hypothesis is that migratory songbirds accumulate OCPs, in particular DDT and its degradation products, while on their wintering grounds in Latin America, where it was used in agriculture and for malarial control and not banned until the late 80's and early 90's (Von Duszeln 1991, Albert 1996, Díaz-Barriga et al. 2003). Although an earlier study by Henny et al. (1982) demonstrated that migrating peregrine falcons (*Falco peregrinus*) accumulated chlorinated pesticides while on their wintering grounds, later studies with Black-crowned night-herons (*Nycticorax nycticorax*) suggested that populations wintering in Mexico had limited exposure to OCPs compared to those wintering in the Southwestern United States (Henny and Blus 1986). A recent paper by Mora et al. (2016) examined temporal and latitudinal contaminant trends in birds from North America. Results from the analyses revealed decreasing concentrations of DDE with increasing latitude for migrant passerines. The authors suggested that these trends could be due to possible hotspots of DDE contamination in the Southwestern United States and Mexico. In contrast to this, the current literature shows relatively low levels of POP residues for resident passerines in Latin America and low exposure in migrants while on their wintering grounds (Fyfe et al. 1990, Harper et al. 1996, Capparella et al. 2003, Klemens et al. 2003, Mora 2008). Given these findings, our study aims to help determine where and when Neotropical migrants are at greatest risk of exposure to contaminants. This is information is important for understanding the broader impacts of pollutants on individuals and populations. Our main objective was to examine seasonal variation in contaminant accumulation of POPs, including OCPs, PCBs, and PBDEs, in migratory and resident songbirds from locations in North and Central America. Due to their year round residency, resident species are good indicators of local contamination levels and can be used to determine pesticide acquisition in migrants.

## Methods

### *Study areas, sample collection, and preparation*

Migrant and resident passerines were collected between 2011 and 2013 during migration and wintering periods from sites located along the central flyway migration corridor in Texas, Mexico, and Costa Rica (Figure 5). Samples collected at three locations in Texas occurred during both the fall (2011 and 2012) and spring (2012 and 2013) migration periods, as birds were moving to and from their wintering grounds. Two of the sites are privately owned properties in Brazos (College Station, TX; 30°32' N, -96°20' W) and Burleson (Hearne, TX; 30°51' N, -96°34' W) counties consisting of Post Oak Savanna habitat. The third site was the Gulf Coast Bird Observatory (GCBO; 29°52' N, -95°28' W) in Lake Jackson, TX, Brazoria County. The GCBO is a ~14 ha property composed of old growth Columbia Bottomland hardwood forest. Samples were also collected while birds were on their wintering grounds in Yucatán, Mexico and Costa Rica between the months of December and May. Birds were collected on property of the Universidad Autónoma de Yucatán in Mérida (20° 58' N, -89°37' W) and approximately 10 km east of the coastal town of Sisal (21°11' N, -89°57' W) during December 2011, October to February of 2012-2013 and spring 2014. This ecoregion is categorized as coastal plain with low tropical deciduous forest. In Costa Rica, birds were collected during February 2012 on properties near La Selva Biological Research Station (10°25' N, -84°0' W), located in the Caribbean lowlands near Puerto Viejo. The area is composed of lowland tropical rain forest in northeastern Costa Rica. Samples were also collected on private properties located near the University of Georgia Athens Costa Rica Station in San Luis (10°16' N, -84°47' W), at the foot of the Monteverde Cloud Forest, in December 2012 and January 2013. Properties were a mixture of coffee agriculture and tropical forest. A total of 153 songbirds were collected from twenty-five different species in the families Parulidae, Troglodytidae, Tyrannidae, and Vireonidae (Table 2). Birds were captured using 6 and 12 m mist nets and playback calls on speakers. Once detected in the net, birds were removed and sacrificed using thoracic compression. In the laboratory, bird carcasses were prepared for analysis by removing the feathers, head, wings, legs, tail feathers, and stomach contents. Bird carcasses were freeze-dried and then homogenized using a mortar and pestle. The Texas A&M University (TAMU) Institutional Animal Care and Use Committee (AUP #2001-155) approved all animal procedures for this study.


**Figure 5.** Map of sampling locations for migrant and resident passerines collected from 2011 to 2013.

**Table 2.** Species information, lipid, and moisture content for passerines collected in Texas, Yucatán, and Costa Rica from 2011-2013[a]

| Location | Common name | Scientific name | N | Migratory status | Lipid (%) | Moisture (%) |
|---|---|---|---|---|---|---|
| Texas | | | | | | |
| | American redstart | *Setophaga ruticilla* | 1 | Migrant | 50.2 | 66.4 |
| | Bewick's wren | *Thryomanes bewickii* | 1 | Resident | 10.9 | 64.0 |
| | Black & white warbler | *Mniotilta varia* | 4 | Migrant | 19.4 (1.60−35.6) | 63.9 (60.1−66.3) |
| | Canada warbler | *Cardellina canadensis* | 1 | Migrant | 35.0 | 63.1 |
| | Carolina wren | *Thryothorus ludovicianus* | 30 | Resident | 7.70 (1.60−18.1) | 69.1 (63.4−75.7) |
| | Common yellowthroat | *Geothlypis trichas* | 15 | Migrant | 29.3 (2.20−64.9) | 61.6 (54.4−68.6) |
| | Hooded warbler | *Setophaga citrina* | 2 | Migrant | 21.4 (19.9−22.9) | 60.0 (58.8−61.2) |
| | Magnolia warbler | *Setophaga magnolia* | 1 | Migrant | 51.8 | 46.4 |
| | Mourning warbler | *Geothlypis philadephia* | 1 | Migrant | 69.6 | 56.6 |
| | Nashville warbler | *Leiothlypis ruficapilla* | 10 | Migrant | 27.3 (14.3−45.4) | 63.7 (58.5−71.2) |
| | Northern parula | *Setophaga americana* | 1 | Migrant | 59.0 | 43.0 |
| | Ovenbird | *Seiurus aurocapilla* | 1 | Migrant | 25.1 | 59.5 |
| | Tennessee warbler | *Leiothllypis peregrine* | 2 | Migrant | 68.4 (50.4−86.3) | 53.8 (52.4−55.2) |
| | Wilson's warbler | *Cardellina pusilla* | 1 | Migrant | 24.0 | 67.3 |
| | Yellow warbler | *Setophaga petechial* | 1 | Migrant | 48.7 | 50.7 |
| | Yellow-rumped warbler | *Setophaga coronata* | 25 | Migrant | 26.7 (5.60−57.5) | 63 (52.4−71.4) |

[a] Lipid content and moisture are shown as arithmetic mean and range in parentheses.
[b] NA = not available.

**Table 2.** (*continued*)

| Location | Common name | Species | N | Migratory status | Lipid (%) | Moisture (%) |
|---|---|---|---|---|---|---|
| Yucatán | | | | | | |
| | Bewick's wren | *Thryomanes bewickii* | 1 | Resident | 15.2 | 67.2 |
| | Carolina wren | *Thryothorus ludovicianus* | 1 | Resident | 6.00 | 68.1 |
| | Common yellowthroat | *Geothlypis trichas* | 6 | Migrant | 23.9 (5.70−77.9) | 64.1 (49.7−68.2) |
| | Dusky-capped flycatcher | *Myiarchus tuberculifer* | 2 | Resident | 8.80 (6.40−11.1) | 72.3 (67.4−77.1) |
| | Hooded warbler | *Setophaga citrina* | 2 | Migrant | 5.10 (3.20−7.00) | N/A |
| | Least flycatcher | *Empidonax minimus* | 2 | Resident | 6.30 (4.30−8.30) | 66.1 (65.7−66.4) |
| | Magnolia warbler | *Setophaga magnolia* | 1 | Migrant | 5.80 | 67.2 |
| | Mangrove vireo | *Vireo pallens* | 4 | Resident | 9.50 (8.30−12.5) | 68.4 (67.8−68.9) |
| | Northern parula | *Setophaga americana* | 3 | Migrant | 16.2 (10.2−22.8) | 67.0 (64.7−69.8) |
| | Ovenbird | *Seiurus aurocapilla* | 3 | Migrant | 6.80 (6.30−7.70) | 68.4 (67.7−69) |
| | Yellow warbler | *Setophaga petechial* | 1 | Migrant | 15.8 | 66.4 |
| | Yellow-rumped warbler | *Setophaga coronata* | 2 | Migrant | 10.7 (8.90−12.5) | 64.7 |
| | Yucatán flycatcher | | 1 | Resident | 2.40 | 75.0 |
| Costa Rica | | | | | | |
| | Black & white warbler | *Mniotilta varia* | 3 | Migrant | 6.90 (5.10−9.30) | 67.9 (67.1−68.4) |
| | Dusky-capped flycatcher | *Myiarchus tuberculifer* | 2 | Resident | 7.00 (2.80−11.1) | 68.2 (66.9−69.5) |

[a] Lipid content and moisture are shown as arithmetic mean and range in parentheses.
[b] NA = not available.

**Table 2.** (*continued*)

| Location | Common name | Species | N | Migratory status | Lipid (%) | Moisture (%) |
|---|---|---|---|---|---|---|
| Costa Rica | | | | | | |
| | House wren | *Troglodytes aedon* | 3 | Resident | 11.1 (4.90−15.8) | 67.0 (66.2−67.5) |
| | Mourning warbler | *Geothlypis philadephia* | 3 | Migrant | 7.70 (5.50−15.8) | 68.2 (63.5−71.1) |
| | Olive-crowned yellowthroat | *Geothlypis semiflava* | 4 | Resident | 6.90 (3.30−10.5) | 65.7 (59.4−69.6) |
| | Ovenbird | *Seiurus aurocapilla* | 1 | Migrant | 13.0 | 68.9 |
| | Rufous & white wren | *Thryophilus rufalbus* | 4 | Resident | 6.80 (4.90−8.60) | 69.7 (69.2−70.1) |
| | Rufous-capped warbler | *Basileuterus rufifrons* | 1 | Resident | 7.70 | 68.8 |
| | Wilson's warbler | *Cardellina pusilla* | 3 | Migrant | 11.7 (5.50−19.7) | 66.7 (64.0−68.1) |
| | Yellow-olive flycatcher | *Tolmomyias sulphurescens* | 3 | Resident | 4.50 (3.20−6.00) | 67.9 (67.8−68.2) |

[a] Lipid content and moisture are shown as arithmetic mean and range in parentheses.
[b] NA = not available.

*Chemical analysis*

Whole body homogenate samples were analyzed for OCPs, PCBs, and PBDEs at the Geochemical & Environmental Research Group (GERG), Texas A&M University, College Station, TX. The following pesticides were measured: DDT isomers (*o,p'*−DDE, *p,p'*−DDE, *o,p'*−DDD, *p,p'*−DDD, *o,p'*−DDT, *p,p'*−DDT), tetrachlorobenzene 1,2,4,5, tetrachlorobenzene 1,2,3,4, pentachlorobenzene, hexachlorobenzene, α−HCH, β−HCH, γ−HCH, δ−HCH, heptachlor, heptachlor epoxide, oxychlordane, α−Chlordane, γ−Chlordane, *cis*− and t*rans*−nonachlor, aldrin, dieldrin, endrin, pentachloroanisole, chlorpyrifos, mirex, endosulfan I and II. The following PCB congeners were quantified: 1, 7, 8, 15, 16/32, 18, 22, 24, 25, 147 26, 28, 29, 31, 33, 39, 40, 41/64, 44, 45, 46, 47/48, 148 48, 49, 52, 53, 60/56, 63, 66, 67, 69, 70, 72, 74, 77, 149 81, 82, 83, 84, 85, 87, 92, 95/80, 97, 99 101, 105, 150 107/108/144, 110, 114, 118/108/149, 119, 126, 128, 151 129, 130, 135, 136, 138, 141, 146, 149, 151, 153, 152 156/171/202, 158, 166, 167, 169, 170, 171/202, 172, 153 174, 175, 177, 178, 180, 183, 185, 187/182/159, 189, 154 191, 193, 194, 195, 196, 197, 199, 200, 201, 205, 155 206, 207, and 209. Congeners that co-eluted during chemical analysis are grouped together by slashes. A total of 38 PBDE congeners (i.e, 1, 2, 3, 7, 8/11, 10, 12, 13, 15, 17, 25, 28, 30, 32, 33, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 116, 118, 119, 126, 138, 153, 154, 155, 166, 181, 183) were investigated. Total PCBs (ΣPCBs) and PBDEs (ΣPBDEs) were the sums of all detectable congeners in a sample.

Chemical analysis followed methods described by GERG standard operating procedures. Briefly, for each sample ~1 g of homogenated tissue was mixed with

Hydromatrix or anhydrous sodium sulfate and solvent extracted with methylene chloride using the Accelerated Solvent Extractor (ASE 200). Prior to extraction, samples, reference material and blanks were spiked with surrogate standards for both chlorinated and brominated compounds. Samples were then concentrated and cleaned with silica/alumina chromatography columns using 200mL of 1:1 pentane/methylene chloride mix as the eluent. High-performance liquid chromatography (HPLC) was used to further purify samples. Finally, extracts were concentrated to 100μL in hexane. The internal standards tetrachloro-meta-xylene (TCMX) and PCB 103 were added before gas chromatographic analysis. Procedural blanks, sample duplicates, and matrix spikes were run with every 20 batch of samples for quality control. The average percent recovery of target analytes in the spiked samples were, with a few exceptions, within the method of acceptable range (40-120%) and the relative percent differences (RDP) between the duplicates were <15% for all compounds. The method detection limits (MDL) were compound and sample specific and based on sample weight. The mean MDLs were 0.52 ± 0.05 (range: 0.44 to 0.86) ng/g dw, 0.52 ± 0.06 (range: 0.25 to 0.86) ng/g dw, and 0.51 ± 0.06 (range: 0.25 to 0.86) ng/g dw, for OCs, PCBs, and PBDEs respectively. Standard reference materials (i.e. SRM2947A and SRM1944) were analyzed for certified PCB and PBDE congeners and organochlorine pesticides for comparison. Quantitative analysis for OCPs, PCBs and PBDEs was accomplished using an Agilent Technologies 6890N Gas Chromatograph coupled to a low resolution 5975C inert Mass Selective Detector in the Selected Ion Monitoring (GC/MSD-SIM) and a 30 m x 0.25 mm i.d. fused silica capillary column with DB-5MS (J&W Scientific Co., USA) bonded phase for organochlorine analytes.

Automatic splitless injections of 2μL were introduced into the column using He as a carrier gas at 1 mL $min^{-1}$. For PCBs, the oven temperature was programmed from 75°C (3 min hold) to 150°C at a rate of 15°C $min^{-1}$ to 260°C at a rate of 2°C $min^{-1}$ and then to 300°C (1 min hold) at a rate of 20°C $min^{-1}$. For OCs, the oven temperature was programmed from 100°C to 200°C at a rate of 10°C $min^{-1}$ and then to 265°C (4 min hold). Injector temperature was held at 270°C and detector temperature at 310°C. For PBDEs, the oven temperature was programmed from 130°C (1 min hold) to 154°C at a rate of 12°C $min^{-1}$, then ramped at 2°C $min^{-1}$ to 210°C, and finally ramped at 3°C $min^{-1}$ to 300°C (5 min hold).

An aliquot of extract was used to calculate the percent extractable organic material (EOM), hereafter referred to as lipids, by gravimetric analysis. Percent moisture was determined by weighing homogenized samples before and after freeze-drying. Percent moisture was calculated as the percent difference between the wet weight and dry weight (Table 1). Contaminant concentrations on a lipid weight (lw) basis were significantly and negatively correlated with lipid content, therefore final concentrations of contaminants are expressed in ng/g on a dry weight (dw) basis.

*Statistical analysis*

Statistical analyses were performed using JMP software program (SAS Institute Inc, 2015). Samples with concentrations at or below the MDL were assigned a value equal to half the MDL. Data were not normally distributed (Shapiro-Wilk test, $p > 0.05$) and were log-transformed to meet the assumptions of normality and homogeneity. One-way analysis of variance (ANOVA) with a post hoc test (Tukey-Kramer HSD) was used to test for differences between migrant and resident birds and among locations. The significance level for all tests was set at $p < 0.05$.

## Results

### *Organochlorine pesticides*

Out of 29 organochlorine pesticides analyzed, only hexachlorobenzene (HCB), *trans*-nanochlor, mirex, and *p,p'*−DDE (hereafter, DDE) were detected above the MDL in 50% or more of the samples (Table 3). DDE was detected in 100% of all samples, low levels of HCB were detected in 91% of samples, and mirex was detected in 77% of all samples. Low levels of *trans*-nanochlor were detected in 59% of all samples, however only in songbirds from Texas. Pentachlorobenzene, heptachlor epoxide, *cis*-nonachlor, alpha- and gamma-chlordane were also detected, but were generally below the MDL. Resident birds from Texas had levels of oxychlordane ranging from 1.29–195 ng/g dw. The metabolite DDE was the most predominant contaminant of the OCPs accounting for 34–94% of the total OCPs burden. Nine birds also contained *p,p*−DDD above the MDL; 6 were from Texas and one individual from Costa Rica.

Mean DDE concentrations for migrant birds collected in Texas, Yucatán, and Costa Rica ranged from 23.2 to 67.6 ng/g dw and for residents from 3.34 to122 ng/g dw (Table 3). Few significant differences in DDE exposure were found. Migrants collected in Texas during the spring migration had significantly greater concentrations ($p = 0.04$) than migrants from Costa Rica, which had the lowest levels of all migrants (Table 3). Residents collected in Texas for both the fall and spring had significantly greater concentrations of DDE compared to residents from Yucatán ($p \leq 0.0007$) and Costa Rica ($p < 0.0001$; Table 3). Additionally, resident species from Yucatán were significantly ($p < 0.0001$) more contaminated with DDE compared to Costa Rican resident species. In general, resident songbirds from Yucatán and Costa Rica had lower concentrations of DDE compared to their migrant counterparts, however only resident birds from Costa Rica were significantly lower than migrants ($p < 0.0001$).

**Table 3.** Concentrations (geometric mean and range ng/g dw) of persistent organic pollutants (POPs) for migrant and resident passerines collected in Texas, Yucatán and Costa Rica from 2011-2013[a]

| | N | *p,p*−DDE[c] | ΣPCBs[c] | ΣPBDEs[c] | HCB | Mirex | *Trans*-nanochlor |
|---|---|---|---|---|---|---|---|
| **Texas** | | | | | | | |
| *Fall* | | | | | | | |
| Migrant | 30 | 49.0[1,2] (14.9−223) | 56.7[1] (17.9−251) | 19.4[1,2] (5.00−96.3) | 3.38 (2ND−14.8) | 2.11 (8ND−8.73) | 2.11 (2ND−7.66) |
| Resident | 13 | 122[1] (21.4−294) | 48.3[1] (20.9−87.5) | 34.9[1] (12.0−460) | 4.47 (0.54−11.6) | 187 (3.80−476) | 25.6 (1ND−120.2) |
| *Spring*[b] | | | | | | | |
| Migrant | 35 | 67.6[1] (3.10−986) | 64.2[1] (17.7−1,479) | 15.0[1,2,3] (3.20−223) | 1.90 (1ND−3.76) | 4.36 (11ND−17.0) | 1.36 (10ND−6.0) |
| Resident | 18 | 93.8[1] (36.8−403) | 41.1[1] (13.0−246) | 25.2[1,2] (7.70−90.7) | 1.39 (1ND−3.20) | 6.58 (3ND−33.8) | 4.91 (1ND−45.1) |
| **Yucatán** | | | | | | | |
| Migrant | 18 | 57.0[1,2] (16.0−1,678) | 79.7[1] (25.9−492) | 12.6[1,3] (2.10−62.9) | 1.14 (3ND−5.08) | 10.9 (5ND−41.4) | – |
| Resident | 11 | 20.0[2] (6.60−129) | 32.1[1] (10.7−210) | 6.00[3] (1.40−17.1) | 0.80 (4ND−1.38) | – | – |
| **Costa Rica** | | | | | | | |
| Migrant | 10 | 23.2[2] (3.70−68.8) | 43.7[1] (13.5−184) | 8.71[2,3] (4.30−18.2) | 1.91 (0.93−3.10) | 19.5 (1.92−76.6) | – |
| Resident | 17 | 3.43[3] (1.00−22.0) | 9.60[2] (3.90−98.0) | 6.42[3] (1.40−194) | 1.46 (1ND−4.25) | 20.5 (0.87−205) | – |

[a] Numbers in front of ND represent total number of samples ≤ MDL
[b] Sample size for ΣPCBs and ΣPBDEs for migrant (n = 36) and resident (n= 17) birds
[c] Within columns, data not sharing the same superscript number are considered significantly different

*Polychlorinated biphenyls*

Mean ΣPCBs concentrations for migrant birds collected in Texas, Yucatán, and Costa Rica ranged from 44 to 80 ng/g dw and for resident birds from 9.60 to 48.3 ng/g dw (Table 3). For ΣPCBs, there were also no significant differences of contaminant levels observed for migrants. In contrast, concentrations of ΣPCBs in resident birds from Costa Rica were significantly lower ($p \leq 0.01$) compared to all other resident groups (Table 3). In general, migrant birds had greater levels of ΣPCB concentrations compared to their resident counterparts, but only migrants from Costa Rica had significantly greater concentrations than resident species ($p = 0.0007$; Table 3).

Congener specific analysis of PCBs revealed that homologues with 5, 6, 7 and 8 chlorine atoms had the highest percent contribution to the total sum of PCBs for both migrants and residents (Figure 7 and Figure 8). Resident birds from Texas tended to have a greater contribution of the heavier chlorinated homologues for 8 (~2.0 times greater) and 9 (~2.2 times greater), whereas resident birds from Yucatán and Costa Rica had a greater percentage of the less chlorinated 3, 4, and 5 homologues. Yucatán residents had 2.3 times greater contribution of tri-, 3.3 times greater proportion of tetra-, and 1.2 times greater proportion of penta- chlorinated homologues compared to Texas residents. In addition, Costa Rican resident birds had 4.4, 2.8, and 2.1 times greater proportion of tri-, tetra-, and penta- chlorinated homologues compared to Texas resident species, respectively. For both migrants and residents, the most abundant PCB congeners were #153, #180, #138, #118, #170 and #187, respectively.

*Polybrominated diphenyl ethers*

Mean ΣPBDEs concentrations for migrant birds collected in Texas, Yucatán, and Costa Rica ranged from 8.71 to 19.4 ng/g dw and for resident birds from 6.0 to 34.9 ng/g dw (Table 3). One single male Carolina wren (*Thryothorus ludovicianus*) collected in Texas during the spring in 2013, had a ΣPBDE concentration of 4,207 ng/g dw and was approximately 9 times greater than the next highest concentration; thus, this value was not included in the statistical analysis or calculation of summary statistics for Table 3. For ΣPBDEs, only resident species showed significant differences in body burdens. Resident birds collected in Texas had greater concentrations of ΣPBDEs compared to resident birds from both Yucatán ($p \leq 0.005$) and Costa Rica ($p \leq 0.002$; Table 3).

PBD-99, 47, 100, 153 and 154 dominated the congener profiles for both migrant and resident songbirds (Figure 8 and 9). These five congeners contributed more than 90% of the total PBDE burden in both migrant and resident birds.

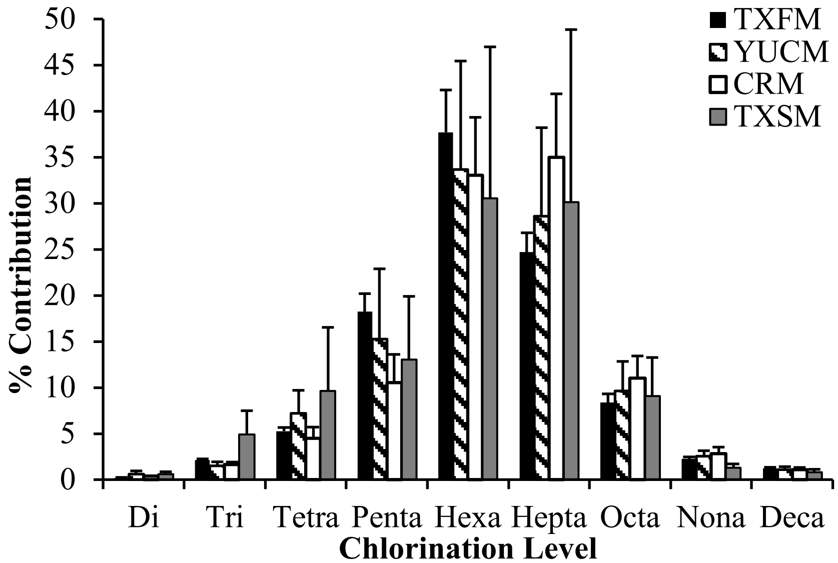



**Figure 6.** Percent contribution and standard error of PCB homologues in migratory passerine birds from different sampling sites and seasons (*TXFM* = Texas fall migrants; *YUCM* = Yucatán migrants; *CRM* = Costa Rica migrants; *TXSM* = Texas spring migrants).

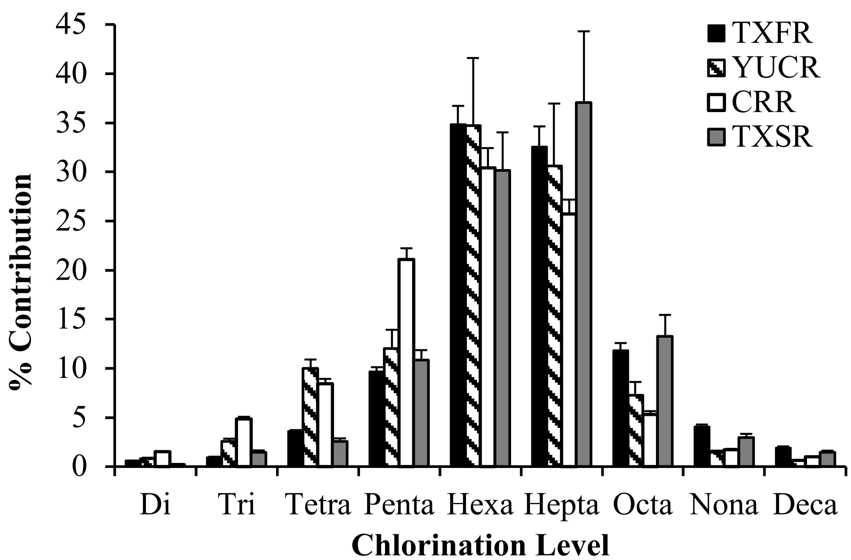



**Figure 7.** Percent contribution of PCB homologues in resident passerine birds from different sampling sites and seasons (*TXFM* = Texas fall migrants; *YUCM* = Yucatán migrants; *CRM* = Costa Rica migrants; *TXSM* = Texas spring migrants).

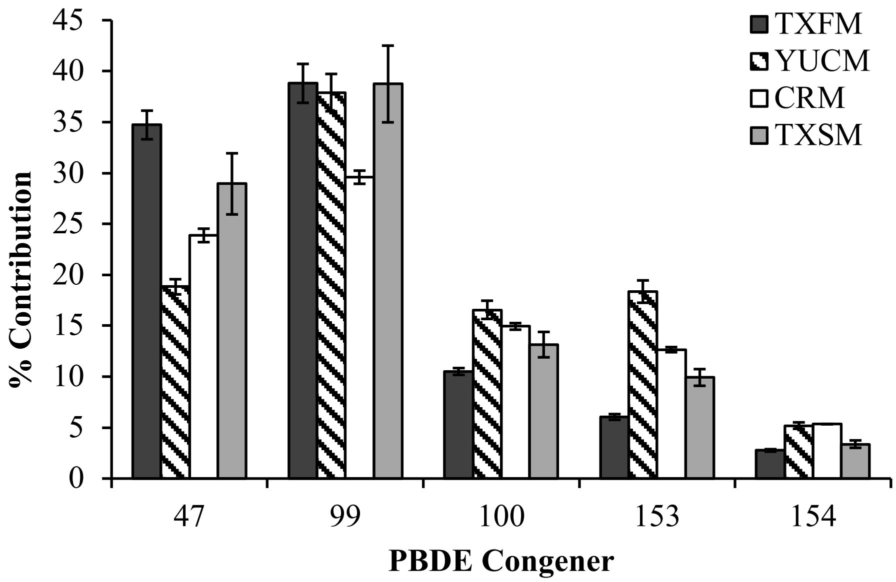



**Figure 8.** Percent contribution and standard error of the six most common PBDE congeners in migrant passerines collected from Texas, Yucatán and Costa Rica (*TXFM* = Texas fall migrants; *YUCM* = Yucatán migrants; *CRM* = Costa Rica migrants; *TXSM* = Texas spring migrant.

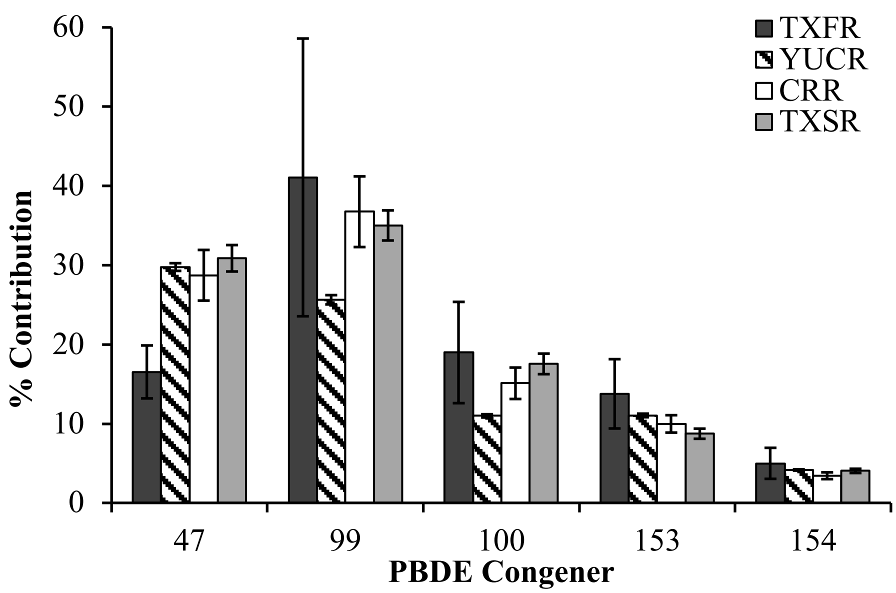



**Figure 9.** Percent contribution and standard error of the six most common PBDE congeners in resident passerines from Texas, Yucatán and Costa Rica (*TXFM* = Texas fall migrants; *YUCM* = Yucatán migrants; *CRM* = Costa Rica migrants; *TXSM* = Texas spring migrants).

*Spatial variation of contaminant profiles for migrants and residents*

The contribution of the different contaminants to the total body burden composition differed slightly between the various sampling locations and migratory status of the birds; in general differences were small (Figure 10). PCBs and OCPs were the dominant contaminants accounting for ≥ 80% of the total contribution for both migrants and residents at each of locations. PBDEs accounted for the least (≤ 16%) to the total body burden of POPs. Texas migrants had ~2.7 times greater contribution of ΣPCBs compared to Texas residents. Migrants collected in Texas during the spring had a very similar composition of ΣOCPs compared to migrants collected in the fall, indicating accumulation and retention of similar contaminants. Migrants collected in Costa Rica had 1.7 times greater contribution of ΣPCBs (~50%) compared to resident species, which had a greater proportion of ΣOCPs (54%). Costa Rican residents had 1.8 times greater contribution of ΣPBDEs (16%), compared to migrants (9%). Additionally, the percent contribution of ΣPCBs resident birds from the Yucatán was 1.7 times greater than in migrant species.

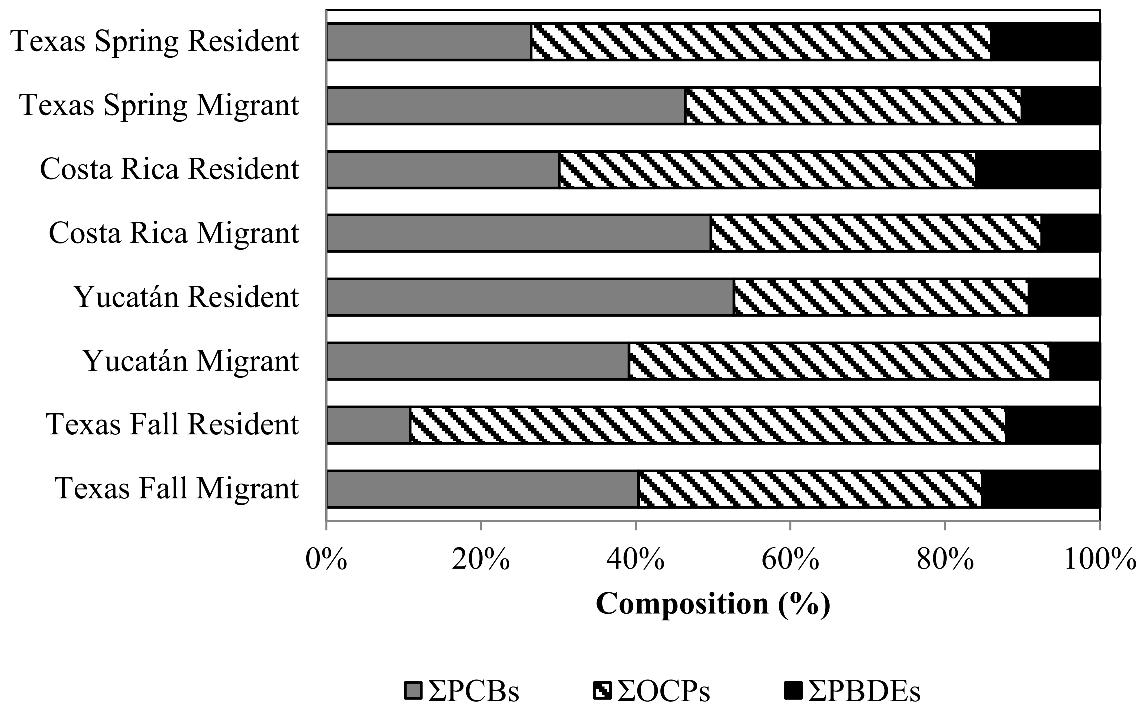



**Figure 10.** Contamination profiles of investigated POPs in passerine birds for migrants and residents from different sampling sites and season.

## Discussion

The data presented in this study did not support the hypothesis that migratory songbirds accumulate contaminants while on their wintering grounds. Furthermore, residents collected in Yucatán and Costa Rica had the lowest concentrations of DDE, indicating low levels of contamination and unlikely pesticide acquisition by migrants during the wintering period in these areas. These findings are in agreement with other studies that have examined contaminant accumulation in Neotropical (Harper et al. 1996, Mora 2008). For example, Harper (1996) investigated organochlorine pesticide levels in Neotropical migrants and found no significant differences between hatch year (HY) and after hatch year (AHY) birds, which would suggest exposure and accumulation of organochlorine pesticides on their breeding grounds or via maternal transfer. Additionally, other researchers have reported relatively low pesticide contaminant levels in passerines from Central and South America (Fyfe et al. 1990, Capparella et al. 2003, Klemens et al. 2003).

I detected relatively low levels of POPs in migratory and resident songbirds (Table 2). The concentrations of DDE, ΣPCBs, and ΣPBDEs in this study were also relatively lower than those reported in other recent ecotoxicology studies (Mora 2008, Mora, Sericano, and Baxter 2012); however, the observed concentration differences for passerine species within Texas may be attributed to sampling location or species and diet differences (Table A-1). All migrant passerines collected in this study were warbler species, which are mainly terrestrial insect gleaners, whereas Mora et al. (2012) examined POP concentrations in cliff swallows (*Petrochelidon pyrrhonota*) from the Rio Grande geographic area of Texas. Cliff swallows feed primarily on aquatic insects, such as odonates, which are carnivorous insects, and may be feeding at a higher trophic level (De Graaf, Tilghman, and Anderson 1985, Brown et al. 1995). In this study, the sampling locations in Yucatán were predominantly rural areas not near traditional agriculture, whereas Mora (2008) sampled birds near agricultural areas in western Mexico, which may explain the greater concentrations of DDE reported in the study compared to values reported here (see Supplementary Table A-1).

Present findings also revealed that migrants collected in Yucatán had slightly, yet not significantly, higher concentrations of DDE compared to resident species (Table 2).

These results are noteworthy given that they are not in agreement with other studies of passerines from Southeastern Mexico. Herrera-Herrera et al. (2013) observed that resident songbirds had significantly greater concentrations of DDT compared to migrants (Table A-1). Levels of ΣPCBs in the migrants we collected in Yucatán (27 ng/g ww) were greater than previously reported concentrations in passerine birds from Southeastern Mexico (8 ng/g ww) (Herrera-Herrera et al. 2015). Resident species, however, were comparable (9 ng/g ww; Table A-1).

In the present study, ΣPBDE concentrations for resident birds from Costa Rica ranged from 1 to 194 ng/g dw (Table 2), this is a tenth fold greater difference compared to migrants from Costa Rica (range: 4 to 18 ng/g dw; Table 2); however, this reported difference could be attributed to a smaller sample size of migrants collected. In addition, the ΣPBDE range for these Costa Rican residents was also greater than the range for migrants collected in Texas during the fall (5 to 96 ng/g dw) and migrants from Yucatán (2 to 63 ng/g dw; Table 2). These findings are noteworthy, because in general birds from North America have greater PBDE burdens compared to birds from other regions, likely due to higher demand of PBDE containing products (Chen and Hale 2010). PBDE contamination in passerines has been shown to be highly correlated to distance from industrialized and urban areas (Sun et al. 2012, Tang et al. 2015). Findings in the present study demonstrate that birds from non-industrialized areas can also accumulate relatively high concentrations of PBDE flame retardants.

Migrant and resident passerines had comparable congener profiles for PCBs and PBDEs suggesting similar acquisition and retention of these contaminants. PCB congener profiles presented in this study are also similar to those reported from studies in China on other passerine species (Yu et al. 2014) and aquatic birds from Texas, South America and India (Mora 1996, Senthilkumar et al. 1999, Colabuono, Taniguchi, and Montone 2012). Additionally, the pattern of dominant PCB congeners observed in present songbirds is consistent with observations for other insectivorous passerines (Winter and Streit 1992, Dauwe et al. 2003, Yu et al. 2014) and aquatic birds from south India (Senthilkumar et al. 1999). It should be noted that, resident birds from Yucatán and Costa Rica had greater contributions of the less chlorinated congeners (3, 4, and 5 chlorines) compared to resident species in Texas, suggesting differences of exposure to various PCB Aroclor mixtures. The PBDE congener profiles in the present study for both migrant and resident birds are consistent with penta-BDE technical mixture patterns (La Guardia, Hale, and Harvey 2006), and congener profiles reported for other passerines from Texas and China (Mora, Sericano, and Baxter 2012, Tang et al. 2015).

The low mean concentrations of PBDEs and PCBs observed in birds from Costa Rica and low PBDE concentrations in birds from Yucatán during this study are similar to the observations made on other environmental media (soil and ambient air) in developing regions (Mochungong and Zhu 2015). Low atmospheric concentrations of PCBs and PBDEs for the Yucatán peninsula and Costa Rica have been reported in the literature (Shen et al. 2006). In addition, Daly et al. (2007) reported low levels of DDT metabolites in soils from Costa Rica. The absence of DDT and low levels of DDE in birds sampled from the Yucatán peninsula and Costa Rica in this study may be attributed in part to the climatic condition of the tropics, which favor volatilization and degradation of organochlorine pesticides (Wania and Mackay 1993, Carvalho et al. 1994). Low levels could also be attributed to low historic usage of DDT in the areas selected as sampling sites in Costa Rica and Mexico.

Levels for DDE, ΣPCBs, and ΣPBDEs in the present study were below those reported to cause adverse health effects in relation to behavior, reproduction and development seen in other passerines (McCarty and Secord 1999b, Neigh et al. 2007, DeLeon et al. 2013, Eng et al. 2014) and raptors (Newton 1988, Henny et al. 2009). However, one male Carolina wren from Texas collected during the spring had a concentration that exceeded levels known to cause reproductive impairments in other birds (Henny et al. 2009). Exposure and accumulation of environmental contaminants is dependent on a large variety of factors including historic use, varying life histories, environmental factors, and physiochemical properties of the contaminant (Jones and de Voogt 1999, Smith et al. 2007). A limitation of this study is the large species variability of the samples collected. Although target species were selected based on likelihood of presence for each sampling location, not all species were collected at each of the sites or in the same proportion. In addition, even within a relatively small geographic area there may be a lot of heterogeneity of contaminant residues in the soil impacting accumulation by songbirds, further complicating the inferences that can be made from exposure and accumulation studies (Reynolds et al. 2001).

## Conclusion

In the present study, relatively low, but detectable levels of POPs were observed in migrant and resident songbirds from Texas, Mexico, and Costa Rica. No significant accumulation of DDE was observed in migrants while on their wintering grounds. Additionally, low levels of DDE observed in resident birds from Yucatán and Costa Rica also suggest limited exposure in these areas. Results from this study are in agreement with others that demonstrate relatively low levels of OCPs observed in passerines from Latin America and no significant contaminant accumulation on wintering grounds. Furthermore, no variation in PBDEs or accumulation of PCBs was observed in migrants throughout the migration period. However, industrial contaminant burdens of PCBs and PBDEs were greater for migrant and resident birds that breed in more northern latitudes compared to resident species in Yucatán and Costa Rica. Though, some resident songbirds from Costa Rica had individual concentrations of ΣPBDEs similar to values seen in the migrant and resident species from developed regions in North America. Concentrations of individual contaminants were relatively low and unlikely to cause adverse health effects. The limited availability of data on POPs in passerines and other wildlife for Latin America makes effective comparisons difficult; future studies should focus on increasing monitoring studies in resident birds for these areas.

# CHAPTER III

# CAN STABLE ISOTOPES ($\delta^{13}C$, $\delta^{15}N$, AND $\delta^{2}H$) PREDICT CONTAMINANT ACCUMULATION IN SONGBIRDS?

## Summary

Contaminant exposure in birds has been shown to be significantly affected by diet and migration. In the present study, stable isotope analyses of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and deuterium ($\delta^{2}H$) were conducted on various tissues in songbirds and compared with concentrations of persistent organic pollutants (POPs) body residues in order to determine if diet or latitudinal gradients could explain pollutant levels. Birds were collected during fall and spring migration in Texas, and on wintering grounds in Mexico and Costa Rica during 2011-2013. In general, there were only minor differences in mean liver $\delta^{13}C$ and $\delta^{15}N$ values between migrants (mean range $\delta^{13}C$: −24 to −23‰; $\delta^{15}N$: +7.9 to +13‰) and residents (mean range $\delta^{13}C$: −24 to −21‰; $\delta^{15}N$: +6.8 to +9.5‰) within a location indicating similar diets. A weak negative latitudinal gradient was observed for sum polybrominated diphenyl ethers (ΣPBDEs) in migrant songbird. However, a significant positive correlation between feather $\delta^{2}H$ and each class POPs contaminants in resident species was observed; the strongest for dichlorodiphenyldichloroethene (DDE). Concentrations of sum polychlorinated biphenyls (ΣPCBs) were significantly correlated with liver $\delta^{15}N$ for residents from Texas. Texas residents also showed distinct $\delta^{13}C$ values amongst sampling locations and were correlated to concentrations of DDE and ΣPBDEs, suggesting more urban environments as a source of pollutants. For birds in Mexico, only

concentrations of ΣPBDEs were related to trophic level ($\delta^{15}N$). No significant relationships between individual POPs and stable isotopes was observed for birds from Costa Rica. The overall mean for $\Delta^{13}C$ was +2.9‰ and +4.6‰ for $\Delta^{15}N$, greater than most values reported in the literature for other bird species.

## Introduction

Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs) are of environmental concern due to their resistance to environmental degradation, ability to undergo long-range atmospheric transport, potential to bioaccumulate and biomagnify (in the food chain), and their adverse health effects on humans and wildlife (Colborn, vom Saal, and Soto 1993). Several factors affect the accumulation of contaminants in birds, including migration, habitat use, and feeding ecology (Cooper et al. 2017, Huertas et al. 2016, Morrissey, Bendell-Young, and Elliott 2004, Peterson, Ackerman, and Eagles-Smith 2017, Sebastiano et al. 2017). Many Neotropical songbirds undergo long-distance migrations from their breeding grounds in North America to their wintering grounds in Latin America. Migration has been shown to have a significant impact on the levels and profiles of contaminant body residues (Minh et al. 2002). Alternatively, resident songbirds are good indicators of local background pollution due to their year-round residency and localized feeding habits. Previously, Maldonado et al. (2017) examined contaminant accumulation in Neotropical songbirds throughout their migratory cycle, in addition to using resident species as a reference group. Significant differences for the metabolite dichlorodiphenyldichloroethene (DDE), ΣPCB, and ΣPBDE concentrations were determined for resident species amongst the different sampling locations in Texas, Yucatán (Mexico), and Costa Rica. Significant differences were also observed between migrant and resident species collected in Costa Rica for the contaminants DDE and ΣPCBs. In addition to migration, I explored other factors, particularly diet through the use of stable isotopes, that could aid in explaining contaminant exposure and acquisition in these songbirds.

The primary source of exposure in terrestrial birds is through consumption of contaminated food items, which is highly dependent on their feeding ecology (Morrissey, Bendell-Young, and Elliott 2004, Smith et al. 2007, Mello et al. 2016). A common characteristic of many POPs is their ability to bioaccumulate in organisms relative to their surrounding environment due to their low water solubility and lipophilic nature, facilitating their partitioning into sediments, organic matter, and living organisms. Therefore, some POPs can also biomagnify with increasing trophic position (Broman et al. 1992, Norstrom 2002, Sørmo et al. 2011). Biomagnification of POPs in organisms occupying higher positions in food webs makes them at greater risk of developing adverse health problems such as endocrine disruption, immune system modulation, and reproductive impairment (Colborn, vom Saal, and Soto 1993, Fernie et al. 2005, DeLeon et al. 2013).

The use of stable isotopes, such as carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), in avian ecology studies is well established and has increased significantly in ecotoxicology studies as a tool to explain differences in contaminant body burdens (Huertas et al. 2016, Roscales et al. 2016, Mello et al. 2016, Sebastiano et al. 2017). Nitrogen isotopes are used as tracers of trophic level due to the preferential loss of $^{14}N$ in nitrogenous waste products (Michener et al. 2007), enriching consumer tissues in $^{15}N$ compared to their diet by 2−5‰ (Peterson and Fry 1987, Gannes, Del Rio, and Koch 1998). Stable isotopes of carbon are useful in tracking carbon sources from marine or terrestrial environments and distinguishing between $C_3$ or $C_4$ plants as the base of the food chain (Peterson and Fry 1987). In addition, stable hydrogen isotopes ($\delta^2H$) correlate with the hydrogen isotopic pattern of meteoric water, which covaries with environmental temperature. Thus, $\delta^2H$ measurements have been applied in animal migration studies and are useful in determining breeding origins and migratory connectivity in songbirds (Hobson 2011); however, its use in ecotoxicological studies is less applied (Mora 2008). Stable isotope mixing models have also become increasingly popular in their application for quantifying consumer diets; however, these models are sensitive to variations in discrimination factors ($\Delta$), which is the difference in isotopic composition between a consumer and their diet (Inger and Bearhop 2008, Bond and Diamond 2011). Therefore, accurate estimations of $\Delta^{13}C$ and $\Delta^{15}N$ are key in the application of stable isotope mixing models.

In this study, I measured stable isotopes of carbon, nitrogen, and deuterium in various bird tissues in order to examine their potential use in explaining contaminant body burdens reported previously (Maldonado, Mora, and Sericano 2017). The aim of the present study was to (i) examine any differences in diet (using stable isotopes of carbon and nitrogen) between migrant and resident birds within a location, (ii) determine discrimination factors ($\Delta$) between liver tissue and stomach contents and, (iii) assess which ecological factors (diet, migratory distance, location) could be useful in explaining contaminant body burdens through the use of stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$).

## Methods

### *Sample collection and preparation*

Samples were collected using mist nets and playback calls during migration and wintering periods (2011-2013) at sites located in Texas (USA), Yucatán (Mexico), and Costa Rica. Once detected in the net, birds were removed and humanely euthanized using thoracic compression, under approved Texas A&M University Institutional Animal Care and Use Committee permit (AUP#2001-155). A total of 153 songbirds were collected from 25 different species (Table 4). The first and second primary feathers were collected from each wing, wrapped in aluminum foil and placed in an envelope until further analysis. In the lab, the first and second primaries from the left wing were cleaned in a 2:1 chloroform:methanol solvent rinse. Feathers were soaked for ~24 hours in the chloroform:methanol solution and then air-dried in a fume hood for another ~24 hours. Wing veins were removed from the rachis and cut using stainless steel scissors and cut until they became homogenous powders. Stomach contents from the ventriculus and a small portion of liver were removed. Stomach contents consisted primarily of small insect parts, however some vegetable matter and seeds were observed. Liver samples were dried in an oven at 47°C for ~72 hr. Dried liver samples and stomach contents were then ground into a powder using a mortar and pestle. Livers were first analyzed for $\delta^{15}N$ and the remaining sample was then lipid extracted, since lipids are isotopically lighter and can affect carbon isotope readings, using successive rinses in 2:1 chloroform:methanol solvent and allowed to air dry under a fume hood to remove any residual solvent.

**Table 4.** Species information for songbirds collected in Texas, Yucatán, and Costa Rica from 2011-2013.

| Location | Common name | Scientific name | N | Status |
|---|---|---|---|---|
| Texas | | | | |
| | American redstart | *Setophaga ruticilla* | 1 | Migrant |
| | Bewick's wren | *Thryomanes bewickii* | 1 | Resident |
| | Black & white warbler | *Mniotilta varia* | 4 | Migrant |
| | Canada warbler | *Cardellina canadensis* | 1 | Migrant |
| | Carolina wren | *Thryothorus ludovicianus* | 30 | Resident |
| | Common yellowthroat | *Geothlypis trichas* | 15 | Migrant |
| | Hooded warbler | *Setophaga citrina* | 2 | Migrant |
| | Magnolia warbler | *Setophaga magnolia* | 1 | Migrant |
| | Mourning warbler | *Geothlypis philadephia* | 1 | Migrant |
| | Nashville warbler | *Leiothlypis ruficapilla* | 10 | Migrant |
| | Northern parula | *Setophaga americana* | 1 | Migrant |
| | Ovenbird | *Seiurus aurocapilla* | 1 | Migrant |
| | Tennessee warbler | *Leiothllypis peregrine* | 2 | Migrant |
| | Wilson's warbler | *Cardellina pusilla* | 1 | Migrant |
| | Yellow warbler | *Setophaga petechial* | 1 | Migrant |
| | Yellow-rumped warbler | *Setophaga coronata* | 23 | Migrant |
| Yucatán | | | | |
| | Bewick's wren | *Thryomanes bewickii* | 1 | Resident |
| | Carolina wren | *Thryothorus ludovicianus* | 1 | Resident |
| | Common yellowthroat | *Geothlypis trichas* | 6 | Migrant |
| | Dusky-capped flycatcher | *Myiarchus tuberculifer* | 2 | Resident |
| | Hooded warbler | *Setophaga citrina* | 2 | Migrant |
| | Least flycatcher | *Empidonax minimus* | 2 | Resident |
| | Magnolia warbler | *Setophaga magnolia* | 1 | Migrant |
| | Mangrove vireo | *Vireo pallens* | 4 | Resident |
| | Northern parula | *Setophaga americana* | 3 | Migrant |
| | Ovenbird | *Seiurus aurocapilla* | 3 | Migrant |
| | Yellow warbler | *Setophaga petechial* | 1 | Migrant |
| | Yellow-rumped warbler | *Setophaga coronata* | 2 | Migrant |
| | Yucatán flycatcher | | 1 | Resident |
| Costa Rica | | | | |
| | Black & white warbler | *Mniotilta varia* | 3 | Migrant |
| | Dusky-capped flycatcher | *Myiarchus tuberculifer* | 2 | Resident |
| | Mourning warbler | *Geothlypis philadephia* | 3 | Migrant |
| | Olive-crowned yellowthroat | *Geothlypis semiflava* | 4 | Resident |
| | Ovenbird | *Seiurus aurocapilla* | 1 | Migrant |
| | Rufous & white wren | *Thryophilus rufalbus* | 4 | Resident |
| | Rufous-capped warbler | *Basileuterus rufifrons* | 1 | Resident |
| | Wilson's warbler | *Cardellina pusilla* | 3 | Migrant |
| | Yellow-olive flycatcher | *Tolmomyias sulphurescens* | 3 | Resident |

*Stable isotope analysis*

Stable isotope analysis for feathers and livers was performed in the Stable Isotopes for Biosphere Science (SIBS) Laboratory, College of Agriculture and Life Sciences, Texas A&M University. Stomach contents were analyzed at the Stable Isotopes Geosciences Facility, College of Geosciences at Texas A&M University. Songbird liver $\delta^{15}N$ values were derived from untreated (chloroform:methanol) samples while $\delta^{13}C$ values were derived from samples with lipids removed. Livers were analyzed using an Elemental Combustion System (EA) (Costech, Valencia, CA) coupled to a Delta V Advance stable isotope ratio mass spectrometer (IRMS) (Thermo Scientific, Waltham, MA) in continuous flow (He) mode. Stomach contents and a subset of feather samples were also analyzed for $\delta^{13}C$ and $\delta^{15}N$ via Thermo Scientific Delta$^{plus}$ isotope ratio mass spectrometer (IRMS) with Carlo Erba NA 1500 Elemental Analyzer (EA). Isotopic values were reported relative to Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$ measurements and atmospheric $N_2$ (AIR) for $\delta^{15}N$ measurements. Feather samples and keratin calibration materials (KHS, CBS, USGS40, and USGS41) were left in the lab for hydrogen exchange between lab air moisture and exchangeable hydrogen in keratin (Wassenaar and Hobson 2003) for 5-7 days. They were then packed in 3.5x5mm silver capsules (Costech, USA), and kept in a desiccator for 3 days until just before analyses. Hydrogen isotope ratios ($\delta^2H$) in feather samples were analyzed using a high temperature conversion elemental analyzer (TC/EA) (Thermo Scientific, Waltham, MA) coupled to a Delta V Advance stable isotope ratio mass spectrometer (Thermo Scientific, Waltham, MA) and Conflo IV (Thermo Scientific, Waltham, MA). Results for $\delta^2H$ are calibrated using Vienna Standard Mean Ocean Water

(VSMOW) and Standard Light Antarctic Precipitation (SLAP) and reported versus VSMOW. The standards used were USGS Glutamic Acid 40 and 41 for the carbon and nitrogen isotope analyses and KHS and CBS (keratin standards from Environment Canada; $\delta^2H = -35.3‰$ for KHS and $-257‰$ for CBS) for the hydrogen isotope analyses. The ratio of isotopes is expressed in the delta (δ) notation in per mil (‰): $[(R_{sample}/ R_{standard})$ -1] x $10^3$, where R is the isotope ratio of the sample relative to the standard (i.e. $^{13}C/^{12}C$ or $^{15}N/^{14}N$). Precision of the isotope ratio analysis, assessed through the standard deviation of replicates of laboratory standard material interspersed among samples in every batch, was ≤ 0.17‰ for carbon, ≤ 0.13‰ for nitrogen, and ≤ 3‰ for hydrogen.

*Statistical analysis*

Summary statistics were calculated using GraphPad Prism version 7.00 (GraphPad 2016). Additional statistical analysis was performed using R Studio (RStudio 2016). Welch's analysis of variance (Welch's ANOVA due to heterogeneity of variances [Levene's test: $p$ = <0.0001]), with a post hoc test (Tukey-Kramer HSD) was used to test for significant differences between liver carbon isotopes based on foraging heights. Pearson's product moment correlation was used to determine the relationship between liver and stomach content stable isotopes of carbon and nitrogen. Correlations between a subset of feather and liver carbon and nitrogen isotope values were also investigated using Pearson's correlation. Reduced major axis (RMA) slopes are reported. I calculated $\Delta^{13}C$ and $\Delta^{15}N$ values from liver tissue and stomach contents (diet) of the songbirds, which primarily consisted of insect parts. Mean discrimination factors were calculated for species with sample sizes ≥ 4. Linear regression models (lm function in R Studio) were also used to assess the relationship between $log_{10}$-transformed POP class concentrations (DDE, ΣPCBs, and ΣPBDEs) and stable isotope values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$). Significance was set at $p < 0.05$.

## Results

### *Stable isotope ratios of C and N*

Summary statistics for nitrogen and carbon stable isotope values for stomach contents and livers of birds are presented in Tables 5 and 6. There was considerable individual variation for both $\delta^{13}C$ and $\delta^{15}N$ in stomach contents and liver tissue for both migrant and resident birds among the different sampling locations (Figure B-1). For migrants, $\delta^{13}C$ values for stomach content and liver tissue ranged from −30‰ to −19‰ and −30‰ to −18‰ respectively (Table 5 and 6). $\delta^{15}N$ values ranged from −3.5‰ to +16‰ for stomach content and +3.1‰ to +19‰ for liver tissue (Table 5 and 6). In resident birds, $\delta^{13}C$ values for stomach content and liver varied between −33 ‰ to −14‰ and −26‰ to −14‰ respectively; and those for $\delta^{15}N$ ranged from −1.6‰ to +9.8‰ and +4.1‰ to 12‰, respectively (Table 5 and 6).

Differences in mean values for both $\delta^{13}C$ and $\delta^{15}N$ between migrant and resident species within a location were generally small (≤ 2‰), with a few exceptions (Table 6 and Figure 11). Within songbirds collected from Texas, residents collected in the fall were more enriched in liver $^{13}C$ compared to residents collected in the spring ($\delta^{13}C$: −21‰ and −24‰, respectively). Texas fall residents were also more enriched in liver $^{13}C$ compared to fall migrants by +3‰ (Table 6 and Figure 11). Only birds from Texas had enough individuals for species comparisons (Figure B-2). Carolina wrens (*Thryothorus ludovicianus*), common yellowthroats (*Geothlypis trichas*), Nashville warblers (*Leiothypis ruficapilla*), and yellow-rumped warblers (*Setophaga coronata*) all had similar mean $\delta^{13}C$ values for livers (−23‰ to −24‰). Mean liver $\delta^{15}N$ values were also similar, around +7‰, except for common yellowthroats, which had a mean of +9.7‰ (Figure B-2). In songbirds collected from the Yucatán (Mexico), migrants were enriched by +3.4‰ in liver $^{15}N$ compared to residents. Mean $\delta^{13}C$ values for livers were similar between locations for both migrants and residents and were ≤ 2‰ (Table 6). However, larger differences (> 4‰) between location means were observed for $\delta^{15}N$ liver values, in particular for migrants from Yucatán (Table 6). Liver $\delta^{13}C$ values based on foraging heights ranging from 0 – 2 m were significantly enriched in $^{13}C$ compared to other foraging heights ($p$ = <0.0001; Figure B-3).

Stable isotope values were significantly and positively correlated between liver and stomach contents for both $\delta^{13}C$ ($r$ = 0.66, $p$ = < 0.0001) and $\delta^{15}N$ ($r$ = 0.69, $p$ = < 0.0001; Figure 12). Additionally, there was a significant positive correlation for both $\delta^{13}C$ and $\delta^{15}N$ between feather and liver samples for resident birds, but not for migrants (Figure 13).

Liver tissue was enriched for both $^{13}C$ and $^{15}N$ on average with a difference of +2.9‰ and +4.6‰ compared to diet (stomach contents), respectively. The isotopic discrimination factor (Δ) for carbon and nitrogen varied considerably depending on the species (Table 7).

**Table 5.** Stomach content carbon and nitrogen stable isotope values (mean ± SE, range) of neotropical migrant and resident songbirds from Texas (USA), Yucatán (Mexico), and Costa Rica.

| | *n* | Migrant | | *n* | Resident | |
|---|---|---|---|---|---|---|
| | | $\delta^{13}C$ (‰) Mean ± SE (range) | $\delta^{15}N$ (‰) Mean ± SE (range) | | $\delta^{13}C$ (‰) Mean ± SE (range) | $\delta^{15}N$ (‰) Mean ± SE (range) |
| Texas (Fall) | 30 | −28 ± 0.3 (−30 to −23) | +1.8 ± 0.4 (−3.5 to +5.2) | 12 | −24 ± 0.5 (−27 to −21) | +3.8 ± 0.4 (+1.5 to +7.0) |
| Yucatán | 14 | −24 ± 0.4 (−26 to −20) | +8.6 ± 1.2 (+3.3 to +16) | 11 | −25 ± 0.5 (−28 to −22) | +5.2 ± 0.4 (+2.9 to + 7.5) |
| Costa Rica | 10 | −26 ± 1.1 (−29 to −19) | +4.5 ± 0.3 (+3.2 to + 6.4) | 16 | −24 ± 1.5 (−33 to −14) | +4.8 ± 0.4 (+2.4 to +8.4) |
| Texas (Spring) | 32 | −27 ± 0.2 (−29 to −23) | +4.0 ± 0.6 (−1.6 to +16) | 18 | −26 ± 0.4 (−28 to −20) | +1.6 ± 0.6 (−1.6 to +9.7) |

**Table 6.** Liver tissue carbon and nitrogen stable isotope values (mean ± SE, range) of neotropical migrant and resident songbirds from Texas (USA), Yucatán (Mexico), and Costa Rica.

| | *n* | Migrant | | *n* | Resident | |
|---|---|---|---|---|---|---|
| | | $\delta^{13}$C (‰) Mean ± SE (range) | $\delta^{15}$N (‰) Mean ± SE (range) | | $\delta^{13}$C (‰) Mean ± SE (range) | $\delta^{15}$N (‰) Mean ± SE (range) |
| Texas (Fall) | 28[a] | −24 ± 0.3 (−30 to −21) | +7.9 ± 0.5 (+5.0 to + 10) | 12 | −21 ± 0.4 (−24 to −19) | +8.6 ± 0.5 (+6.5 to +9.5) |
| Yucatán | 16[b] | −23 ± 0.4 (−27 to −18) | +13 ± 2.0 (+8.6 to +19) | 8[b] | −23 ± 0.7 (−25 to −20) | +9.5 ± 0.8 (+7.28 to + 11.1) |
| Costa Rica | 9[c] | −24 ± 0.3 (−25 to −22) | +8.5 ± 0.7 (+6.5 to + 9.7) | 16[c] | −22 ± 1.1 (−26 to −14) | +9.5 ± 0.6 (+7.1 to + 12) |
| Texas (Spring) | 32[d] | −23 ± 0.2 (−26 to −21) | +8.2 ± 0.8 (+3.1 to 14) | 19 | −24 ± 0.1 (−25 to −23) | +6.8 ± 0.8 (+4.1 to +9.3) |

[a] Sample size for $\delta^{15}$N in migrants ($n$ = 27)
[b] Sample size for $\delta^{15}$N in migrants ($n$ = 14) and ($n$ = 9) for residents
[c] Sample size for $\delta^{15}$N in migrants ($n$ = 10) and ($n$ = 17) for residents
[d]Sample size for in migrants ($n$ = 34)

**Table 7.** Diet-tissue isotope discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$; mean) for liver from six families of passerines and comparisons with the results of other studies.

| Common name | N | $\Delta^{13}C$ (‰) | N | $\Delta^{15}N$ (‰) | Reference |
|---|---|---|---|---|---|
| Black & white warbler | 6 | 2.7 | 6 | 5.0 | Present study |
| Carolina wren | 30 | 2.4 | 30 | 4.9 | Present study |
| Common yellowthroat | 19 | 3.5 | 20 | 4.6 | Present study |
| Dusky-capped flycatcher | 4 | 2.3 | 4 | 4.1 | Present study |
| Rufous & white wren | 4 | 4.4 | 4 | 5.5 | Present study |
| Yellow-rumped warbler | 8 | 2.9 | 8 | 6.2 | Present study |
| Dunlin | 4 | 1.1 | 4 | 4.0 | Ogden et al. 2004[a] |
| Japanese Quail | 3 | -1.0 | – | – | Hobson and Clark 1992*a* |
| Japanese Quail | 3 | 0.9 | – | – | Hobson and Clark 1992*a* |
| Domestic chicken | 8 | 0.4 | 8 | 1.7 | Hobson and Clark 1992*b*[a] |
| Japanese Quail | 5 | 0.2 | 5 | 2.3 | Hobson and Clark 1992*b*[a] |
| Ringbilled Gull | 14 | -0.4 | 14 | 2.7 | Hobson and Clark 1992*b*[a] |
| Common cormorant | 1 | 1.3 | 1 | 2.3 | Mizutani et al. 1991[a] |

[a]Lipids were not extracted.

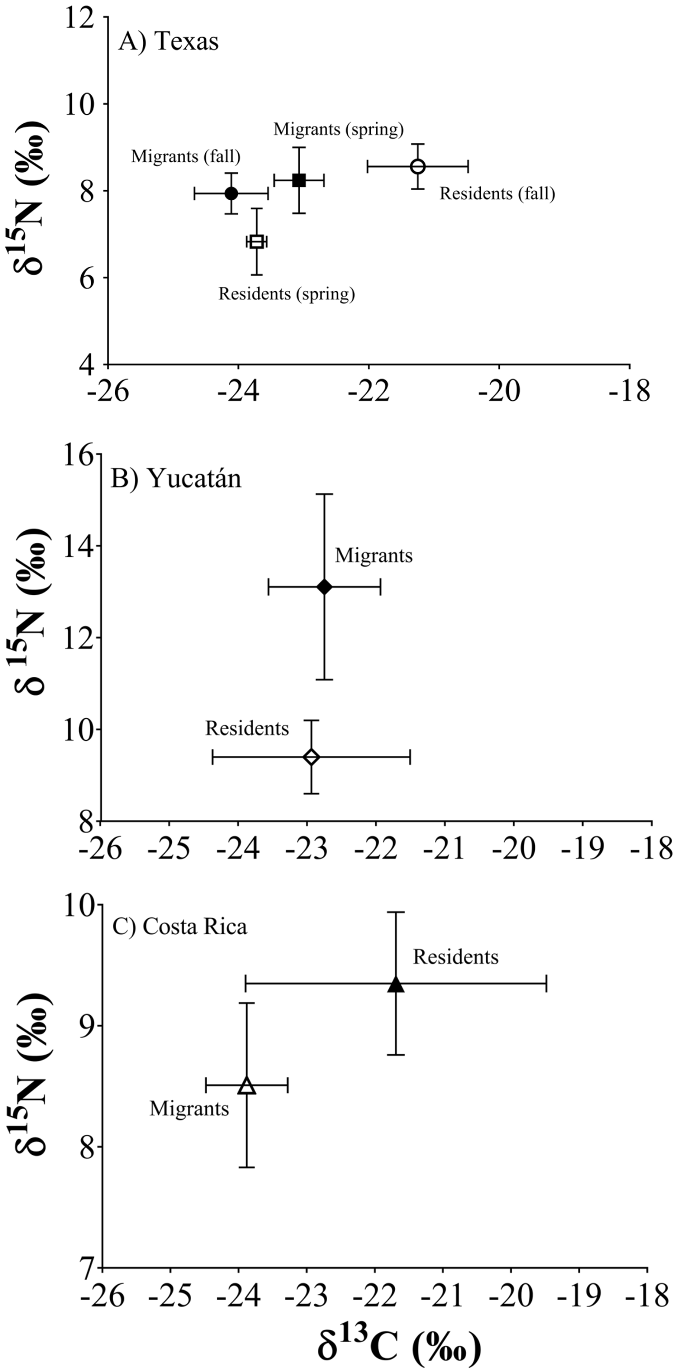



**Figure 11.** Biplot of liver tissue $\delta^{13}C$ vs $\delta^{15}N$ (‰) for migrant (open) and resident (filled) songbirds from sites located in Texas (A), Yucatán (B), and Costa Rica (C). Standard error bars are 2*SE.

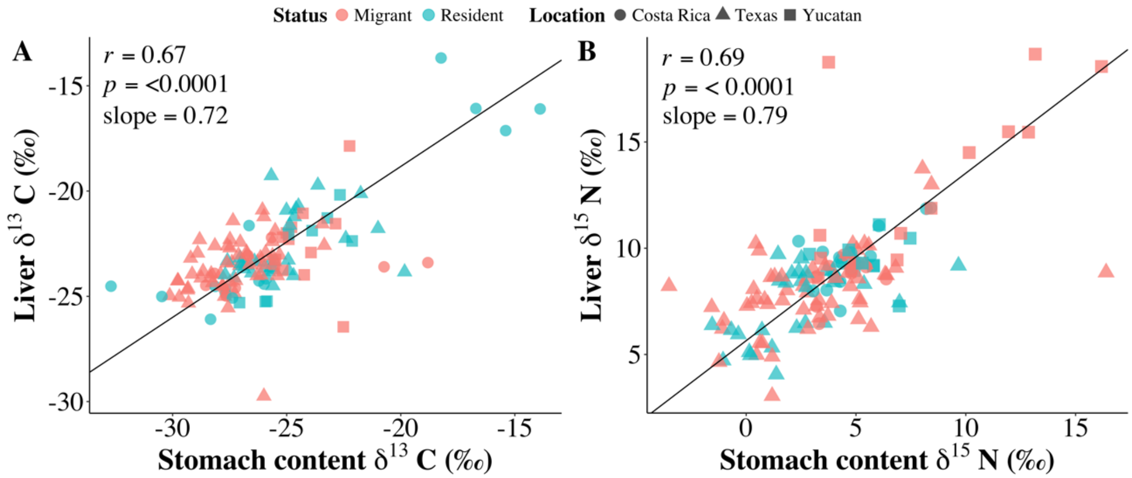



**Figure 12.** Correlation between stomach content and liver tissue stable isotope ratios of carbon (A) and nitrogen (B) for migrant (red) and resident (blue) songbirds from Texas (triangle), Yucatán (square), and Costa Rica (circle). Test statistics reflect Pearson correlation analyses. *R* values and slopes of the lines are derived from reduced major axis regressions.

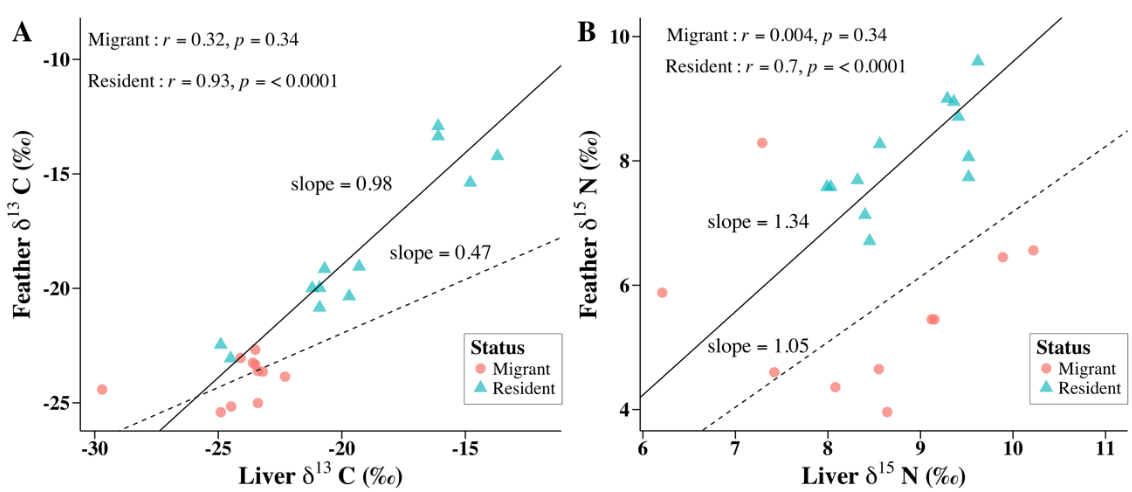



**Figure 13.** Correlation between feather and liver values for $\delta^{13}C$ and $\delta^{15}N$ (‰) for migrant (circle) and resident (triangle) songbirds collected from Texas and Costa Rica. Test statistics reflect Pearson correlation analyses. *R* values and slopes of the lines are derived from reduced major axis regressions.

*Feather $\delta^2H$*

Feather deuterium values ranged considerably for migrants with less variation seen in resident species (Figure 14 and Figure B-2). Even within species there was considerable variation for migratory birds (Figure 15). Within migrants, variation within and between species was also wide (Figure B-2). Migrant birds collected in Texas had the greatest range (−155‰ to −3.1‰) and a median $\delta^2H_f$ value of −108‰. For residents, the median was −25‰ (range: −51‰ to −1.2‰). Migrant species collected in Yucatán had a median of −71‰ (range: −130‰ to −25‰), whereas residents had a median of −35‰ (range: −65‰ to −25‰). Songbirds from Costa Rica had median values of −76‰ (range: −137 to −33‰) and −54‰ (range: −67‰ to −43‰) for migrants and residents respectively.

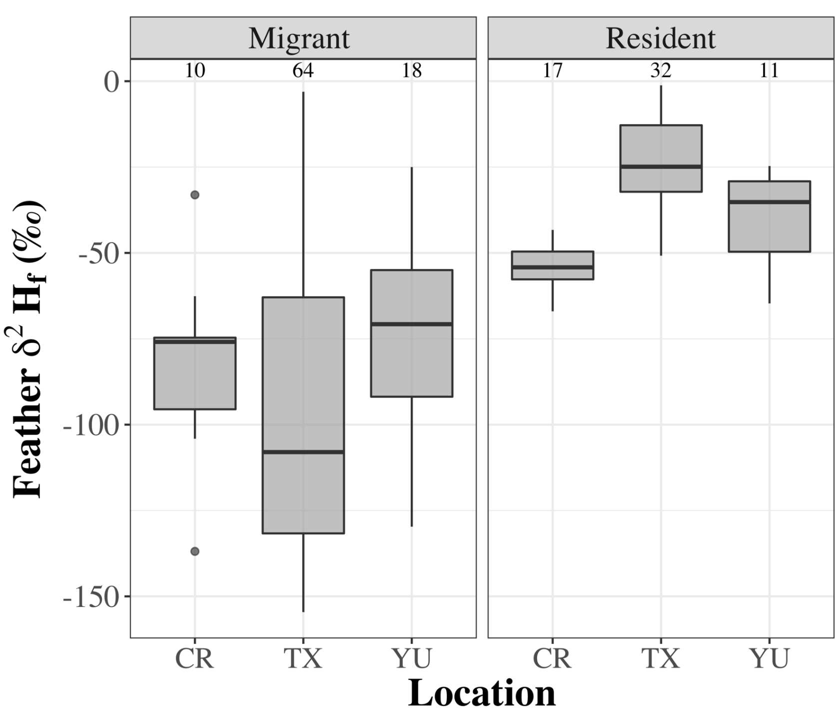



**Figure 14.** Box plots of deuterium for feather samples of songbirds. Numbers at top of boxplots indicate the sample size. *CR* = Costa Rica, *TX* = Texas, *YU* = Yucatán.

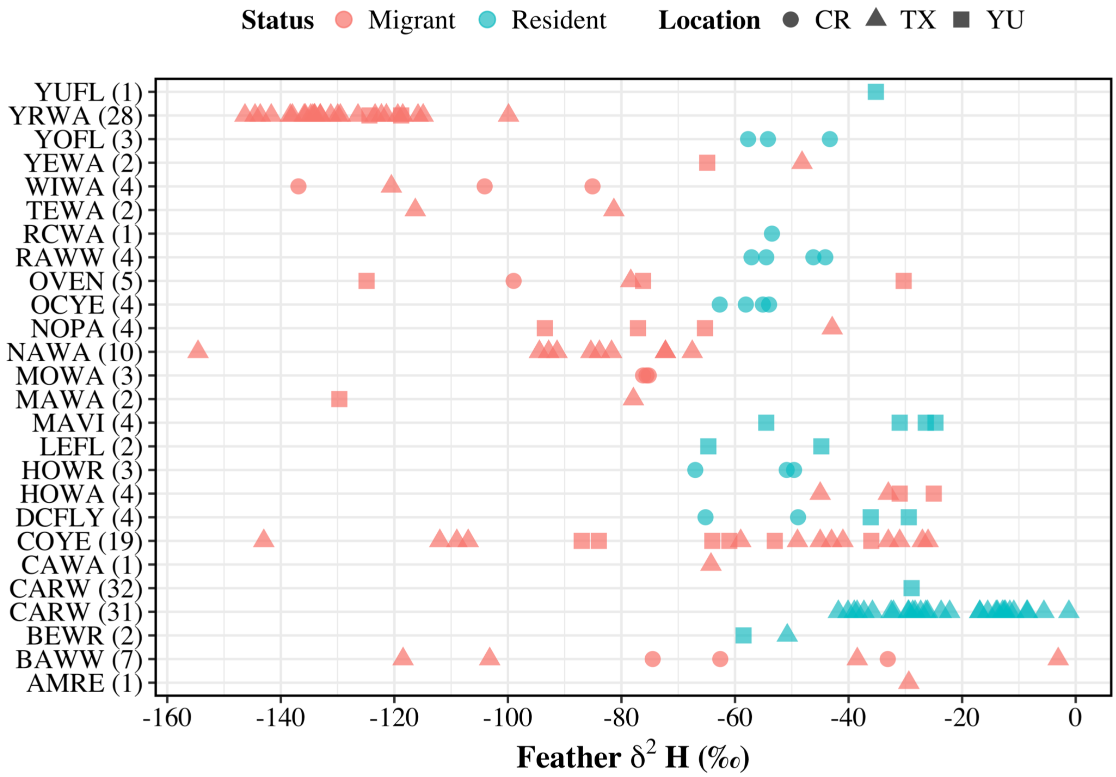



**Figure 15.** Distribution of feather $\delta^2H$ (‰) values for neotropical migrant and resident songbirds sampled during the fall and spring migration in Texas, and at wintering grounds in Yucatán (Mexico) and Costa Rica. Sample sizes for each species are given in parentheses. See Table B-1 for species abbreviation codes. *CR* = Costa Rica, *TX* = Texas, *YU* = Yucatán.

*Relationships between stable isotopes and contaminant burdens*

**Deuterium ($\delta^2H$)**

A significant and positive correlation between feather $\delta^2H$ values and the three major classes of contaminants ($p$ = DDE: <0.0001, ΣPCBs: 0.001, and ΣPBDEs: 0.007; Figure 16 and Table B - 2) was observed for all resident species. For migrants, a significant negative relationship was observed between ΣPBDEs and $\delta^2H_f$ ($p$ = 0.003; Figure 15); however, no relationship for DDE or ΣPCBs was found (Figure 16).

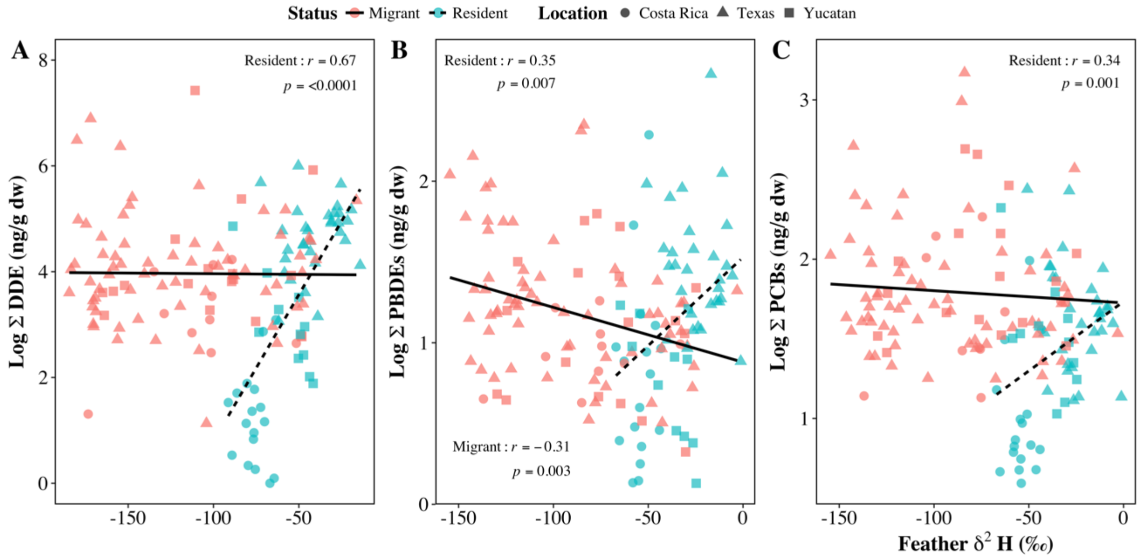



**Figure 16.** Correlations between individual POPs contaminants with feather $\delta^2$H (‰) values for migrant (red) and resident (blue) songbirds from Texas (triangle), Yucatán (square), and Costa Rica (circle).

## Carbon ($\delta^{13}$C) and Nitrogen ($\delta^{15}$N)

*Texas*

When examining all songbirds, there was a significant negative relationship between DDE concentrations and liver $\delta^{15}$N values ($p$ = 0.03; Table 8); however, a significant positive correlation was observed for liver $\delta^{13}$C values ($p$ = 0.01; Table 8). However, both relationships were relatively weak ($\delta^{15}$N: $r = -0.23$; $\delta^{13}$C: $r = 0.27$). For resident birds from Texas, almost all of which were Carolina wrens, a significant and positive correlation was also found for liver $\delta^{15}$N and ΣPCBs levels ($p$ = 0.01; Figure 17). There was also a significant and positive relationship between liver $\delta^{13}$C and concentrations of DDE ($p$ = 0.01; Table 8 and Figure 17) and ΣPBDEs ($p$ = 0.03; Table 8 and Figure 17).

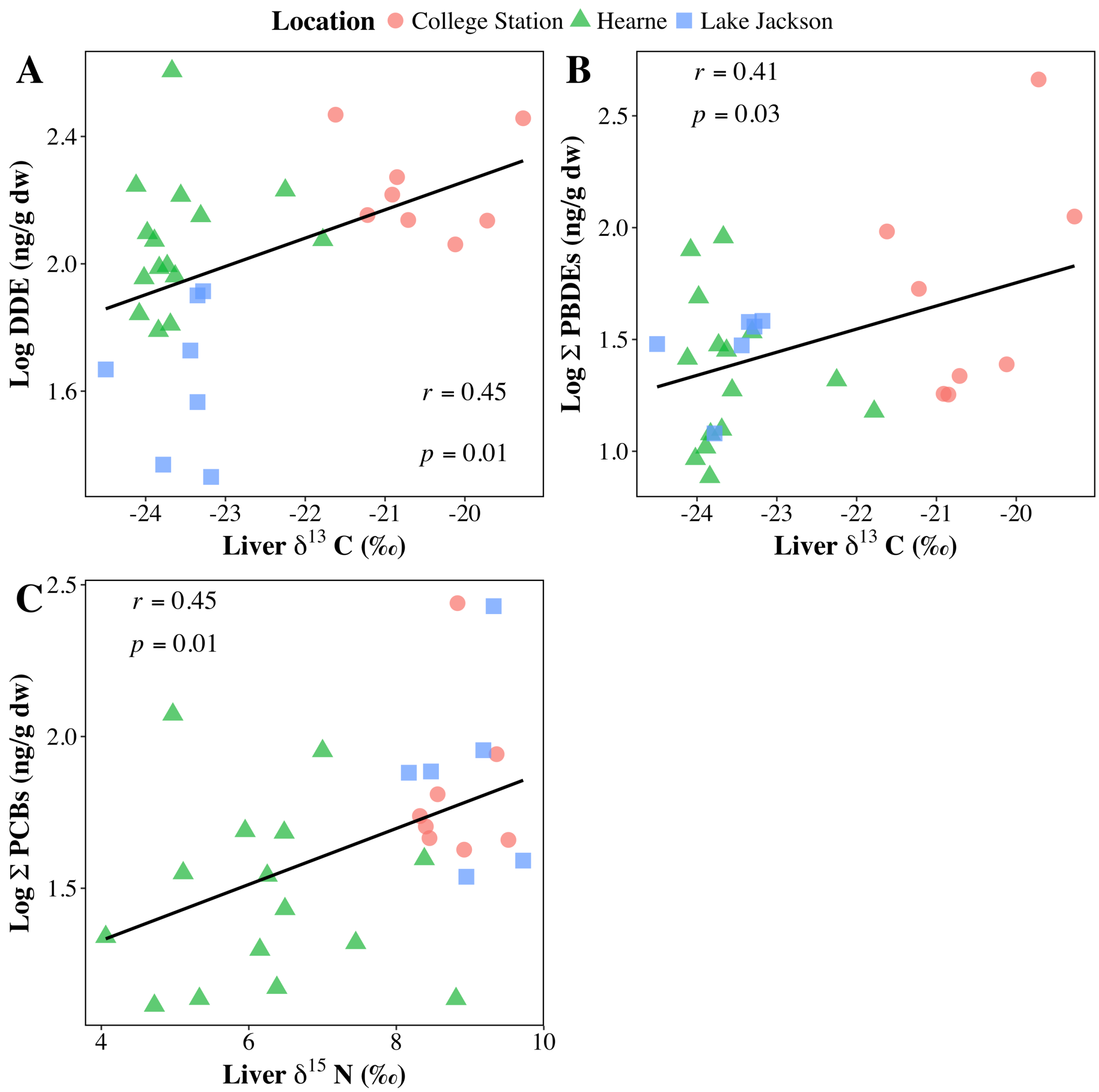



**Figure 17.** Relationship between liver $\delta^{13}C$ (‰) and (A) DDE, (B) ΣPBDEs, and between liver $\delta^{15}N$ (‰) and (C) ΣPCBs (ng/g dw) in resident songbirds from College Station (circles), Hearne (triangles), and Lake Jackson (squares), Texas, USA.

**Table 8.** Parameter estimates for linear regression models used to assess the relationship between persistent organic pollutants and stable isotopes of carbon and nitrogen in songbirds. Significant P-values are bolded.

| | | | Texas | | | Yucatán | | | Costa Rica | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Slope (SE) | *t*-value | *P* | Slope (SE) | *t*-value | *P* | Slope (SE) | *t*-value | *P* |
| DDE & $\delta^{15}N$ | All | (intercept) | 2.29 (0.19) | 11.8 | **<.0001** | 1.16 (0.35) | 3.35 | **0.003** | 1.93 (0.79) | 0.79 | **0.02** |
| | | $\delta^{15}N$ | −0.05 (0.02) | −2.23 | **0.03** | 0.04 (0.03) | 1.40 | 0.18 | −0.12 (0.09) | −1.39 | 0.18 |
| | Migrant | (intercept) | 2.17 (0.26) | 8.38 | **<.0001** | 1.55 (0.52) | 3.00 | **0.01** | 2.29 (1.15) | 1.99 | 0.08 |
| | | $\delta^{15}N$ | −0.05 (0.03) | −1.54 | 0.13 | 0.02 (0.04) | 0.43 | 0.68 | −0.11 (0.13) | −0.81 | 0.44 |
| | Resident | (intercept) | 2.35 (0.25) | 9.34 | **<.0001** | 1.12 (1.15) | 0.97 | 0.36 | 0.26 (0.73) | 0.36 | 0.73 |
| | | $\delta^{15}N$ | −0.04 (0.03) | −1.37 | 0.18 | 0.03 (0.12) | 0.24 | 0.81 | 0.03 (0.08) | 0.38 | 0.71 |
| DDE & $\delta^{13}C$ | All | (intercept) | 3.81 (0.73) | 5.19 | **<.0001** | 2.52 (1.29) | 1.95 | 0.06 | 0.36 (0.73) | 0.50 | 0.62 |
| | | $\delta^{13}C$ | 0.08 (0.03) | 2.64 | **0.01** | 0.04 (0.06) | 0.67 | 0.51 | −0.02 (0.03) | −0.69 | 0.50 |
| | Migrant | (intercept) | 2.89 (1.18) | 2.45 | **0.02** | 3.12 (1.67) | 1.88 | 0.08 | −0.24 (2.80) | −0.09 | 0.93 |
| | | $\delta^{13}C$ | 0.05 (0.05) | 0.93 | 0.36 | 0.06 (0.07) | 0.81 | 0.43 | −0.07 (0.12) | −0.61 | 0.56 |
| | Resident | (intercept) | 4.04 (0.76) | 5.32 | **<.0001** | 0.59 (1.69) | 0.35 | 0.73 | 0.90 (0.52) | 1.72 | 0.11 |
| | | $\delta^{13}C$ | 0.09 (0.03) | 2.67 | **0.01** | −0.04 (0.07) | −0.48 | 0.65 | 0.02 (0.02) | 0.72 | 0.48 |
| ΣPCBs & $\delta^{15}N$ | All | (intercept) | 1.47 (0.19) | 7.88 | **<.0001** | 1.22 (0.32) | 3.87 | **0.001** | 1.71 (0.73) | 2.35 | **0.03** |
| | | $\delta^{15}N$ | 0.04 (0.02) | 1.52 | 0.13 | 0.05 (0.03) | 1.80 | 0.09 | −0.05 (0.08) | −0.67 | 0.51 |
| | Migrant | (intercept) | 1.75 (0.24) | 7.30 | **<.0001** | 1.59 (0.45) | 3.55 | **0.004** | 1.97 (1.22) | 1.61 | 0.15 |
| | | $\delta^{15}N$ | 0.01 (0.03) | 0.19 | 0.85 | 0.03 (0.03) | 0.76 | 0.46 | −0.04 (0.14) | −0.27 | 0.79 |
| | Resident | (intercept) | 0.96 (0.27) | 3.52 | **0.002** | 0.95 (1.24) | 0.76 | 0.47 | 0.31 (0.70) | 0.45 | 0.66 |
| | | $\delta^{15}N$ | 0.09 (0.04) | 2.61 | **0.01** | 0.06 (0.13) | 0.49 | 0.64 | 0.07 (0.07) | 0.97 | 0.35 |
| ΣPCBs & $\delta^{13}C$ | All | (intercept) | 1.95 (0.76) | 2.56 | **0.01** | 2.26 (1.12) | 2.03 | 0.06 | 0.24 (0.63) | 0.38 | 0.71 |
| | | $\delta^{13}C$ | 0.01 (0.03) | 0.26 | 0.79 | 0.02 (0.05) | 0.42 | 0.68 | −0.04 (0.03) | −1.59 | 0.13 |
| | Migrant | (intercept) | 1.13 (0.92) | 1.22 | 0.23 | 2.14 (1.33) | 1.61 | 0.13 | −3.21 (3.35) | −0.96 | 0.37 |
| | | $\delta^{13}C$ | −0.03 (0.04) | −0.74 | 0.47 | 0.01 (0.06) | 0.17 | 0.86 | −0.21 (0.14) | −1.47 | 0.19 |
| | Resident | (intercept) | 2.8 (0.97) | 2.90 | **0.007** | 1.73 (1.88) | 0.92 | 0.39 | 0.68 (0.51) | 1.33 | 0.21 |
| | | $\delta^{13}C$ | 0.05 (0.04) | 1.99 | 0.24 | 0.01 (0.08) | 0.10 | 0.92 | −0.01 (0.02) | −0.58 | 0.57 |
| ΣPBDEs & $\delta^{15}N$ | All | (intercept) | 1.32 (0.20) | 6.49 | **<.0001** | 0.33 (0.29) | 1.14 | 0.27 | 0.30 (0.68) | 0.44 | 0.66 |
| | | $\delta^{15}N$ | 0.0003 (0.03) | 0.01 | 0.99 | 0.06 (0.02) | 2.47 | **0.02** | 0.06 (0.07) | 0.82 | 0.42 |
| | Migrant | (intercept) | 1.33 (0.25) | 5.34 | **<.0001** | 0.35 (0.46) | 0.77 | 0.45 | 0.83 (0.64) | 1.28 | 0.23 |
| | | $\delta^{15}N$ | −0.01 (0.03) | −0.31 | 0.75 | 0.06 (0.03) | 1.66 | 0.12 | 0.01 (0.07) | 0.17 | 0.87 |
| | Resident | (intercept) | 1.06 (0.33) | 3.19 | **0.004** | −0.46 (0.88) | −0.51 | 0.62 | −0.29 (1.04) | −0.28 | 0.78 |
| | | $\delta^{15}N$ | 0.05 (0.04) | 1.26 | 0.22 | 0.14 (0.1) | 1.53 | 0.17 | 0.12 (0.11) | 1.06 | 0.31 |
| ΣPBDEs & $\delta^{13}C$ | All | (intercept) | 2.59 (0.81) | 3.20 | **0.002** | 1.22 (1.08) | 1.13 | 0.27 | 0.06 (0.60) | 0.10 | 0.92 |
| | | $\delta^{13}C$ | 0.05 (0.03) | 1.56 | 0.12 | 0.01 (0.05) | 0.17 | 0.87 | −0.04 (0.03) | −1.34 | 0.19 |
| | Migrant | (intercept) | 0.89 (0.96) | 0.93 | 0.36 | 2.16 (1.45) | 1.49 | 0.16 | −2.03 (1.60) | −1.27 | 0.25 |
| | | $\delta^{13}C$ | −0.02 (0.04) | −0.39 | 0.67 | 0.05 (0.06) | 0.73 | 0.47 | −0.13 (0.07) | −1.87 | 0.10 |
| | Resident | (intercept) | 3.82 (1.01) | 3.78 | **0.001** | −0.97 (1.26) | −0.76 | 0.48 | 0.16 (0.77) | 0.21 | 0.84 |
| | | $\delta^{13}C$ | 0.10 (0.04) | 2.33 | **0.03** | −0.08 (0.06) | −1.47 | 0.19 | −0.03 (0.03) | −0.84 | 0.41 |

*Mexico*

A significant and positive relationship was found between ΣPBDEs concentrations and liver $\delta^{15}N$ values when examining all birds ($p = 0.02$; Table 8 and Figure 18); however, when migrant and resident species were examined separately, no significant relationship was observed (Table 8). For all other classes of contaminants and stable isotopes, no significant relationships were found.

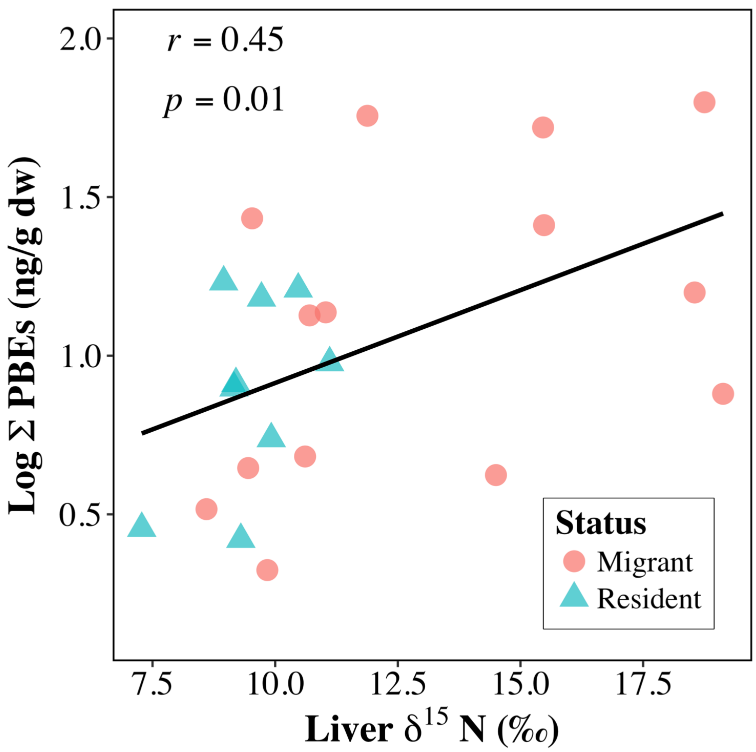



**Figure 18.** Relationship between liver $\delta^{15}N$ (‰) values and ΣPBDEs (ng/g dw) body burdens in migrant and resident songbirds from Yucatán, Mexico.

*Costa Rica*

Songbirds collected in Costa Rica showed no significant relationships between any of the contaminants and liver stable isotope values of carbon and nitrogen (Table 8). Additionally, when migrants and residents were examined separately, no significant relationships were found for either $\delta^{13}C$ or $\delta^{15}N$ and contaminant levels.

## Discussion

### *Songbird diets*

The significant correlation in $\delta^{13}C$ and $\delta^{15}N$ values observed between liver tissue and stomach contents for all songbirds shows recent assimilation of local dietary food items (Figure 12). However, only resident species had a significantly strong and positive correlation between feather and liver $\delta^{13}C$ and $\delta^{15}N$ values (Figure 13). This suggests that diets of resident songbirds are consistent over a longer period of time with little variation in resource shifts. Stable isotope ratios in feathers reflect the diet of the bird during the period of feather growth and are metabolically inactive once grown, making their isotopic values relatively fixed (Hobson and Clark 1992a); therefore, it was not surprising that in migrant birds there was not a significant correlation between stable isotope values in liver and feathers stable isotope values. In contrast, metabolically active tissues are continuously incorporating elements, such as carbon and nitrogen, resulting in isotopic changes depending on diet and tissue-specific metabolic rates, referred to as isotopic turnover (Bearhop et al. 2002, Pearson et al. 2003, Hobson and Bairlein 2003). Liver tissue has a relatively high turnover rate (8.3 – 9.8 d) and therefore is reflective of very recent diet (Hobson and Clark 1992a, Carleton et al. 2008, Bauchinger and McWilliams 2009). The vast majority of wood warbler species (Parulidae) undergo an annual pre-basic molt, growth of new feathers, in the fall while on their breeding grounds (Leu and Thompson 2002) and local baseline isotope values may be significantly different in breeding areas compared to migratory stopover sites and wintering habitats (Shearer, Kohl, and Chien 1978). Therefore, a significant relationship between feather and liver isotope values in migratory species may be confounded due to variations in baseline isotope values or dietary differences while on their breeding grounds.

Although mean liver $\delta^{15}N$ values were generally similar for songbirds (Table 6), there was high variability in the mean isotope values for stomach contents (Table 5) and large individual ranges for liver tissue and stomach contents (Figure B - 1). The high individual variability observed in $\delta^{15}N$ for both liver and stomach content samples is likely due to the large number of different bird species collected and the prey items they consume (Figure B-1). The large diversity of common prey items for many songbirds include arthropods from the orders Araneae, Coleoptera, Diptera, Ephemeroptera, Homoptera, Hymenoptera, Lepidoptera, Odonata, and Orthoptera (Rodewald 2015). In a study of arthropod communities, Bennett and Hobson (2009) found significant differences in $\delta^{15}N$ values for eight different orders that was consistent with their known feeding ecology. For instance, beetle families (Coeleoptera) occupied a variety of trophic levels, which corresponded to the large diversity of feeding strategies from herbivores to carnivores seen in this family (Bennett and Hobson 2009).

Mean liver $\delta^{13}C$ values in songbirds were similar between locations for both migrants (−24‰ to −23‰) and residents (−24‰ to −21‰), with birds from Costa Rica having the largest individual range (−26 to −14‰; Figure B-2). Resident birds collected in Texas during the fall were more enriched in $^{13}C$ compared to residents collected in the spring by +3‰. These differences appear to be due to resident species of wrens that were collected during the fall in College Station, however, not during the spring. In general, wrens collected in College Station were more enriched in $^{13}C$ compared to wrens collected in Hearne and Lake Jackson (Figure 11). Mean $\delta^{13}C$ values for all birds were within the typical range for $C_3$ plants (−20 to −34‰), which is consistent with the known foraging behavior for most species in the present study (Pate 2001). Trees and shrubs are classified as $C_3$ plants, based on their photosynthetic pathway, and the vast majority of songbirds in this study are classified as lower canopy gleaners (De Graaf, Tilghman, and Anderson 1985). Foraging height did not appear to effect $\delta^{13}C$ values in birds from Texas, as differences amongst species were negligible (Figure B-2). Common yellowthroats and wren species are lower-canopy or ground gleaners (≤ 4 m); whereas Nashville and yellow-rumped warblers are mid to upper-canopy and shrub gleaners (5 − 10 m) (De Graaf, Tilghman, and Anderson 1985, Poole 2005, Sabo and Holmes 1983). In contrast, the large individual range of $\delta^{13}C$ values for Costa Rican residents appears to be species related. Although all are insectivorous, differences in $\delta^{13}C$ values may be attributed to varying foraging habits. Olive-crowned yellowthroats (*Geothlypis semiflava*) are resident wood-warblers in the Caribbean lowlands of Costa Rica and like other warblers forage in the lower canopy, but also in dense grasses (Curson 1994). By foraging in dense grasses, olive-crowned yellowthroats may be preying on insects that are foraging on tropical grasses, which are classified as $C_4$ plants (−13‰) and are more enriched in $^{13}C$ (Peterson and Fry 1987).

### *Isotopic discrimination factors (Δ)*

Discrimination factors for both carbon and nitrogen in the present study were greater than those reported for other avian species from controlled laboratory feeding studies also examining liver tissue (Mizutani, Kabaya, and Wada 1991, Hobson and Clark 1992a, b, Ogden et al. 2004). In general, consumer tissue is only slightly enriched in $^{13}C$ relative to the diet, usually less than 2‰ (DeNiro and Epstein 1978, Hobson and Clark 1992b); for nitrogen, the difference is larger (~3.2‰) (Peterson and Fry 1987). In a meta-analysis, Caut et al. (2009) examined variation in discrimination factors and estimated mean $\Delta^{13}C$ and $\Delta^{15}N$ for liver tissues in birds was 0.35 ± 0.83‰ and 2.6 ± 0.86‰, respectively. Mean $\Delta^{13}C$ and $\Delta^{15}N$ values for individual species (Table 7) in our study were much greater than those reported in the literature (Caut, Angulo, and Courchamp 2009), however, sample sizes for individual species were relatively small and taken from wild birds not under controlled experiments.

Discrimination factors for liver tissue in songbirds are not well established; however, a few studies have examined discrimination factors between blood and diet (Hobson and Bairlein 2003, Pearson et al. 2003). Researchers reported values that ranged from −1.2 to 2.2 for $\Delta^{13}C$ and 1.7 to 2.7 for $\Delta^{15}N$; both lower compared to values in the present study for liver, +2.9‰ and +4.6‰ respectively (Hobson and Bairlein 2003, Pearson et al. 2003).

Diet composition, in particular protein quantity and quality, has been shown to have an effect on discrimination factors (Pearson et al. 2003, Robbins, Felicetti, and Sponheimer 2005, Del Rio and Wolf 2005). Pearson et al. (2003) found that tissues were generally more enriched in yellow-rumped warblers (*Setophaga coronata*) that were fed on high insect diets (97%); isotopic discrimination factors for both $\Delta^{13}C$ and $\Delta^{15}N$ also increased with carbon and nitrogen concentrations of diets. However, Hobson and Bairlein (2003) did not find a positive trend between discrimination factors and nitrogen concentration in garden warblers (*Sylvia borin*). Also, Robbins et al. (2005) found a negative relationship between $\Delta^{15}N$ and nitrogen content (%) in the diet; birds fed high protein diets had smaller nitrogen discrimination factors. Yellow-rumped warblers collected in Texas in the present study were the only birds that contained seeds from fruits in their stomach contents; the vast majority of the stomach contents were composed of insect parts, which are high in protein and may help to explain the larger discrimination factors observed. However, there is some bias in stomach content observations since some fruit material may already be digested.

Other studies have also shown that digestion can result in isotopic enrichment of stomach contents (Pinnegar and Polunin 1999, Guelinckx, Dehairs, and Ollevier 2008), which may also help explain the larger discrimination factors observed in the present study. Guelinckx et al. (2008) found that enrichment was more pronounced in the hindgut than in the foregut. This may be due to the high acidity of the digestive process affecting the $\delta^{15}N$ values of samples; however, no effect of acidity was observed for $\delta^{13}C$ (Pinnegar and Polunin 1999). In another study conducted by Grey et al. (2002), the authors found the effect of digestion to be negligible on the stability of nitrogen isotopes.

*Stable isotopes and contaminant body burdens*

To test if contaminant body burdens in songbirds could be explained with stable isotopes, we used contaminant data previously reported on the same species (Maldonado, Mora, and Sericano 2017). Results from linear regression models showed a significant negative relationship between ΣPBDEs and $\delta^2H_f$ in migrants, indicating that birds from more northern latitudes are slightly more contaminated; however, the relationship was weak ($r$ = –0.31). No latitudinal gradient of contaminant levels was observed in migrants for either DDE or ΣPCBs. Conversely, significant and positive correlations between $\delta^2H_f$ and each class of contaminant were observed for resident species with the strongest relationship observed for DDE ($r$ = 0.67; Figure 15). However, the strength of relationships for ΣPCBs ($r$ = 0.34) and ΣPBDEs ($r$ = 0.35) was much weaker. In an earlier study Mora (2008) found a significant and negative relationship between DDE concentrations and tail feather $\delta^2H$ values in migrant and resident songbirds from Mexico indicating birds from more northern latitudes were more contaminated. However, in a more recent study Mora et al. (2016) observed a clear trend of decreasing DDE concentrations in insectivorous passerines with latitude.

For resident birds from Texas, there was a significant and positive relationship between DDE and ΣPBDEs concentrations with liver $\delta^{13}C$ (Figure 16 and Table 8); however, not for ΣPCBs. The significant relationship appears to be due to sampling location with resident Carolina wrens from College Station having greater contaminant concentrations and more enriched liver $^{13}C$ values. These results suggest that contaminant burdens for DDE and ΣPCBs are likely influenced by proximity to more urban environments. Birds collected from Hearne and Lake Jackson were further removed from residential areas compared to birds from College Station. POPs contaminant levels in birds have previously been shown to be related to use and proximity of urban habitats (Focardi et al. 1996, Muñoz and Becker 1999).

In general, contaminant concentrations did not increase with liver $\delta^{15}N$ for migrants or residents, with the exception of ΣPCBs in resident species from Texas and ΣPBDEs in songbirds from Yucatán (Figure 16), as was expected based on previous studies in the literature (Table 8). Although Texas resident birds had greater POPs contaminant burdens compared to residents in Yucatán and Costa Rica, they had slightly lower mean liver $\delta^{15}N$ values compared to residents from Yucatán and Costa Rica (Table 2). This could be due to (1) resident species from Yucatán and Costa Rica eating a more enriched protein diet, (2) species differences, or (3) differences in baseline nitrogen values (Kelly 2000, Pearson et al. 2003). Furthermore, foliar $\delta^{15}N$ from tropical lowland forests has been documented as being more enriched in $^{15}N$ (Martinelli et al. 1999).

Resident birds from Costa Rica had significantly lower ΣPCB concentrations than migrants and also resident birds from Texas and Yucatán (Maldonado et al. 2017). However, mean liver $\delta^{15}N$ values were similar between migrants and residents from Costa Rica indicating that birds were feeding at similar trophic levels. Similarly, differences between mean liver $\delta^{15}N$ values for residents from each of the locations were less than

3−4 ‰, the average enrichment value for each increase in trophic level (Kelly 2000). Only migrant birds collected from Yucatán were feeding at a higher trophic level compared to resident species as indicated by a +3.4‰ difference in liver tissue.

Although stable isotopes have shown to be useful tools in studying the accumulation of environmental contaminants in birds, interpretation of contaminant residues is complicated due to differences in tissue turnover rates and clearance rates of contaminants. Liver tissue in songbirds has a relatively high turnover rate (~ 9 d), which is a good indicator of recent diet, however, half-lives of many POPs are much longer in bird tissues (Loganathan 2011, Stickel et al. 1984b, a). The lack of a significantly positive relationship between most contaminant body burdens and liver $\delta^{15}N$ values in the present study may be due to the large differences in residence times between stable isotopes and contaminants; therefore, recent diets may not relate well to bioaccumulation of POPs in migratory songbirds. In addition, although most migratory songbirds exhibit dietary plasticity during migration (Parrish 1997), there may insufficient dietary variance among individuals to observe significant relationships between POPs levels and $\delta^{15}N$ values.

These results indicate that contaminant body burdens in songbirds may be better explained by foraging locations, and likely historic use, rather than by diet alone. Given these results, it is important to note that soil and terrestrial plants can vary widely in their $\delta^{15}N$ values and given the large spatial scale of the different sampling locations in the present study it is difficult to accurately compare trophic positions between groups (Kelly 2000). The large species variation and small samples sizes also makes interpretation of the present results difficult. Species differences may be a confounding factor in examining the relationship between contaminants and stable isotope values due to differences in biotransformation rates or metabolic rates. Therefore, these observations must be interpreted with caution. Determining historic use of legacy pollutants and establishing an accurate assessment of the potential environmental exposure patterns in geographic areas is a difficult task due to lack of information on pesticide use.

## Conclusion

Stable isotope analyses revealed that there were only minor differences in the diets of migrant and resident songbirds, within each sampling location. Trophic level, as predicted by $\delta^{15}N$ values, was only useful in explaining contaminant concentrations of ΣPCBs for resident species from Texas and ΣPBDEs for all songbirds from Yucatán. Resident species from Texas showed distinct liver $\delta^{13}C$ values amongst the different sampling locations; furthermore, significant relationships between $\delta^{13}C$ and POPs (DDE and ΣPBDEs) concentrations were observed, suggesting location and proximity to more residential environments as a source of contaminant exposure. No latitudinal gradient of contaminant concentrations was observed for migratory songbirds through use of feather $\delta^{2}H$ values; however, a significant relationship for resident species was observed. Isotope discrimination factors between liver tissue and stomach contents in songbirds for $\Delta^{13}C$ and $\Delta^{15}N$ are more enriched compared to values reported in the literature for other avian species which is important for diet reconstruction using mixing models.

CHAPTER IV

# A REVIEW OF PERSISTENT ORGANIC POLLUTANTS (POPS) IN BIRDS FROM LATIN AMERICA: 1980 – 2018

## Summary

Persistent organic pollutants (POPs) are a group of toxic chemicals that are highly persistent and ubiquitous in the environment. They have received much attention over the years, however, most studies and reviews have focused on regions of the Northern Hemisphere. Data on POPs residues in birds is summarized for reported studies in Latin America from 1980 – 2018. The geographic spread of the data reveals that the distribution of POPs data is uneven. Studies conducted in Mexico accounts for 43% of all data, followed by Brazil (19%); large data gaps for Central America and parts of South America remain. In addition, most studies have focused on coastal ecosystems and on aquatic species. The pesticide DDE was the most frequently reported contaminant, followed by ΣPCBs, and generally accounted for the majority of total pollutant burdens. Very little data for PBDEs or PCDD/Fs was available highlighting the need for future studies to include them in analyses. For Mexico, DDTs ranged from <0.01–25,000, for ΣPCBs 0.3–10,600, and ΣPBDEs 0.2–62 ng/g ww, respectively. In Central America, DDTs ranged from 0.4–4,590, ΣPCBs 1.8–360, and ΣPBDEs 0.7–23 ng/g ww, respectively. For South America, DDTs ranged from <0.01–70,700, ΣPCBs <0.01–7,423, and ΣPBDEs <0.8–73 ng/g ww, respectively. Levels of POPs are decreasing in birds and the most recent reported levels are generally below those known to cause adverse health effects.

## Introduction

Persistent Organic Pollutants (POPs) are a group of toxic contaminants that share similar characteristics and have been shown to negatively impact humans and wildlife (Colborn, vom Saal, and Soto 1993). POPs generally do not occur in nature and environmental contamination by them is due mainly to anthropogenic activities such as agriculture and industrial processes. As their name suggest, these compounds are persistent in the environment due to their highly stable structures (Miniero and Iamiceli 2008). Their persistence in the environment has led to their ubiquity across the globe and potential long-term exposure of these compounds in both humans and wildlife. Furthermore, many of these chemicals are highly lipophilic and can accumulate in the fatty tissues of organisms, which can lead to bioaccumulation within an organism and biomagnification through food webs (Furness 1993). Of particular concern are organochlorine (OCs) pesticides, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) or collectively "dioxins". Many organochlorine pesticides, including dichlorodiphenyldichloroethane (DDT), chlordane, dieldrin, aldrin, endrin, heptachlor, and mirex were widely produced and used throughout Latin America in agriculture and for control of vector borne diseases, such as malaria (Albert 1996, Barra et al. 2006). PCBs are industrial chemicals that were produced as mixtures with the trade name of Aroclor. They had a wide variety of commercial applications, but were mainly used as insulation for electrical equipment (Grube et al. 2011). PBDEs are another type of industrial contaminant which are used as fire retardants in a variety of materials including electronics, upholstery, and building materials (de Wit 2002, Alaee 2003). Unlike the previous POPs, dioxins and furans have no commercial applications and are produced as unintentional byproducts through manufacturing and combustion processes of other chlorinated chemicals (Allsopp and Erry 2000). In spite of the widespread use of many POPs throughout Latin America and the continued global pollution problem they pose, the majority of scientific research and available data is concentrated in developing regions of the Northern Hemisphere (Whylie et al. 2003).

Birds were among the first wildlife species to play a major role in creating awareness of the environmental pollution problems posed by POPs. Birds are found in all habitat types, occupy all trophic levels, and are sensitive to environmental changes, thus they can act as biomonitors of POPs pollution across many ecosystems. Nearly three fourths of North American birds the breed across the United States (U.S.) and Canada migrate to the neotropics during the non-breeding season. Despite this, most studies and reviews have focused on birds in arctic or temperate regions of North America and Europe, with much less attention focused on the tropics and Latin America (Braune et al. 2005, Chen and Hale 2010, Lacher and Goldstein 1997, Walker 1990). A handful of reviews have focused on overviews of POPs levels in different regions of Latin America and tropical ecotoxicology (Albert 1996, Allsopp and Erry 2000, Lacher and Goldstein 1997). In addition, a review focusing on persistent organochlorine pesticide levels in birds from the southwestern United States and Mexico was conducted by Mora in (1997); however, no systematic reviews have focused solely on birds in Latin America.

This review focuses on the current state of POPs contamination for OC pesticides, PCBs, PBDEs, and PCDD/Fs in birds from Latin America (Mexico, Central and South America). The specific aims of this review paper were to determine: 1) where and what data is available for POPs levels in birds from Latin America, 2) what are the temporal trends, if any, of POPs levels in birds, and 3) what are the knowledge gaps and priorities for future research.

## Methods

A quantitative literature review was conducted on POPs studies for birds in Latin America (Mexico, Central and South America). The resulting quantitative assessment documents the geographical and temporal spread of the literature, as well as the current status of POPs levels in birds for Latin America. Original research papers, theses, and dissertations were obtained from searches of several electronic databases (Table C-1). Databases were searched for the following key words: ‘birds’, ‘chlorinated pesticides’, ‘contaminants’, ‘dichlorodiphenyltrichloroethane (DDT)’, ‘dichlorodiphenyldichloroethene (DDE)’, ‘organic pollutants’, ‘organochlorine pesticides’, ‘organochlorines’, ‘persistent organic pollutants (POPs)’, ‘pesticides’, ‘pollutants’, ‘polybrominated diphenyl ethers (PBDEs)’, ‘polychlorinated biphenyls (PCBs)’, ‘dioxins (PCDDs)’, ‘furans (PCDFs)’, ‘Latin America’, ‘Central America’, ‘South America’, ‘Mexico’, ‘Belize’, ‘Costa Rica’, ‘El Salvador’, ‘Guatemala’, ‘Honduras’, ‘Nicaragua’, ‘Panama’, ‘Argentina’, ‘Bolivia’, ‘Brazil’, ‘Chile’, ‘Columbia’, ‘Ecuador’, ‘Guyana’, ‘Peru’, ‘Suriname’, ‘Uruguay’ and ‘Venezuela’. Any refereed papers, academic theses, or government reports published between 1980-2018 were included; however, some studies were conducted prior to 1980 with papers published after 1980.

Coordinates for sampling locations were used to generate maps in ArcGIS. If coordinates were unavailable, authors were contacted to obtain information or georeferenced data based on descriptions of study areas or references to nearby municipalities.

Contaminant values for POPs were obtained from data tables in the literature and include means (arithmetic or geometric), individual values, and ranges. All data points were converted to a wet weight (ww) basis in ng/g using 'OrgMassSpec' package in R statistical software using the "ConvertConcentration" function (RStudio 2016). If moisture content was not provided by the authors, 70% moisture was assumed in tissues for conversion to wet weight. For lipid content, 5% for eggs and 20% for liver was assumed (Focardi et al. 1996, Porter and Jenkins 1988). For sample collection year(s), studies that were conducted over several years, an average of the range was used (i.e. 2011–2013 = 2012 used). All contaminant concentrations referenced in this paper are in ng/g ww units unless otherwise indicated.

## Statistical analysis

Non-parametric Kendall's rank correlation test was performed in R statistical software to test if there was a significant decrease in POPs (DDE/ΣDDT and PCBs) levels in birds over time (treated as a continuous variable; see list of references included in analyses in Appendix C, Table C-2). R squared values were also calculated based on general linear regression analysis using "lm" function in R. Significance levels was set at $p < 0.05$.

## Results and Discussion

### *Geographic and temporal spread of the studies*

Since 1980, there have only been a limited number of studies conducted in Latin America that have examined POPs in birds (Figure 19). On average, ≤ 8 studies were conducted every five years; only since about 2006 has there been a recent increase in the numbers of studies. Of the available research, the vast majority of studies have been conducted in Mexico and Brazil (Figure 20 and 21). Mexico leads substantially accounting for 43% of all studies, more than double for Brazil (19%), the second country with the most studies. Within Mexico, the Baja California and the Gulf of California region have received considerable attention (Figure 20). Most of Central America, with the exception of Costa Rica and Panama, had no data available (Figure 20). For South America, Brazil had a considerably greater number of studies followed by Chile and Peru. Most countries in South America only had one to two references with no available information for Bolivia or Paraguay. In addition, almost all of the studies were concentrated in coastal regions with few located in interior sites. These trends in avian ecotoxicology studies mirror the overall trend of environmental toxicology and chemistry studies in Latin America and have remained consistent over the last two decades (Allsopp and Erry 2000, Carriquiriborde and Bainy 2012). Approximately 90% of studies conducted in Latin

America are concentrated in Brazil (40%), Mexico (26%), Argentina (14%), Chile (8%) and Colombia (3%) (Carriquiriborde and Bainy 2012). An analysis of papers published in 2011 from researchers in Argentina, Brazil, and Chile revealed that environmental research on metal and pesticide contamination to be a primary focus for all three countries. Although publications on POPs was evenly distributed between the three countries, it only accounted for approximately 3% of all environmental issues being addressed in Latin America. This data suggests that studies on POPs and other contaminants continues to be a challenge for most of Latin America, particularly for Central America.

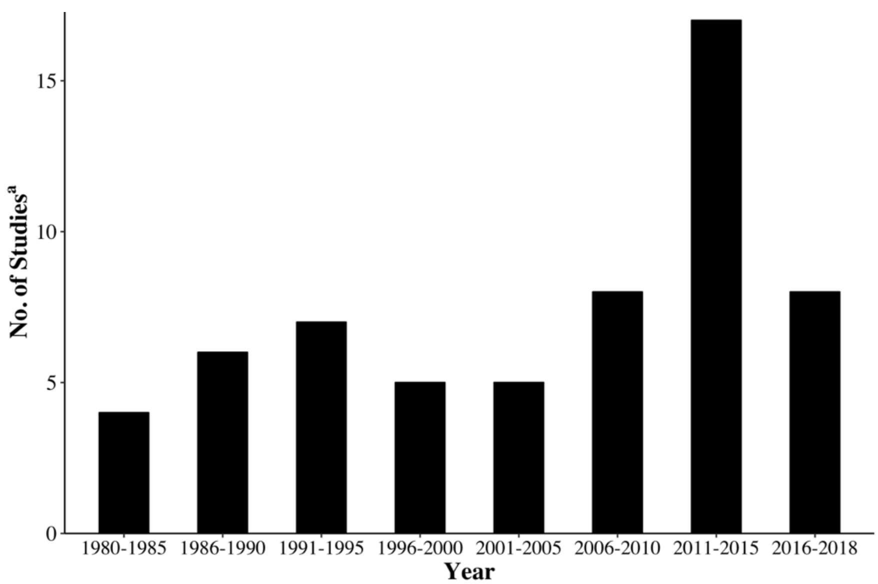



**Figure 19.** Number of studies on persistent organic pollutants in birds conducted in Latin America published or reported since 1980.
[a]Includes publications, theses, dissertations, and government reports.


**Figure 20.** Map of Mexico and Central America with locations of data for persistent organic pollutants (POPs) in birds and the frequency of studies per country published or reported from 1980 – 2018.


**Figure 21.** Map of South America with locations of data for persistent organic pollutants (POPs) in birds and the frequency of studies per country published or reported from 1980–2018.

*Characteristics of POPs data*

Approximately 89% of available data for POPs in birds was comprised of three orders (Figure 22). Shorebirds in the order Charadriiformes (snipe, sandpipers, dowitchers and phalaropes, jacanas, noddies, gulls, skimmers and terns, stilts, plovers and lapwings), and seabirds in the order Suliformes (frigatebirds, boobies, and cormorants) were the most represented order of aquatic birds each accounting for 23% of available POPs data (Figure 22). For terrestrial birds, songbirds in the order Passeriformes were the most predominant and also accounted for 23% of POPs data for Latin American birds. These results likely reflect the large diversity of species in each order.

Eggs, including eggshells, were the most analyzed tissue, followed by whole carcasses, and livers (Figure 23). DDTs, mainly in the form of the metabolite DDE, were the most detected and frequently reported of the different POPs contaminants (Figure 24); in general, it also contributed to the greatest contaminant body burdens. Less data was available for PCBs, however, when measured or detected, was generally the second most abundant contaminant measured (Figure 24). These results may be slightly biased, as not all studies reported the suite of POPs contaminants that were measured and analyzed, which could influence the frequency of detected contaminants. Congener patterns of PCBs tended to be similar across locations and species; congeners of the penta-, hexa-, and hepta- homologues were the most predominant (Adkesson et al. 2018a, Baldassin et al. 2016, Cardoso et al. 2014, Colabuono, Taniguchi, and Montone 2012, Cunha et al. 2012, Dias et al. 2018, Focardi et al. 1996, Maldonado, Mora, and Sericano 2017, Mora et al. 2011, Vander Pol et al. 2012). PCB levels also tended to be lower in resident species compared to migrant species from North America. These contaminant patterns are similar to what has been observed in many other species of aquatic and terrestrial birds from North America, China, and India and appear to be influenced by sources of Arochlor mixtures more so than by differences in metabolic pathways (Colabuono, Taniguchi, and Montone 2012, Harris and Elliott 2011, Maldonado, Mora, and Sericano 2017, Mora 1996, Senthilkumar et al. 1999, Yu et al. 2014). Less information was available for PCDD/Fs, PBDEs and other flame retardants. Only seven studies have reported on PBDEs and two for PCDD/Fs and are therefore less frequent compared to other POPs contaminants (Figure 24).

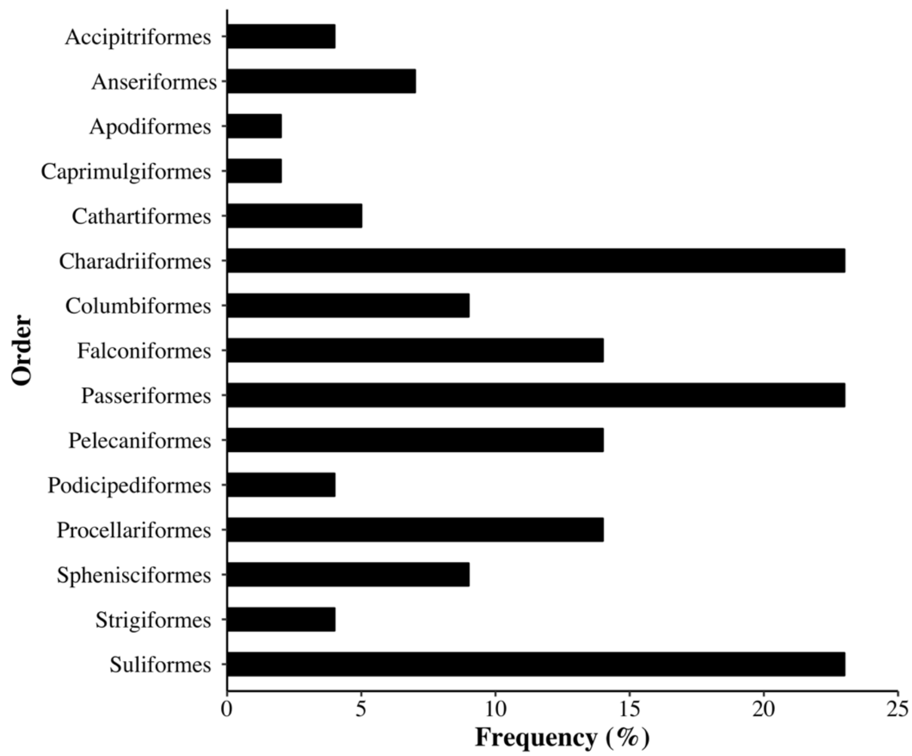



**Figure 22** Frequency of persistent organic pollutant data for different orders of birds from studies conducted in Latin America from 1954–2014.

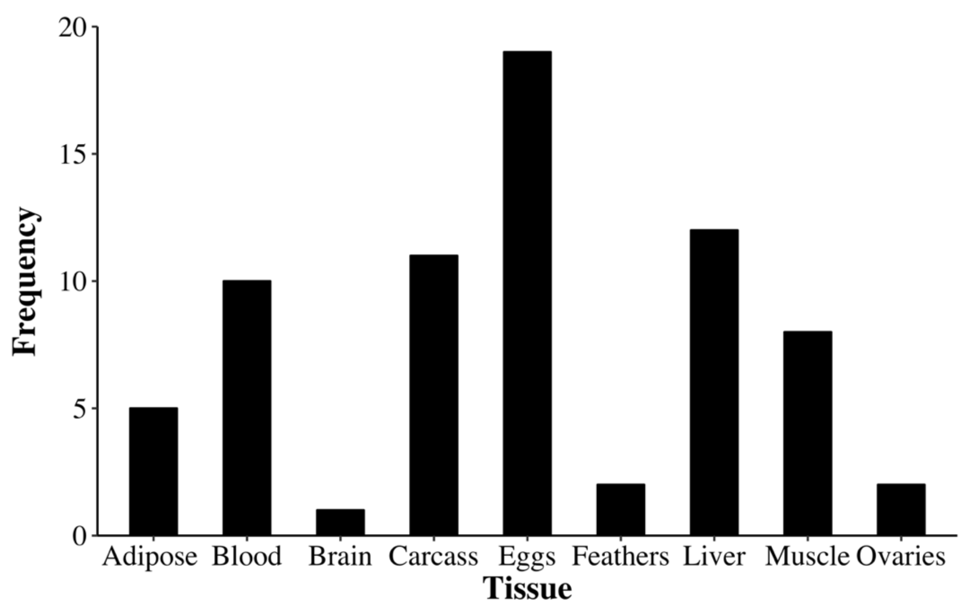



**Figure 23.** Frequency of avian tissues analyzed for persistent organic pollutants in studies conducted in Latin America sampled from 1954–2014.

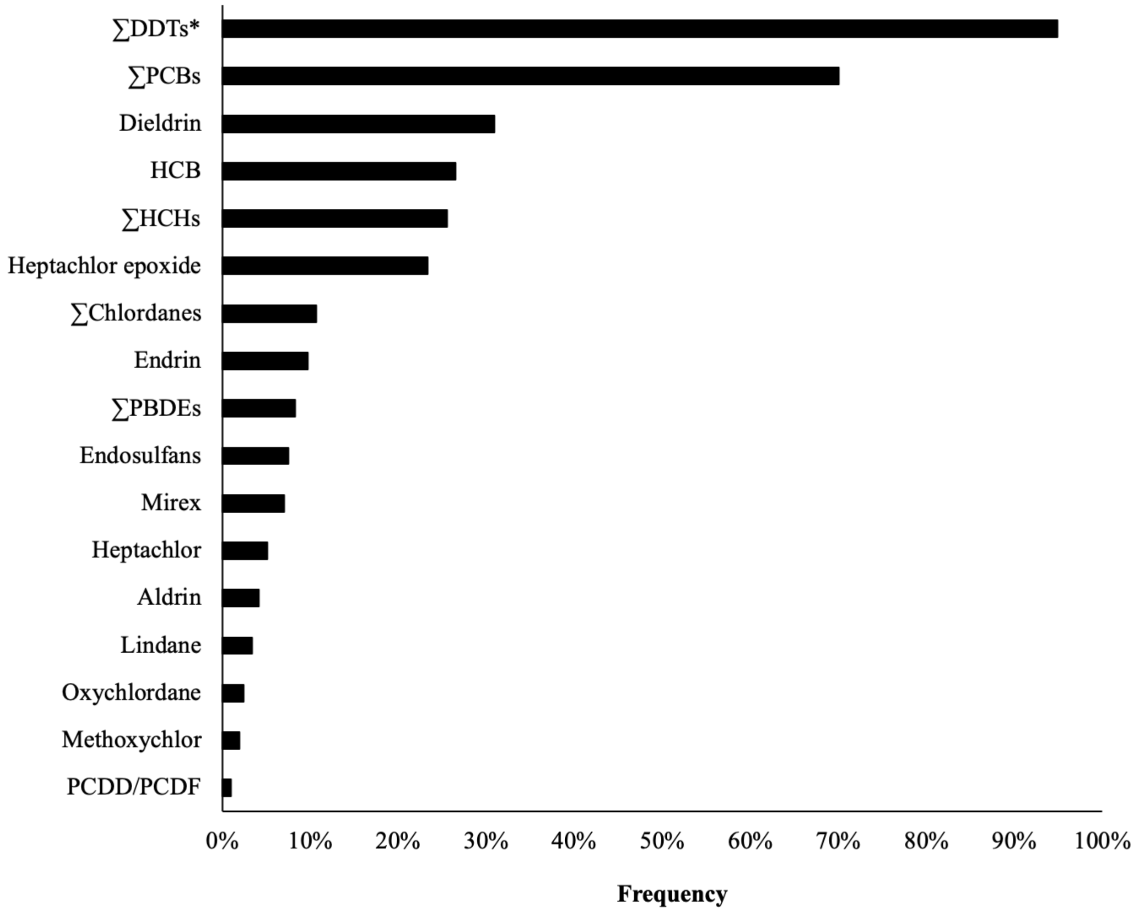



**Figure 24.** Frequency of POPs contaminants measured and detected in bird samples from Latin America from 1954–2014.
*Primarily in the form of the metabolite DDE.

*Summary of POPs levels in birds from Latin America*

## Mexico

### *Aquatic birds*

Table 9 provides summary data for POPs levels in bird tissues for studies conducted in Mexico. ΣDDTs ranged from <0.01 to 11,460, non-DDT pesticides ranged from 0.03–9,936, ΣPCBs ranged from 0.25–1,400, and ΣPBDEs from 0.2–62 ng/g ww in tissues of aquatic birds (Banasch et al. 1992, Carvalho et al. 2002, Covantes-Rosales 2014, Gamble, Blankinship, and Jackson 1994, Harwani et al. 2011, Huysman 1995, Jimenez-Castro, Mellink, and Villaescusa-Celaya 1995, Mellink, Riojas-Lopez, and Luevano-Esparza 2009, Mora, Anderson, and Mount 1987, Mora and Anderson 1991, Pina-Ortiz 2014, Porter and Jenkins 1988, Vallarino and von Osten 2017, Vander Pol et al. 2012, White, Mitchell, and Stafford 1985). The large range of values in birds can be attributed to the wide variety of tissues analyzed, large species diversity, the habitats they occupy, and their varied diets. Double-crested cormorants (*Phalacrocorax auritus*) from Mexicali, near the US – Mexico border, had the greatest concentrations of DDE in carcasses (11,460 ng/g ww) (Mora and Anderson 1991). There was a wide range in the levels and types of other organochlorine pesticides detected, but were generally lower in most tissues compared to DDE (Table 9). Sooty terns (*Onychoprion fuscatus*) from the Alacranes reef were recently reported to have the highest levels of heptachlor (9,936 ng/g ww; Table 9) measured in eggs (Vallarino and von Osten 2017). The highest residue levels of ΣPCBs were found in muscle tissues of the heermann's gull (*Larus heermanni*; mean: 6,120 ng/g ww) from the Gulf of California (Porter and Jenkins 1988). Only two studies have measured ΣPBDEs in aquatic birds from Mexico; the highest residues (62 ng/g ww) were from brown pelican (*Pelecanus occidentalis*) eggs collected from three colonies in the Gulf of California (Vander Pol et al. 2012).

Ranges of ΣDDTs and ΣPCBs in birds from Mexico were greater compared to values reported for Costa Rica, but were comparable to levels in birds from South America. This may be attributed to the larger data sets available for Mexico and South America that include larger numbers of fish-eating birds and data for raptors. Overall, approximately 19% of aquatic birds had residues of DDE ≥ 3,000 ng/g ww, mostly fish-eating species. For piscivorous birds, levels of DDE in brown pelicans were below those known to cause adverse health effects (3,000 ng/g ww) (Blus 1982); however, several values in cormorants exceeded concentrations associated in increased egg breakage (4,000 ng/g ww) (Dirksen et al. 1995). For ΣPCBs, concentrations were generally lower than reported values observed to reduce hatching success in cormorants (13,600 ng/g ww) (Custer et al. 1999), herons (4,100 ng/g ww) (Hoffman et al. 1986), and gulls (8,000–21,000 ng/g ww) (Hario et al. 2004).

**Table 9.** POPs levels (min[a]–max ng/g ww) in bird tissues from Mexico collected between 1954 – 2014.

| | Tissue | ΣDDTs[b] | Non-DDT pesticides[c] | ΣPCBs | ΣPBDEs |
|---|---|---|---|---|---|
| Aquatic birds | | | | | |
| | Adipose | 6,400 | – | 1,330 | – |
| | Blood[d] | 0.03–43 | 0.08–30 | 45 | 0.2 |
| | Carcass | 6.6–11,460 | 4.4–381 | 10–1,400 | – |
| | Eggs | 1.5–6,300 | 0.06–9,936 | 0.25–348 | 62 |
| | Liver | 0.33–1,157 | 0.12–100 | 6.7–42 | – |
| | Muscle | <0.01–6,120 | 0.03–84 | 4.2–1,670 | – |
| Granivores[e] | | | | | |
| | Carcass | 19–60 | 6–191 | – | – |
| | Eggs | 11–27 | 2.7–37 | 968 | – |
| | Liver | 1.4–34 | – | 503 | – |
| Passerines | | | | | |
| | Carcass | 0.2–6,300 | 2.0–72 | 2.7–500 | 0.7–18 |
| | Liver | 0.5–3,669 | – | 103 | – |
| Raptors | | | | | |
| | Adipose[f] | 40,530 | | | |
| | Blood[d] | 0.07–50 | 0.03–310 | – | – |
| | Brain | 51 | 12–18 | – | – |
| | Eggs | 18–25,000 | 5.6–6,000 | 60–10,600 | 5.1–22 |
| | Feathers | 40 | 10–630 | – | – |
| | Liver | 521 | 29–55 | – | – |

[a]Does not include non-detects (ND)
[b]Includes DDT metabolites
[c]Includes chlordanes, oxychlordane, HCB, HCHs, lindane, heptachlor, heptachlor epoxide, mirex, endosulfans, aldrin, dieldrin, endrin
[d]Units are in ng/mL wb
[e]Birds from order Columbiformes
[f]Units are in ng/g lw

*Passerines and granivores*

Large ranges for ΣDDTs in carcasses (0.2–6,300 ng/g ww) and liver (0.5–3,669 n/g ww) were reported for passerines (Table 9) (Banasch et al. 1992, García-Hernández et al. 2006, Henry 1993, Jiménez et al. 2005, Maldonado, Mora, and Sericano 2017, Mora

2008). The highest DDE concentrations were found in great-tailed grackle (*Quiscalus mexicanus*) carcasses from southern Mexico on the western side of the country (Figure 24) (Henry 1993); which were high enough to be of concern for raptors preying on them. Elevated DDE levels were also measured in songbirds from the US – Mexico border (4,060 ng/g ww) (Mora and Anderson 1991) and the southern tip of the Baja California peninsula (3,669 ng/g ww; Figure 24) (Jiménez et al. 2005). Residues of ΣPCBs were lower compared to DDTs with the highest levels reported by Banasch et al. (1992) in barn swallows (*Hirundo rustica*; 500 ng/g ww) from the southern Gulf of Mexico region (Table 9). Low levels of non-DDT chlorinated pesticides (2.0–72 ng/g ww) and ΣPBDEs (0.7–18 ng/g ww) were also found (Table 9). Although smaller in size, insectivorous passerines had greater concentrations of POPs contaminants compared to granivorous species of Columbiformes (ΣDDTs range: 1.4–60 ng/g ww; Table 9); however, PCBs levels were greater in granivorous birds (range: 503–968 ng/g ww). Levels of contaminant residues in relation to diet are well established in passerines; differences between passerines and doves from Mexico can likely be attributed passerines feeding at a higher trophic level compared to doves.

For DDE, 25% of passerines exceeded levels ≥ 1,000 ng/g ww, however, most studies of passerines show no adverse health effects well above this value. No effects on reproductive success in tree swallows (*Tachycineta bicolor)* was observed for DDE levels of 2,570 ng/g ww and American robins (*Turdis migratoius*) at levels of 83,000 ng/g ww (Elliott et al. 1994). Additionally, levels of ΣPCBs were well below those shown to impair reproductive behavior (5,000–25,000 ng/g ww) in other songbirds

(McCarty and Secord 1999a). In addition, most elevated concentrations in songbirds (≥ 1,000 ng/g ww) are from studies published in the early 90's, with the exception of Jimenez et al. in 2005, where researchers reported DDE levels of 3,669 ng/g in house sparrows (*Passer domesticus*) from Baja California (Figure 24).

*Raptors*

Seven studies have measured POPs levels in falcons (Kiff and Peakall 1980, Mora et al. 2008, Mora et al. 2011, Porter and Jenkins 1988), ospreys (Rivera-Rodríguez and Rodríguez-Estrella 2011), owls (Arrona-Rivera et al. 2016, García-Hernández et al. 2006), and vultures (Albert et al. 1989, Albert, Rendon, and Inigo 1989) in Mexico. The highest levels of ΣDDTs (25,000 ng/g ww; Table 9) and ΣPCBs (10,600 ng/g ww) for all birds from Mexico were measured in addled peregrine falcon (*F. peregrinus*) eggs from the Gulf of California region in an early study by Porter and Jenkins (1988). One of the earliest studies conducted in Mexico measured 18% and 25% eggshell thinning in bat (*Falco rufigularis*) and Aplomado falcons (*F. femoralis*) from preserved museum specimens (Kiff and Peakall 1980). DDE was the major contaminant detected and mean concentrations were lower in bat falcons (6,650 ng/g ww) compared to Aplomado falcons (14,850 ng/g ww). In a similar study, Albert et al. (1989) found slight eggshell thinning in black vulture (*Coragyps atratus*) eggs from Chiapas, Mexico, but no significant correlation with DDE was observed; however sample size was low (n = 4) as were mean DDE values (521 ng/g ww). Two later studies were conducted on Aplomado falcon populations in the states of Chihuahua and Veracruz by Mora et al. in (2008) and later in

(2011). By 2011, mean DDE levels had decreased in Aplomado eggs from Chihuahua by 1.5–6 times and dropped substantially by 17–40 times compared to values reported by Kiff and Peakall in 1980. Low mean levels of quantifiable OCs were also measured in feathers (ΣDDT: 40 ng/g dw; ΣHCHs: 630 ng/g dw) and blood (ΣDDT: 50 ng/mL; ΣDrienes: 310 ng/mL ww) of the ferrunginous pygmy owl (*Glaucidium brasilianum*) from the southern state of Chiapas (Arrona-Rivera et al. 2016) and in burrowing owl (*Athene cunicularia*) eggs (22 ng/g ww) from the Colorado River Delta (García-Hernández et al. 2006).

Only DDE levels in falcons (≥ 2,000 ng/g ww) collected prior to 2001 were high enough to be adversely affecting reproductive success; ranges of DDE levels associated with reproductive impairment in other raptors are generally from 2,000–10,000 ng/g ww (Cade et al. 1971, Newton 1988, Noble and Elliott 1990, Wiemeyer, Bunck, and Stafford 1993). For ΣPCBs, only two individuals had levels ≥ 3,000 ng/g ww which have been associated with reduced parental breeding behavior and physiology in American kestrels (*F. sparvrius*) (Harris and Elliott 2011).

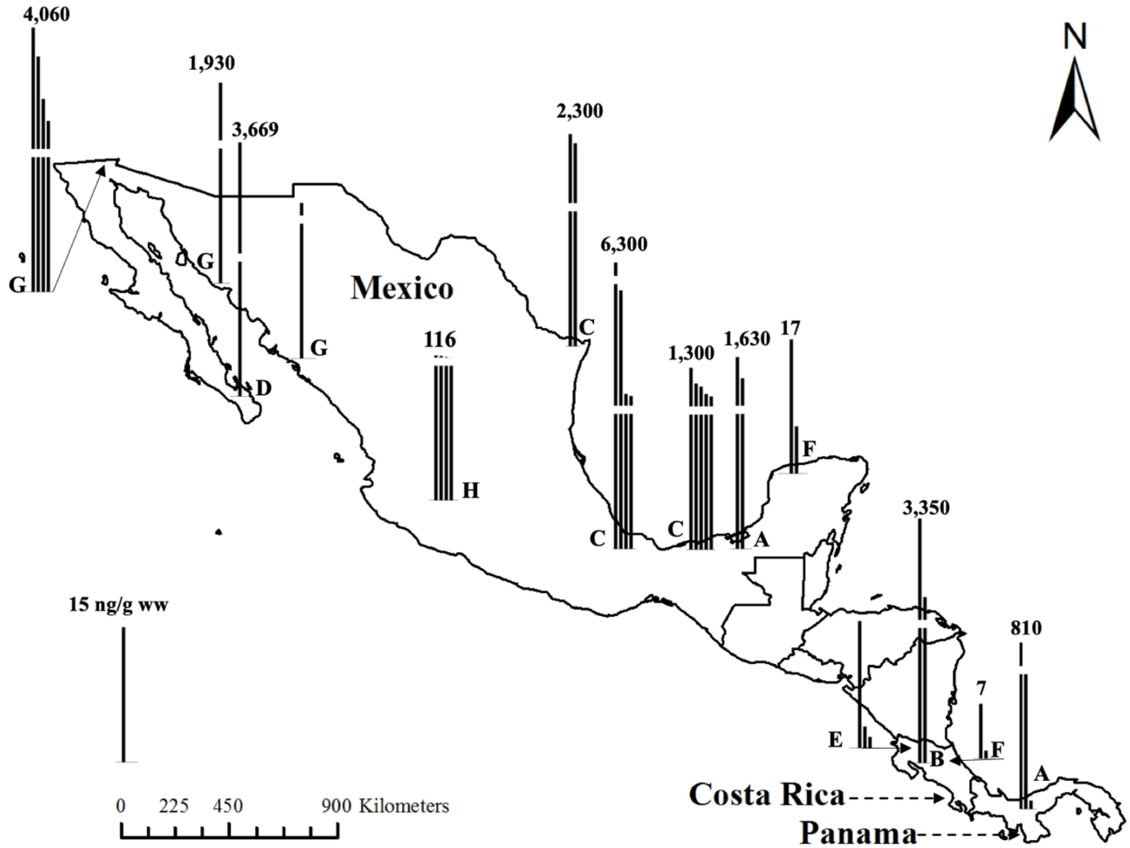



**Figure 25.** DDE[a] concentrations (ng/g ww) in passerine tissues from birds in Mexico and Central America. Axis breaks are from: 1) 0 – 17, 2) 40 – 4,000, and 3) 4,200 – 6,300. Data for passerines from Banash et al., 1992 (A); Fyfe et al., 1990 (B); Henry 1992 (C); Jimenez et al., 2005 (D); Klemens et al., 2003 (E); Maldonado et al., 2017 (F); Mora and Anderson 1991 (G); and Mora 2008 (H).
[a]Includes ΣDDTs and metabolites.

## Central America

*Aquatic birds*

Only four papers have measured POPs in birds from Central America (Banasch et al. 1992, Fyfe et al. 1990, Klemens et al. 2003, Maldonado, Mora, and Sericano 2017) and data is only available for Costa Rica and Panama. Two large scale studies in the early 90's surveyed potential prey species of peregrine falcons for OC body residues across several countries in Latin America (Banasch et al. 1992, Fyfe et al. 1990) in order to determine if dietary exposure to DDT was from contaminated species on wintering grounds. Levels of DDE ranged from 20–4,590 ng/g ww (Table 10). The highest concentration of DDE reported was for a resident black-crowned night heron (*Nycticorax nycticorax*) from northwestern Costa Rica (Figure 25) (Fyfe et al. 1990). Four species of migrants arrived in Costa Rica in the fall with DDE burdens exceeding 1,000 ng/g ww and most showed declining levels of DDE while there (Fyfe et al. 1990). Additionally, Banasch et al. (1992) reported greater mean DDE levels for all species of migrants (520 ng/g ww) compared to residents (30 ng/g ww). Non-DDT pesticides ranged from 10–790 ng/g ww in carcasses (Table 10). Low levels of PCBs were also detected in carcass tissues ranging from 20 ng/ww in a least sandpiper (*Calidris minutilla*) from Costa Rica to 880 ng/g ww in a long-billed dowitcher (*Limnodromus scolopaceus*) from Panama (Banasch et al. 1992, Fyfe et al. 1990).

Levels of DDE $\geq$ 3,000 ng/g ww have been associated with reproductive effects in species of aquatic birds (Blus 1982). Only one black-crowned night heron had DDE levels $\geq$ 3,000 ng/g ww compared to 19% for birds from Mexico and 13% for birds from

South America exceeding this value. This is likely due to the smaller number of available data points for Central America and the greater proportion of wading birds represented. Most wading birds feed on a diversity of aquatic invertebrates (i.e. insects, molluscs, crustaceans) as well as smaller vertebrates such as amphibians and fish (De Graaf, Tilghman, and Anderson 1985, Rodewald 2015). Levels of POPs may not bioaccumulate to the same degree as seen in higher trophic level fish-eating species. The highest DDE concentration for the black-crowned night heron was also still below levels that have resulted in reduced reproductive succes in the same species from the northwestern United States (8,000 ng/g ww) (Henny et al. 1984). Some levels of ΣPCBs were within ranges shown to reduce hatching success in gulls (289 ng/g ww) (Bustnes et al. 2003), but lower for other adverse effects observed in herons (1,000–6,000 ng/g ww) (Champoux et al. 2006).

**Table 10.** POPs levels (min[a]–max ng/g ww) in bird tissues from Central America collected between 1984 – 2013.

| | Tissue | DDE | Non-DDT pesticides[b] | ΣPCBs | ΣPBDEs |
|---|---|---|---|---|---|
| Aquatic birds | | | | | |
| | Carcass | 30–4,590 | 10–790 | 20–880 | – |
| | Liver | 20 | – | – | – |
| | Muscle | 20–80 | – | – | – |
| Nightjars | Carcass | 3.7–145 | 0.7–2.8 | – | – |
| Passerines | | | | | |
| | Carcass | 0.4–3,350 | 0.7–180 | 1.8–360 | 0.7–23 |

[a]Does not include non-detects (ND)
[b]Includes chlordanes, oxychlordane, HCB, HCHs, lindane, heptachlor, heptachlor epoxide, mirex, endosulfans, aldrin, dieldrin, endrin

*Passerines and other insectivores*

DDE levels in songbirds were wide ranging from 0.4–3,350 ng/g ww (Table 10); the highest concentration was for a migrant barn swallow (*Hirundo rustica*) from Costa Rica (Figure 24) (Fyfe et al. 1990). Low levels of non-DDT pesticides (0.7–180 ng/g ww) and ΣPCBs (1.8–360 ng/g ww) were also found (Table 10). Similar to the findings by Fyfe et al. (1990) and Banasch at el. (1992) for aquatic birds, migrant passerines from Costa Rica also had elevated levels of DDE (7.1 ng/g ww) and ΣPCBs (14.1 ng/g ww) compared to residents (DDE: 1.1 ng/g ww; PCBs: 3.1 ng/g ww, respectively); ΣPBDEs burdens, however, were similar (2.8 ng/g ww and 2.1 ng/g ww, respectively) (Maldonado, Mora, and Sericano 2017). Low levels of DDE in songbirds found by Maldonado et al. (2017) were comparable to levels in other songbird species from Costa Rica reported by Klemens et al. (2003). Levels of DDE ranged from 1.4 ng/g ww in a brown-crested flycatcher (*Myiarchus tyrannulus*) to 15 ng/g ww in a yellow-bellied elaenia (*Elaenia flavogaster*). Nightjars, also terrestrial insectivores, from Costa Rica had levels of DDE within ranges reported for passerines (Table 10). No data for birds was found for Belize, Guatemala, Honduras, or Nicaragua. However, a study on morelet's crocodiles (*Crocodylus moreletii*) in Belize found methoxychlor present in 100% of samples followed by *p,p*–DDE found in only 56% of the samples (Sherwin et al. 2016). Higher concentrations of pesticides were observed in crocodiles inhabiting lagoon estuaries compared to river habitats. In Nicaragua, DDTs were measured in sediment samples up to 270 ppb in the coastal lagoon Estero Naranjo-Pasao on the Pacific Coast (Carvalho et al. 1999). Oysters were analyzed for POPs from an estuary in the northern coast of El Salvador near the town of Acajutla;

OC pesticides were below the detection limit and PCBs were also low (Michel and Zengel 1998). This data suggest that avian species are likely also exposed to detectable levels of POPs in other countries in Central America but may have different contaminant profiles compared to birds from Costa Rica and Panama.

Similar to results for aquatic species, levels of DDE reported for songbirds in Central America were less compared to those for Mexico and South America (Figure 25 and 26); however, levels of ΣPCBs were more comparable. Concentrations of POPs were also generally below those known to cause adverse health effects in songbirds (Bishop et al. 1999, Custer et al. 1998, DeLeon et al. 2013, Eng et al. 2014, McCarty and Secord 1999a, Neigh et al. 2007). Present findings, however, limit interpretation of risk to individuals and populations from POPs contamination due to the limited data available for Central America.

**South America**

*Aquatic birds*

Most data for South America has focused on aquatic species of birds, in particular shorebirds and wading birds (charadriiformes, pelicaniformes, suliformes, and procellariiformes) (Baldassin et al. 2012, Baldassin et al. 2016, Banasch et al. 1992, Botero et al. 1996, Cardoso et al. 2014, Cid, Antón, and Caviedes-Vidal 2007, Cifuentes et al. 2003, Colabuono, Taniguchi, and Montone 2012, Cunha et al. 2012, Dias et al. 2013, Dias et al. 2018, Ferreira 2013, Pacheco Ferreira and Dias Wermelinger 2013, Ferreira 2014, Focardi et al. 1996, Fyfe et al. 1990, Muñoz and Becker 1999, Cipro et al. 2013).

Table 11 summarizes POPs data in various tissues of aquatic birds from South America. ΣDDTs ranged from 0.04 ng/g ww in blood from magnificent frigratebirds (*Fregata magnificens*) in French Guiana to 70,700 ng/g ww for resident killdeers (*Charadrius vociferus*) from Peru (Table 11). This was not only the greatest level found in birds for South America, but for all birds from Latin America since 1980. Non-DDT pesticides ranged from 0.1–1,770 ng/g ww, ΣPCBs from 0.1–7,423 ng/g ww, and ΣPBDEs from <0.8–73 ng/g ww (Table 11). Ranges for non-DDT pesticides and ΣPCBs in carcasses were greater compared to those for Mexico and Costa Rica, but were similar for other tissues. The range for ΣPCBs in liver tissue was also greater compared to values in birds from Mexico. Albatrosses from Brazil, peregrine falcons from Argentina, and wading birds from Peru and Ecuador had DDE levels ≥ 3,000 ng/g ww, accounting for approximately 13% of all birds from South America. However, most of the data for these elevated DDE levels was from samples collected in the 1980's and 90's with one recent study in 2007 (Colabuono, Taniguchi, and Montone 2012, Ellis 1985, Fyfe et al. 1990).

Several studies have examined contaminant accumulation in relation to diet and trophic position through the use of stable (Colabuono et al. 2014, Dias et al. 2018, Sebastiano et al. 2016, Sebastiano et al. 2017). In French Guiana, Sebasatiano et al. (2016) found significant differences in POPs levels between adults and nestling of different seabird species. Stable isotopes of $\delta^{15}N$ showed that adults and nestlings were feeding at similar trophic levels, however, significant differences in $\delta^{13}C$ values were found indicating that adults may forage at a different location than where they forage for nestlings or exhibited changes in diet between incubation and chick rearing period

(Sebastiano et al. 2016). In another study, seabirds from Brazil that were more enriched $^{15}N$ had lower concentrations of POPs leading authors to postulate that $\delta^{15}N$ values may actually reflect oceanic foraging habits rather than trophic relationships (Colabuono et al. 2014).

A handful of studies have looked at contaminant residues in penguins from Argentina, Brazil, Chile, Peru, and Uruguay (Adkesson et al. 2018b, Baldassin et al. 2012, Baldassin et al. 2016, Smith et al. 2008). Smith et al. (2008) found no detectable levels of OC pesticides or ΣPCBs in blood plasma of humboldt penguins (*Spheniscus humboldti*) in Peru; however, in a more recent analysis low levels of ΣDDTs (10 ng/g ww), ΣPCBs (20 ng/g ww), and ΣPBDEs (3.81 ng/g ww) were detected by Adkesson et al. (2018). Rockhopper penguins (*Eudyptes chrysocomes*) in Argentina and magellanic penguins (*Spheniscus magellanicus*) from Brazil, Chile, and Uruguay all had low to moderate detectable levels of ΣDDTs (2.3–275 ng/g ww), ΣPCBs (16–1,983 ng/g ww), and ΣPBDEs (3.9–9.5 ng/g ww) (Baldassin et al. 2012, Baldassin et al. 2016, Karesh et al. 1999).

**Table 11.** POPs levels (min[a]–max ng/g ww) in bird tissues from South America collected between 1979 – 2014.

| | Tissue | ΣDDTs[b] | Non-DDT pesticides[c] | ΣPCBs | ΣPBDEs |
|---|---|---|---|---|---|
| Aquatic birds | | | | | |
| | Adipose | 153–4,795 | 0.1–1,770 | 667–7,423 | 73 |
| | Blood | 0.04 | – | – | – |
| | Carcass | <10–70,700 | 20–1,100 | 10–4,520 | – |
| | Eggs | 0.6–1,800 | 0.1–227 | 48–840 | – |
| | Liver | 2.6–1,127 | <0.2–190 | 0.1–6,519 | <0.8–9.5 |
| | Muscle | 8.7–430 | <1.0–80 | 10–1,040 | – |
| Granivores[d] | Carcass | <10 | – | 40 | – |
| Passerines | | | | | |
| | Carcass | 1.9–17,600 | 6.3–1,750 | 10–870 | – |
| | Eggs | 6.6–189 | – | 29–65 | – |
| | Muscle | 10 | 10 | 30–160 | |
| Raptors | | | | | |
| | Eggs | <0.01–7,500 | <0.01–950 | <0.01–1,050 | – |
| | Feathers | 870–1,410 | 10–2990 | – | – |
| | Muscle | 10–2,680 | 10–390 | 10–2,680 | – |

[a]Does not include non-detects (ND)
[b]Includes values of ΣDDTs and metabolites (DDE, DDD)
[c]Includes chlordanes, oxychlordane, HCB, HCHs, lindane, heptachlor, heptachlor epoxide, mirex, endosulfans, aldrin, dieldrin, endrin
[d]Birds from order Columbiformes

*Passerines, granivores, and nectivores*

Passerines are not as well represented in South America as in Mexico (Figure 26), however a few studies have measured POPs in songbirds (Banasch et al. 1992, Capparella et al. 2003, Cid, Antón, and Caviedes-Vidal 2007, Fyfe et al. 1990, Nadal et al. 1987). Concentrations of ΣDDTs ranged from 1.9–17,600, non-DDTs ranged from 6.3–1,750, and ΣPCBs ranged from 10–870 ng/g ww (Table 11). The highest levels of DDE were found in barn swallows from Ecuador (17,600 ng/g ww) and Peru (1,490 ng/g ww; Figure 26) (Fyfe et al. 1990). However, Nadal et al. (1987) found much lower levels of DDE (4–

181 ng/g ww) in passerine eggs from the province of Calca in Peru, an area with mixed agriculture and forest remnants. DDE ranges for other passerines from Argentina, Guyana, Peru, Suriname and Venezuela were much lower, ranging from 3–77 ng/g ww (Banasch et al. 1992, Capparella et al. 2003, Cid, Antón, and Caviedes-Vidal 2007, Fyfe et al. 1990). Low levels of ΣDDTs were also measured in oasis hummingbirds (*Rhodopis vesper*; 35–96 g/g ww) from Peru and eared doves (*Zenaida auriculate*; <10 ng/g ww) from Venezuela (Banasch et al. 1992, Nadal et al. 1987). In general, 89% of reported values for DDE and ΣPCBs were < 200 ng/g ww; only one value for non-DDTs exceeded 1,000 ng/g ww. Levels were generally well below concentrations observed for adverse health effects in songbirds (Bishop et al. 1999, Custer et al. 1998, DeLeon et al. 2013, Eng et al. 2014, Eng et al. 2018, McCarty and Secord 1999a, Neigh et al. 2007).

*Raptors*

Since 1980, only three studies have measured chlorinated contaminants for various species of raptors from Argentina, Ecuador, and Suriname (Ellis 1985, Fyfe et al. 1990, Jenny et al. 1983, Martínez-López et al. 2015). ΣDDTs ranged from <0.01–7,500 ng/g ww with the highest levels in peregrine falcon eggs from Argentina which had returned to pre-DDT thickness (Ellis 1985). Non-DDT pesticides ranged from <0.01–2,990 ng/g ww, and PCBs ranged from <0.01–1,050 ng/g ww (Table 11). Jenny et al. (1983) measured low levels chlorinated pesticides in peregrine eggs from Ecuador and found no evidence of eggshell thinning at DDE concentration of 1,045 ng/g ww which is in agreement with previous studies where reproductive effects are observed at ≥ 3,000 ng/g ww in peregrines

(Newton 1988). In Suriname, Fyfe et al. (1990) found moderate levels of DDE and ΣPCBs in a resident bat falcon (DDE and ΣPCBs: 2,680 ng/g ww). Snail kites (*Rostrhamus sociabilis*) from the same area however had substantially lower body residues of DDE (10 ng/g ww) and ΣPCBs (10 ng/g ww), likely due to their diets of invertebrates (Fyfe et al. 1990). The most recent publication to examine contaminant exposure in raptors measured POPs in feathers of three species of scavengers from Argentinean Patagonia (Martínez-López et al. 2015). Turkey vultures (*Cathartes aura*) had the highest concentrations of ΣDDTs in feathers (1,410 ng/g dw), while American black vultures (*Coragypus atratus*) and Southern crested caracaras (*Polyborus plancus*) were comparable (880 ng/g dw and 870 ng/g dw, respectively). Interestingly, *p,p*–DDT was found in higher concentrations compared to *p,p*–DDE for all species. In addition, ΣHCHs, heptachlor epoxide, and aldrin exceeded levels observed for DDE. The authors postulate that this may be due feathers accumulating more polar and less persistent OCs from the bloodstream during periods of feather growth (Martínez-López et al. 2015). With the exception of peregrine falcon eggs from Argentina, levels of DDE and ΣPCBs were generally below values known to impair reproductive success or result in embryo lethality in other raptors (Fernie, Bortolotti, and Smits 2003, Fernie et al. 2000, Fernie et al. 2001, Helander et al. 2002, Wiemeyer, Bunck, and Stafford 1993).


**Figure 26.** DDE[a] concentrations (ng/g ww) in passerine tissues from birds in South America. Axis breaks are from: 1) 0 – 130, 2) 500 – 2230, and 3) 17,000 – 17,600. Data for South America from Banasch et al., 1992 (A); Capparella et al., 2003 (B); Cid et al., 2007 (C); Fyfe et al., 1990 (D); and Nadal et al., 1987 (E).
[a]Includes ΣDDTs and metabolites.

*Temporal trends of POPs in birds for Latin America*

Combined data for all birds showed a significant decrease in the levels of DDE in tissues (carcass, muscle, liver, fat, and eggs) since the 1960's ($p$ = <0.0001; $r^2 = 0.10$; Figure 27). When birds were analyzed based on diets, insectivorous passerines ($p = 0.002$; $r^2 = 0.20$; Figure 30), piscivorous waterbirds ($p = 0.0004$; $r^2 = 0.12$; Figure 29), and raptors ($p = 0.0002$; $r^2 = 0.36$; Figure 30) all showed significant decreasing levels of DDE; however, a significant and slightly increasing trend for wading birds that feed primarily on aquatic invertebrates was observed ($p = 0.006$; $r^2 = 0.14$; Figure 31). For most models of DDE trends, $r^2$ values were low, indicating generally weak correlations. A significant negative correlation was also found for ΣPCBs when looking at all birds, however, the $r^2$ value was very low indicating a very weak decreasing trend ($p = 0.001$; $r^2 = 0.07$; Figure 32). In addition, there is a large variety in the types of tissues analyzed and not all tissues are represented equally for different classes of birds. Contaminant burdens in tissues can vary greatly between species making comparisons difficult (Voorspoels et al. 2006). Overall, most studies reported decreases in the level of pollutants when data was available for comparison with earlier studies. The general decreasing levels for DDE and PCBs are consistent with decreasing trends of POPs in wildlife and the environment not only in South America (Barra et al. 2006), but also for birds in Europe (Fasola et al. 1987, Fimreite, Brevik, and Torp 1982, Helgason et al. 2008, Law et al. 2006, Newton, Haas, and Freestone 1990), Canada and the U.S. (Elliott, Norstrom, and Keith 1988, Henny, Yates, and Seegar 2009, Mora et al. 2016). Although significant decreases in the level of DDE and PCBs were observed for most birds, the limitations of the data set, including small sample sizes along with confounding biological and ecological variables, does not allow for the careful and robust time trend data analysis necessary to avoid bias or unreasonable conclusions in the present chapter.

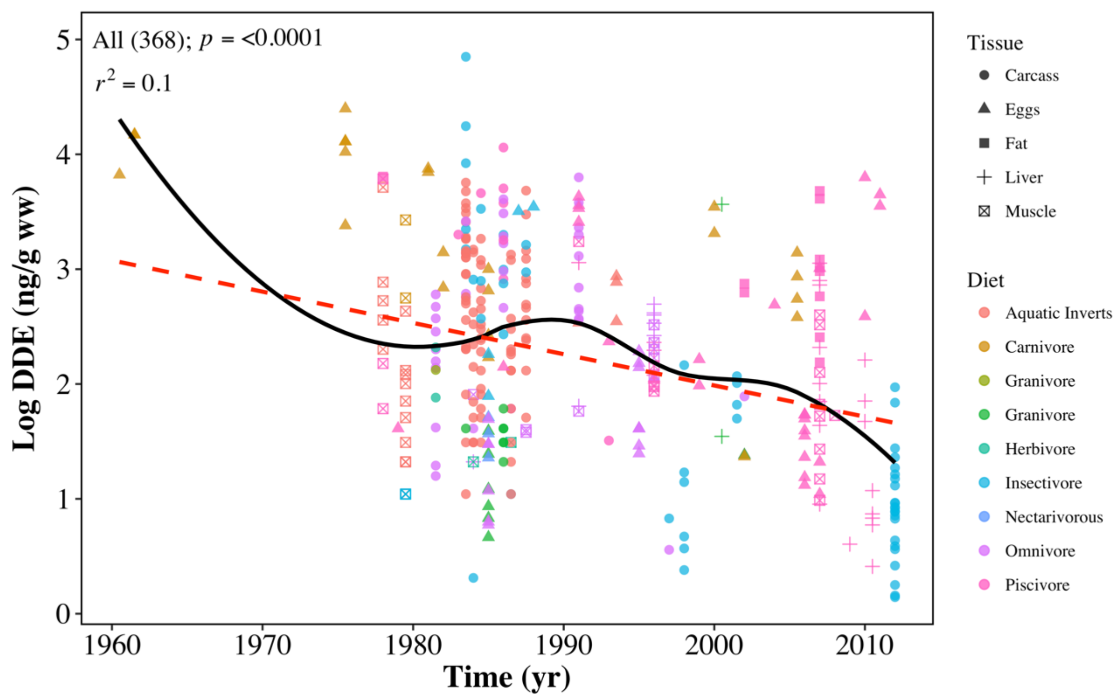



**Figure 27.** Log DDE (ng/g ww) in birds, bird tissues, and eggs vs year samples were collected in studies from Latin America. The red dashed line indicates the regression line and the solid black line represents the locally weighted scatterplot smoother (LOESS) line. Number in parentheses is sample size (n).

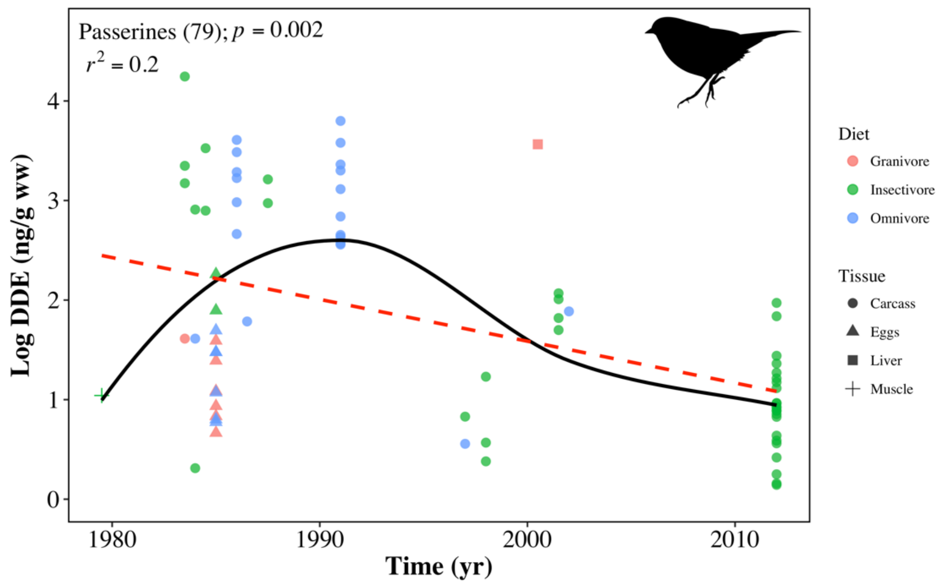



**Figure 28.** Log DDE (ng/g ww) in birds, bird tissues, and eggs for passerines vs year samples were collected from studies conducted in Latin America. The red dashed line indicates the regression line and the solid black line represents the locally weighted scatterplot smoother (LOESS) line. Number in parentheses is sample size (n).

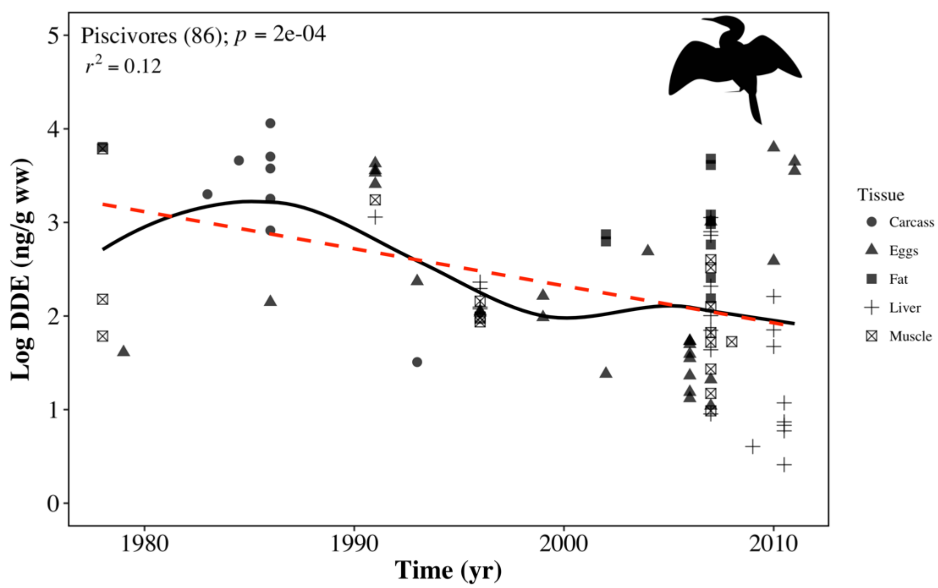



**Figure 29.** Log DDE (ng/g ww) in birds, tissues, and eggs for piscivorous species vs year samples were collected in studies from Latin America. The red dashed line indicates the regression line and the solid black line represents the locally weighted scatterplot smoother (LOESS) line. Number in parentheses is sample size (n).

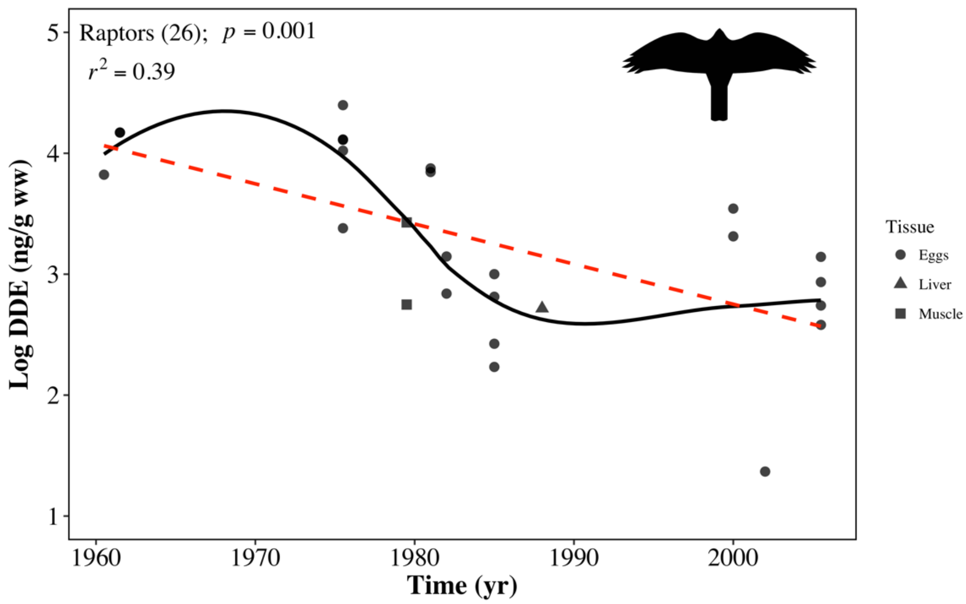



**Figure 30.** Log DDE (ng/g ww) in eggs and muscle tissue for raptors vs year samples were collected in from studies conducted in Latin America. The red dashed line indicates the regression line and the solid black line represents the locally weighted scatterplot smoother (LOESS) line. Number in parentheses is sample size (n).

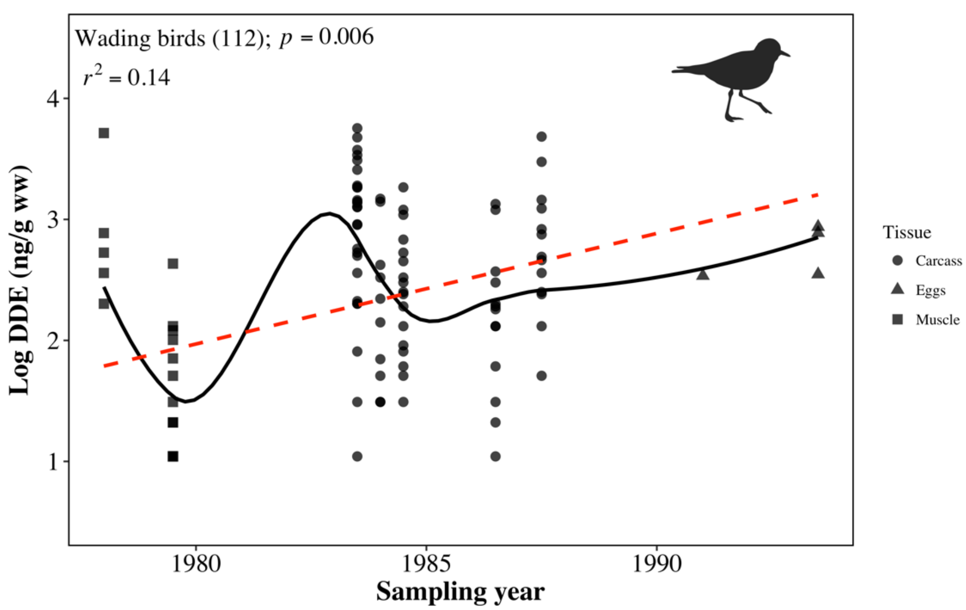



**Figure 31.** Log DDE (ng/g ww) in birds, tissues, and eggs for wading birds vs year samples were collected in studies for Latin America. Number in parentheses is sample size (n).

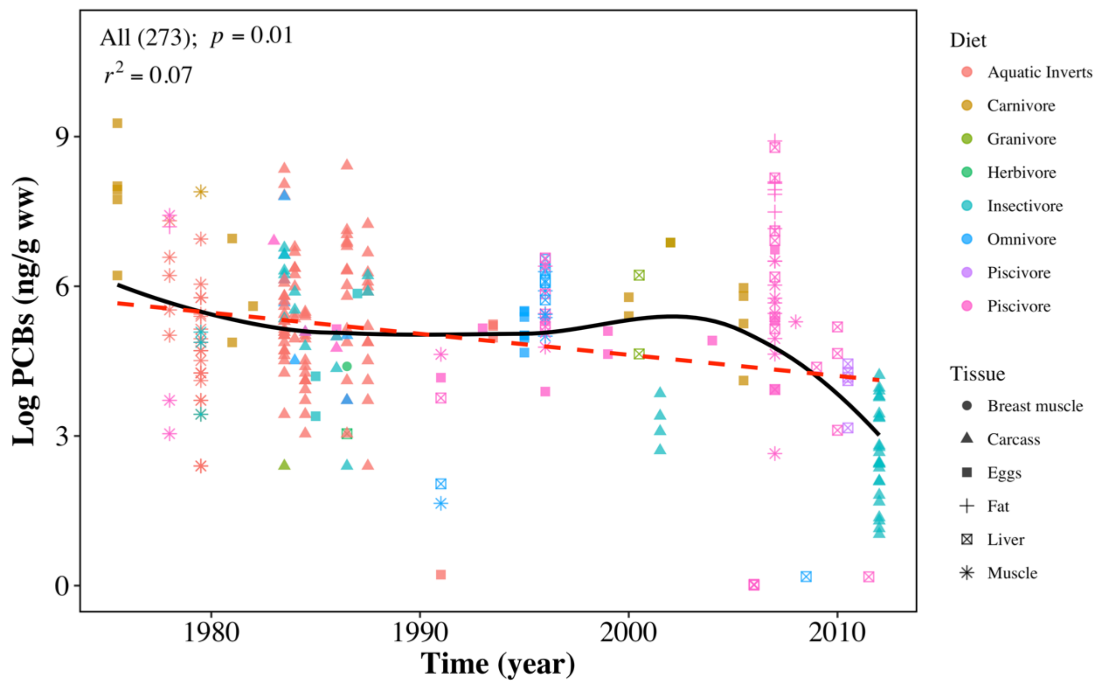



**Figure 32.** Log ΣPCBs (ng/g ww) in birds, bird tissues, and eggs vs year samples were collected from studies conducted in Latin America. The red dashed line indicates the regression line and the solid black line represents the locally weighted scatterplot smoother (LOESS) line. Number in parentheses is sample size (n).

*Accumulation Studies: What do we know about contaminant accumulation on wintering grounds in Latin America?*

Several studies have been conducted over the years to address the long-held theory that migratory birds are exposed to and accumulate organochlorine contaminants on their wintering grounds in Latin America due to the later use of DDT in agriculture and for malarial control. Earlier studies by Henny et al. (1982) and Botero et al. (1996) showed peregrine falcon and blue-winged teal migrants returning to their breeding grounds from Latin America with elevated levels of pesticides. Exposure and accumulation of DDE was also observed for migrants wintering in Peru and Ecuador with resident species having elevated levels compared to migrants suggesting a local contaminant source (Fyfe et al. 1990). In addition, resident species in these areas showed elevated levels of DDE suggesting wintering grounds as a contaminant source. However, in contrast to these findings other studies have not supported a general theory of high pesticide exposure and accumulation on wintering grounds in Latin America. In the studies mentioned above by White et. al (1985), Mora (1987, 2008), and Maldonado et al. (2017) no clear pattern of organochlorine pesticide accumulation in migratory birds was observed. In addition, the studies by Banasch (1992), Fyfe (1990), Capparella (2003), and Klemens (2003) demonstrate generally low levels of contaminants in resident and migratory species from certain parts of Latin America. As there are significant data gaps on POPs levels for much of Latin America, it is still possible that birds may be exposed to and acquire pesticides in certain hot spots and exposure in the neotropics is localized rather than a generalized trend. Additionally, several recent studies have found elevated levels of the parent compound of DDT in air samples of Mexico (Alegria et al. 2008), eggs and biota from Brazil (Cunha et al. 2012, Liebezeit et al. 2011), suggesting potential continued illegal use. These reports of recent DDT input into the environment are cause for concern and warrant further investigation.

## Knowledge gaps and priorities for future research

Most migratory birds spend their annual cycle either migrating or on their wintering grounds, however despite this, limited baseline data on POPs exists for birds in most of Latin America. As mentioned above, most countries in Central America have no information for migrant or resident bird species despite the fact that many species of North American songbirds winter in these areas. Studies have shown that different habitats can result in varying pathways of exposure to environmental pollutants in organisms. Currently the regional distribution of the data is uneven, with studies heavily centered in coastal environments and on aquatic species. Future research should focus on providing not only baseline levels for terrestrial habitats and species, but understanding how exposure to contaminants in birds may be different in tropical ecosystems. The vast majority of the published data corresponds to chlorinated pesticides and to a much lesser extent PCBs. Data for PBDEs and PCDD/Fs is scarce with only 9 papers reporting on PBDEs and only 2 for PCDD/Fs. Although these compounds have generally been found at low levels, some dioxins and furans are known to be highly toxic; without much information on the current levels in birds it is difficult to determine what effects, if any, they have on individuals and populations in Latin America. In order to facilitate understanding of global levels of POPs, future studies should be concerned with the monitoring of a wider range of persistent organic pollutants and include analyses of PBDEs and PCCD/Fs when examining POPs in birds for Latin America. Such data would not only facilitate understanding of the current state of POPs at the global scale but allow for more robust assessments and comparisons to other regions. Additionally, analyses of museum specimens may allow for more comprehensive time trend data analyses. Most studies included toxic effects benchmarks of individual POPs, however only a few studies examined links to population level effects. An increasing number of papers have focused on linking contaminant profiles to differences in trophic ecology and habitat use through use of stable isotopes. Continued research in this direction will be useful in elucidating sources of exposure and understanding accumulation patterns of POPs in birds.

## Conclusion

In summary, it has been shown from the literature that DDE is the most frequently detected and reported organochlorine pesticide; in general, it also contributes to the greatest contaminant body burdens. Large variations in the ranges and types of non-DDT chlorinated pesticides were reported, but usually at lower levels than DDE and detected less frequently. Less data was available for PCBs, however when measured, was generally the second most abundant contaminant reported. Congener patterns of PCBs tended to be similar across locations and species; congeners of the penta-, hexa-, and hepta-homologues were the most predominant. Of the available data, PCDD/Fs were considerably low (0.1–21 ng/g ww) and ΣPBDE levels (0.2–73 ng/g ww) were also lower compared to concentrations reported for piscivorous and terrestrial-feeding birds in North America, Europe, and Asia (Chen and Hale 2010). In general, POPs concentrations have been declining over the last few decades.

In most of the present studies, levels of DDE were near or below those known to cause adverse health effects in birds. Only two out of the thirteen papers that reported levels ≥ 2,000 ng/g ww were from samples collected in the 2000's, the remainder were from the 1960's to 1990's. For PCBs only four studies had concentrations above ≥ 2,000 ng/g ww, generally below harmful levels, and only one of the studies occurred after

2000. However, additive or synergistic effects of overall POPs burdens are less well known.

The geographic distribution of POPs data is uneven with the vast majority of studies conducted in Mexico and Brazil, with fewer data available for other South American countries and scarce information for most of Central America. Studies have also been focused primarily on aquatic species and coastal habitats with much less information available for terrestrial ecosystems and species.

# CHAPTER V

# CONCLUSIONS

The aim of the present study was to investigate accumulation features of persistent organic pollutants (POPs) in neotropical songbirds during migration. Despite the decades long ban on legacy contaminants such as DDT and PCBs, detectable levels of POPs continue to be measured in migratory songbirds; however, the sources of contaminants are less well understood. Migratory songbirds spend a large portion of their annual cycle either migrating or on their wintering grounds in Latin America and a common hypothesis is that exposure to contaminants may occur in Latin America. In order to address this question, I measured POPs in migrant and resident songbirds in Texas during the fall and spring migration, and while birds were on their wintering grounds in Mexico and Costa Rica. I found low, but detectable levels of persistent organic pollutants in all songbirds and of all the POPs analyzed, *p,p'*–DDE, ΣPCBs, and ΣPBDEs were the most predominant contaminants. OC pesticides and PCBs accounted for the greatest proportion contaminant burdens for both migrants and residents. PCB homologue and PBDE congener profiles were also similar between migrants and residents and between locations indicating similar sources of Aroclor and PBDE mixtures. No significant accumulation of contaminants was observed in migratory songbirds during their migration or while on wintering grounds. Furthermore, the greatest differences in contaminant levels were amongst the resident species. Resident wrens from Texas had significantly greater concentrations of DDE compared to resident species from Yucatán and Costa Rica suggesting low exposure and acquisition of contaminants for migrants in these wintering areas. Levels of POPs were below those known to cause any adverse health effects on songbirds, however, one male Carolina wren from Texas exceeded values known to cause reproductive impairments in ospreys. These results represent the first reported values for PBDE in songbirds from Latin America.

In addition to migration, several factors can affect the accumulation of contaminants in birds, including habitat use and feeding ecology. I used stable isotopes of carbon, nitrogen, and deuterium to assess which ecological factors best explained contaminant accumulation in birds. In general, I found only minor differences (≤ 2 ‰) in mean values of $\delta^{13}C$ and $\delta^{15}N$ between migrants and residents suggesting that birds were feeding at similar trophic levels. Results from linear regression models found that stable isotopes of $\delta^{15}N$, as a measure of trophic level, was only useful in explaining contaminant concentrations of ΣPCBs for resident species from Texas and ΣPBDEs for all songbirds from Yucatán. However, a significant positive correlation was found for $\delta^{13}C$ and POPs (DDE and ΣPBDEs) concentrations in resident birds from Texas. Wrens sampled in College Station (Texas) were more enriched in $^{13}C$ and more contaminated compared to wrens from Hearne and Lake Jackson (Texas), suggesting location and proximity to more residential environments as a source of POPs exposure. No latitudinal gradient of contaminant concentrations was observed DDE or ΣPCBs for migratory songbirds through use of feather $\delta^{2}H$ values; however, a weak negative latitudinal gradient for ΣPBDEs was observed. In general, stable isotopes were poor predictors of contaminant burdens which may be attributed to insufficient dietary differences among individuals in order to observe significant relationships between POPs levels and $\delta^{15}N$ values. In addition, contaminants are accumulated throughout a birds lifetime and rapid changes in diet and location during migration may not relate well to contaminant levels.

Although birds represent one of the most well studied taxa in regards to POPs contamination, most studies have been conducted in temperate regions in the Northern Hemisphere. In addition, nearly three fourths of North American birds migrate to the neotropics during the winter. Despite this, the data on POPs levels in birds from Latin America is limited. In order to understand the current state of POPs contamination in birds from Latin America, I conducted a systematic analysis of the available literature from 1980 – 2018. Results from the review showed that data on POPs for birds is highly concentrated towards studies conducted in Mexico and Brazil, and in coastal habitats and aquatic species. Data is lacking for much of Central America and terrestrial ecosystems. The mostly frequently reported contaminant was DDTs, primarily in the form of the metabolite DDE, followed by ΣPCBs. Scant data is available for PBDEs or PCDD/Fs. Time trend analyses indicate that, in general, contaminant levels of DDE and ΣPCBs across different orders of birds are decreasing, which is consistent with studies conducted in temperate regions. Furthermore, levels of DDE and ΣPCBs from the most recent studies were generally below those known to cause adverse health effects in birds. Data for POPs in birds from Latin America is limited in not only the number of studies, but for species inhabiting terrestrial ecosystems, and the diversity of POPs that have been researched. In order to facilitate understanding of global levels of POPs pollution in birds, future studies should include a wider range of POPs analyzed, inclusion of terrestrial ecosystems and species, and in regions where little or no data is currently available.

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# APPENDIX A

## SUPPORTING INFORMATION FOR CHAPTER II

**Table A-1** Comparison of mean concentrations of persistent organic pollutants (POPs) for passerines between the present study with others (ng/g wet weight)

| Migratory Status | Location | N | Season | DDE | ΣPCBs | ΣPBDEs | Reference |
|---|---|---|---|---|---|---|---|
| Migrant | Texas, USA | 30 | Fall | 18.2 | 20.9 | 7.1 | Present study |
| Residents | Texas, USA | 13 | Fall | 39.0 | 15.5 | 11.2 | Present study |
| Migrant | Texas, USA | 35 | Spring | 26.2 | 24.9 | 5.8 | Present study |
| Residents | Texas, USA | 18 | Spring | 28.3 | 12.4 | 10.1 | Present study |
| Migrant | Yucatán, MX | 18 | Winter | 19.5 | 27.2 | 4.3 | Present study |
| Residents | Yucatán, MX | 11 | Winter | 6.2 | 10.0 | 1.9 | Present study |
| Migrant | Costa Rica | 10 | Winter | 7.5 | 14.1 | 2.8 | Present study |
| Residents | Costa Rica | 17 | Winter | 1.1 | 3.1 | 2.1 | Present study |
| Migrant | Southeastern, MX | – | Winter | ND[a,b] | 7.6 | – | Herrerra-Herrerra et al. 2015 |
| Resident | Southeastern, MX | – | Winter | 14.5[b] | 9.1 | – | Herrerra-Herrerra et al. 2015 |
| Migrant | Texas, USA | 4 | – | 8.31 | 114 | – | Mora et al. 2012 |
| Migrant | Texas, USA | 4 | – | 8511 | 698 | 258 | Mora et al. 2012 |
| Migrant | Texas, USA | 16 | – | 6182 | 128 | 196 | Mora et al. 2012 |
| Migrant | Texas, USA | 8 | – | 642 | 193 | 25 | Mora et al. 2012 |
| Migrant | Texas, USA | 4 | – | 987 | 175 | 18 | Mora et al. 2012 |
| Migrant | Michoacán, MX | 10 | Fall | 49.0 | 49.0 | – | Mora 2008 |
| Resident | Michoacán, MX | 11 | Fall | 65.0 | 21.0 | – | Mora 2008 |
| Migrant | Michoacán, MX | 11 | Spring | 101.0 | 101.0 | – | Mora 2008 |
| Resident | Michoacán, MX | 7 | Spring | 116.0 | 29.0 | – | Mora 2008 |

[a]ND = not detected

[b]*p,p*−DDT is the measured DDT isomer

[c]*p,p*−DDD is the measured DDT isomer

**Table A-1 (*continued*)**

| Migratory Status | Location | N | Season | DDE | ΣPCBs | ΣPBDEs | Reference |
|---|---|---|---|---|---|---|---|
| Resident | Costa Rica | 5 | Summer | 1.4 | — | — | Klemens et al. 2003 |
| Resident | Costa Rica | 4 | Summer | 16 | — | — | Klemens et al. 2003 |
| Resident | Costa Rica | 5 | Summer | 2.7 | — | — | Klemens et al. 2003 |
| Resident | Argentina | 1 | Winter | 1.9[c] | — | — | Capparella et al. 2003 |
| Resident | Suriname | 2 | Fall | 10 | 7.5 | — | Fyfe et al. 1990 |
| Migrant | Peru | 9 | Fall | 2230 | 550 | — | Fyfe et al. 1990 |
| Migrant | Peru | 9 | Spring | 1760 | 870 | — | Fyfe et al. 1990 |
| Migrant | Ecuador | 10 | Fall | 1490 | 210 | — | Fyfe et al. 1990 |
| Resident | Ecuador | 10 | Spring | 40 | 10 | — | Fyfe et al. 1990 |
| Migrant | Costa Rica | 10 | Fall | 3350 | 160 | — | Fyfe et al. 1990 |
| Migrant | Costa Rica | 10 | Spring | 790 | 120 | — | Fyfe et al. 1990 |

[a]ND = not detected

[b]*p,p*−DDT is the measured DDT isomer

[c]*p,p*−DDD is the measured DDT isomer

# APPENDIX B

## SUPPORTING INFORMATION FOR CHAPTER III

**Table B - 1** Species abbreviation codes for neotropical songbirds.

| Abbreviation code | Common name |
|---|---|
| AMRE | American redstart |
| BAWW | Black-and-white warbler |
| BEWR | Bewick's wren |
| CARW | Carolina wren |
| CAWA | Canada warbler |
| COYE | Common yellowthroat |
| DCFLY | Dusky-capped flycatcher |
| HOWA | Hooded warbler |
| HOWR | House wren |
| LEFL | Least flycatcher |
| MAVI | Mangrove vireo |
| MAWA | Magnolia warbler |
| MOWA | Mourning warbler |
| NAWA | Nashville warbler |
| NOPA | Northern parula |
| OCYE | Olive-crowned yellowthroat |
| OVEN | Ovenbird |
| RAWW | Rufous-and-white wren |
| RCWA | Rufous-crowned warbler |
| TEWA | Tennessee warbler |
| WIWA | Wilson's warbler |
| YEWA | Yellow warbler |
| YOFL | Yellow-olive flycatcher |
| YRWA | Yellow-rumped warbler |
| YUFL | Yucatán flycatcher |

**Table B - 2** Parameter estimates for linear regression models used to assess the relationship between persistent organic pollutants and stable isotopes of deuterium in feathers for migrant and resident passerines. Significant P-values are bolded.

| | | | Slope (SE) | *t*-value | *P* |
|---|---|---|---|---|---|
| DDE & $\delta^2H_f$ | Migrants | (intercept) | 1.68 (0.12) | 13.6 | **<.0001** |
| | | $\delta^2H_f$ | –0.0004 (0.001) | –0.33 | 0.75 |
| | Residents | (intercept) | 2.44 (0.16) | 15.12 | **<.0001** |
| | | $\delta^2H_f$ | .03 (0.004) | 6.74 | **<.0001** |
| PCBs & $\delta^2H_f$ | Migrants | (intercept) | 1.73 (0.11) | 16.12 | **<.0001** |
| | | $\delta^2H_f$ | –0.001 (0.001) | –0.69 | 0.49 |
| | Residents | (intercept) | 1.73 (0.13) | 13.48 | **<.0001** |
| | | $\delta^2H_f$ | 0.009 (0.003) | 2.69 | **0.01** |
| PBDEs & $\delta^2H_f$ | Migrants | (intercept) | 0.88 (0.11) | 8.29 | **<.0001** |
| | | $\delta^2H_f$ | –0.003 (0.001) | –3.11 | **0.003** |
| | Residents | (intercept) | 1.53 (0.16) | 9.85 | **<.0001** |
| | | $\delta^2H_f$ | 0.01 (0.004) | 2.79 | **0.01** |

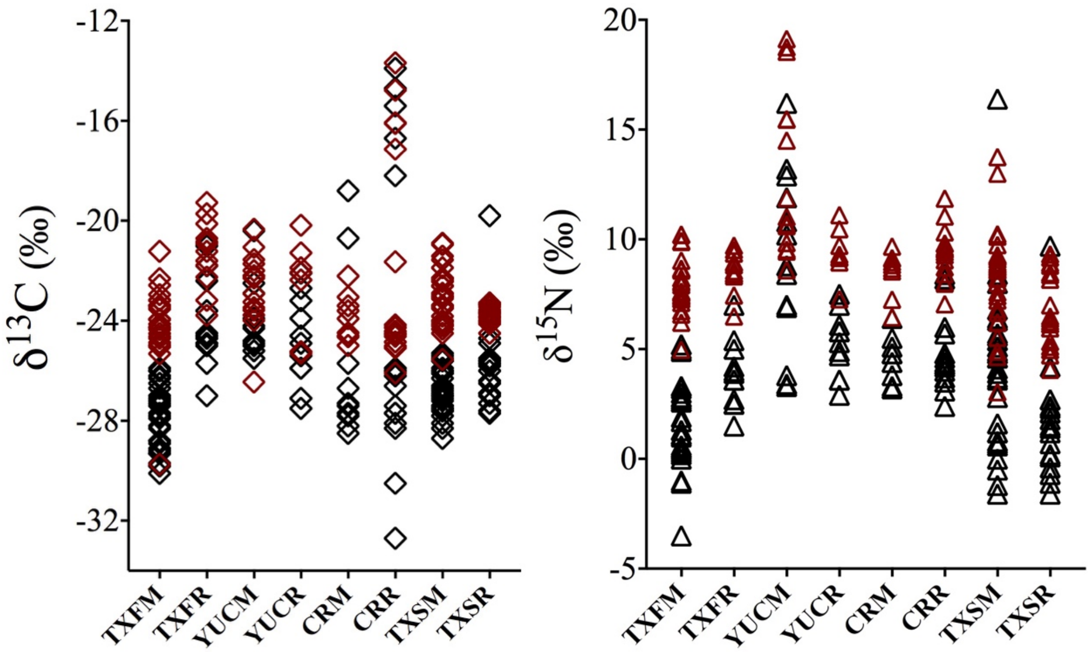


**Figure B - 1** $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) values from liver tissues (red) and stomach contents (black) of migrant and resident songbirds collected in Texas, Yucatán and Costa Rica. TXFM = Texas fall migrant, *TXFR* = Texas fall resident, *YUCM* = Yucatán migrant, *YUCR* = Yucatán resident, *CRM* = Costa Rica migrant, *CRR* = Costa Rica resident, *TXSM* = Texas spring migrant, and *TXSR* = Texas spring resident.

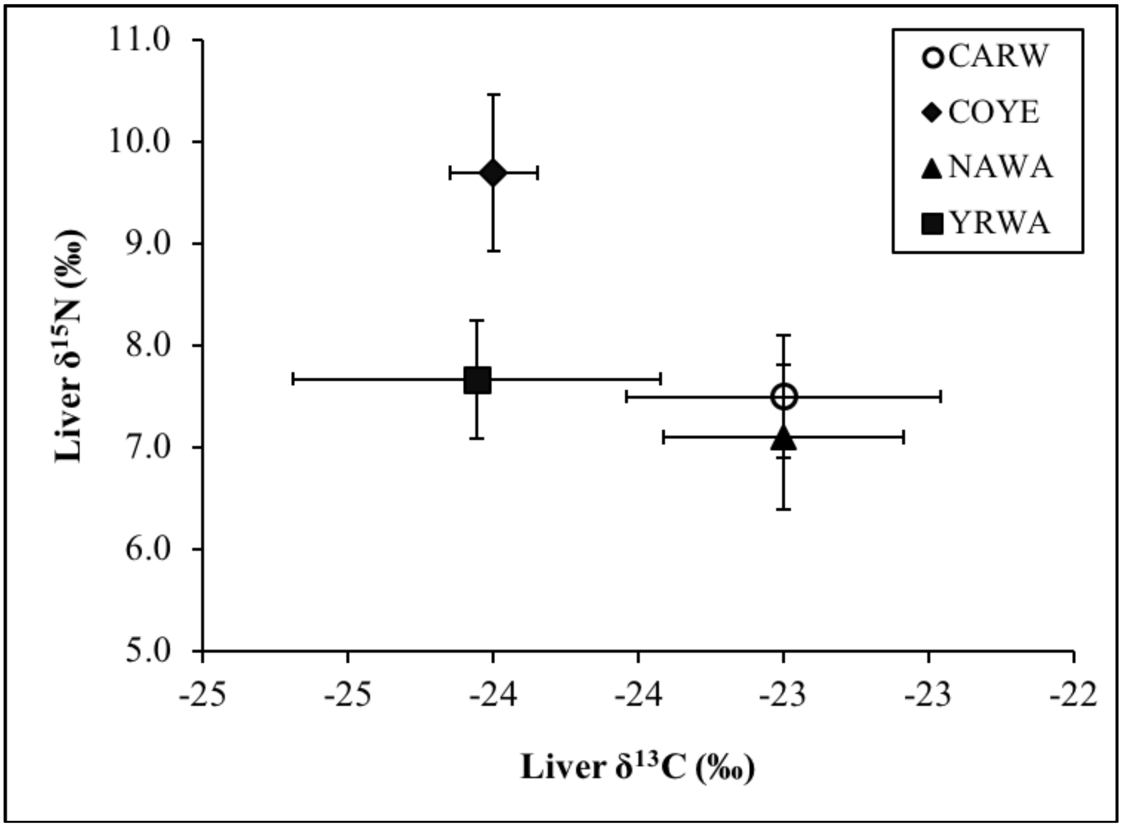


**Figure B - 2** Biplot of liver tissue $\delta^{13}C$ vs $\delta^{15}N$ (‰) for migrant and resident species of songbirds collected from Texas from 2011-2013. Error bars represent 2*SE of the mean. *CARW* = Carolina wren, *COYE* = common yellowthroat, *NAWA* = Nashville warbler, *YRWA* = yellow-rumped warbler.

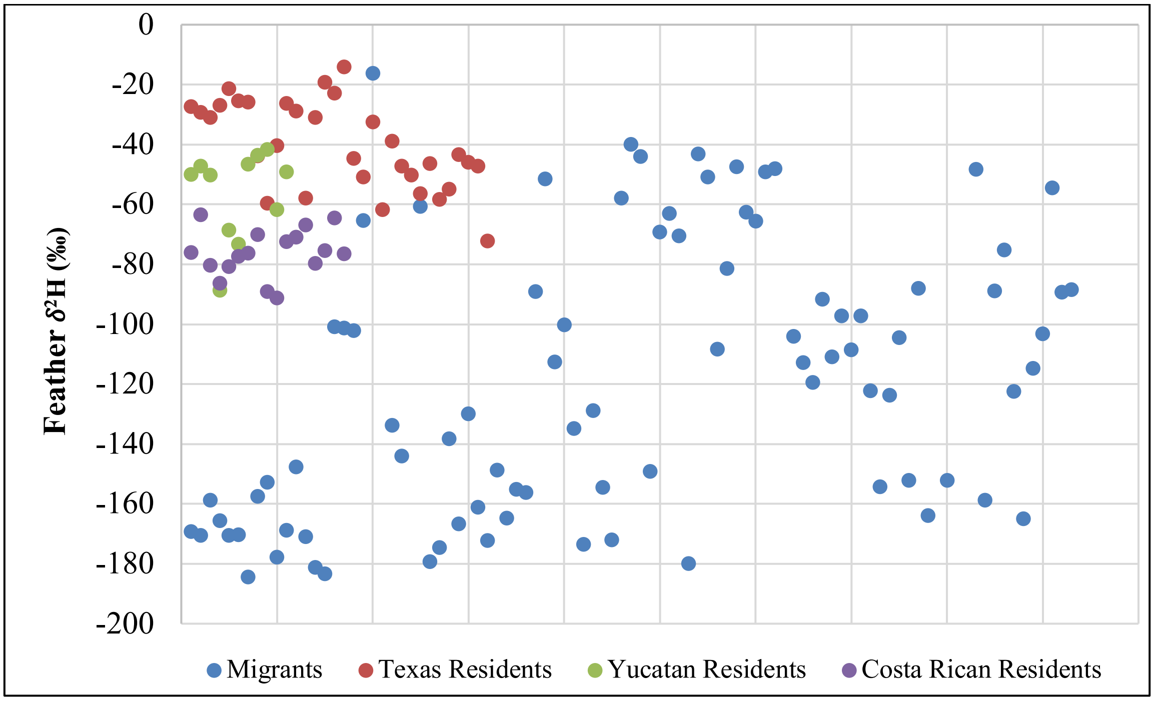


**Figure B - 3** Feather deuterium values for migrant and resident songbirds collected in Texas, Mexico (Yucatán), and Costa Rica from 2011–2013.

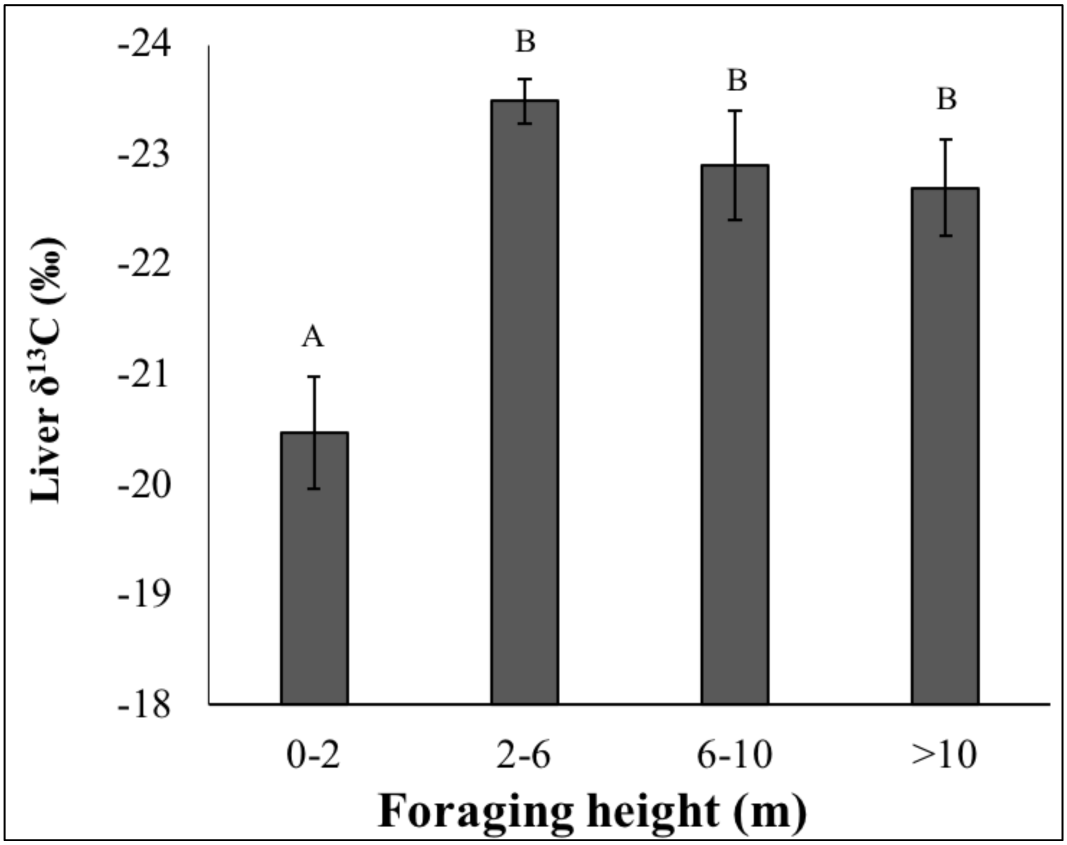


**Figure B - 4** Bar plot of means for liver carbon isotope values for songbirds based on foraging heights (m). Error bars indicate standard error of the mean. Bars sharing the same letter are not significantly different according to Tukey's HSD test.

APPENDIX C

SUPPORTING INFORMATION FOR CHAPTER IV

**Table C - 1.** List of databases searched.

| |
|---|
| AGRICOLA |
| AGRIS |
| Biological and Agricultural Index Plus |
| BioOne |
| BIOSIS Previews |
| CAB Abstracts |
| Environment Complete |
| Google Scholar |
| SCOPUS |
| Web of Science |
| Wildlife and Ecology Studies Worldwide |

**Table C - 2.** List of references from which persistent organic pollutant data was collected for time trend analysis.

| Reference | Collection Year | Country | Tissue Type |
|---|---|---|---|
| Albert et al. 1989 | 1985 | Mexico | Eggs |
| Albert and Inigo 1989 | – | Mexico | Liver |
| Baldassin et al. 2016 | 2008-2012 | Brazil | Liver |
| Banasch et al. 1992 | 1984-1989 | Mexico | Carcass; Liver |
| Banasch et al. 1992 | 1984-1989 | Panama | Carcass; Liver |
| Banasch et al. 1992 | 1984-1989 | Venezuela | Carcass; Liver |
| Botero et al. 1996 | 1987-1988 | Colombia | Breast muscle |
| Cardoso et al. 2014 | 2005-2011 | Brazil | Muscle |
| Capparella et al. 2003 | 1996 | Argentina | Carcass |
| Capparella et al. 2003 | 1996 | Peru | Carcass |
| Capparella et al. 2003 | 1997 | Guyana | Carcass |
| Carvalho et al. 2002 | 1989-1993 | Mexico | Muscle; Liver; Eggs |
| Cid et al. 2007 | 2002 | Argentina | Adipose tissue; carcasses |
| Cifuentes et al. 2003 | 1990's | Chile | Eggs |
| Cipro et al. 2013 | | Brazil | Adipose tissue |
| Colabuono et al. 2012 | 2006-2008 | Brazil | Adipose tissue; Liver; Muscle |
| Cunha et al. 2012 | 2007 | Brazil | Eggs |
| Dias et al. 2013 | 2009 | Brazil | Liver |
| Dias et al. 2018 | 2010-2011 | Brazil | Liver |
| Ellis 1985 | 1981 | Argentina | Eggs |
| Ferreira 2013 | 2005-2010 | Brazil | Liver |
| Ferreira 2014 | 2009-2014 | Brazil | Liver |
| Ferreira and Wermelinger 2013 | 2006-2011 | Brazil | Liver |
| Focardi et al. 1996 | | Chile | Muscle; Liver |
| Fyfe 1990 | 1984-1985 | Costa Rica | Carcass |

**Table C – 2.** (*continued*)

| Reference | Collection Year | Country | Tissue Type |
|---|---|---|---|
| Fyfe 1990 | 1983-1984 | Ecuador | Carcass |
| Fyfe 1990 | 1983-1984 | Peru | Carcass |
| Fyfe 1990 | 1979-1980 | Suriname | Breast muscle |
| Garcia-Hernandez et al. 2006 | 2002 | Mexico | Eggs |
| Harwani et al. 2011 | 2005 | Mexico | Blood plasma |
| Huysman, A.P. 1995 | 1994 | Mexico | Eggs |
| Jenny et al. 1983 | 1982 | Ecuador | Eggs |
| Jimenez et al. 2005 | 2000 & 2001 | Mexico | Liver |
| Jimenez-Castro et al. 1995 | 1991 | Mexico | Eggs |
| Kiff and Peakall 1980 | | Mexico | Eggs |
| Klemens et al. 2002 | 1998 | Costa Rica | Carcass |
| Maldonado et al. 2017 | 2011-2013 | Mexico | Carcass |
| Maldonado et al. 2017 | 2011-2013 | Costa Rica | Carcass |
| Mellink et al. 2009 | 2006 | Mexico | Eggs |
| Mora et al. 1987 | 1981-1982 | Mexico | Carcass |
| Mora 1991 | 1987-1988 | Mexico | Eggs |
| Mora and Anderson 1991 | 1986 | Mexico | Carcass |
| Mora 2008 | 2001-2002 | Mexico | Carcass |
| Mora et al. 2008 | 1997-2003 | Mexico | Eggs |
| Mora et al. 2011 | 2004-2007 | Mexico | Eggs |
| Munoz and Becker 1998 | 1994-1996 | Chile | Eggs |
| Nadal et al. 1987 | 1985 | Peru | Eggs |
| Porter and Jenkins 1988 | 1967-1984 | Mexico | Eggs; Breast muscle |
| Vallarino and von Osten 2017 | 2010-2011 | Mexico | Eggs |
| Vander Pol et al. 2012 | 2004 | Mexico | Eggs |
| White et al. 1985 | 1982-1983 | Mexico | Carcass |