PERSISTENT ORGANIC POLLUTANTS IN NEOTROPIC CORMORANTS (*PHALACROCORAX BRASILIANUS*) NESTING ALONG THE TRINITY RIVER, TEXAS

A Thesis

by

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ABSTRACT

The Trinity River is a historically polluted river with records of various contaminants including organochlorine pesticides and their metabolites (OCPs), polychlorinated biphenyls (PCBs), and Mercury (Hg) detected in contaminant studies of the Trinity River and its watershed. Polybrominated Diphenyl Ethers (PBDEs) are a persistent contaminant group that has been shown to cause endocrinological changes, and some of its congeners have been banned in the US and in the whole EU. Fish eating birds are good indicators of contaminant accumulation in aquatic environments, as they accumulate lipophilic contaminants from eating fish and are typically top predators of their food web. Neotropic cormorants (*Phalacrocorax brasilianus*) are good indicators for local contamination and accumulate OCPs, PCBs, Hg, and PBDEs efficiently as the base levels of their food webs include aquatic invertebrates which regularly uptake lipophilic compounds from ambient water and sediment. The objectives of this research are to measure the persistent contaminant burden in Neotropic cormorants, a top piscivore, and to determine contaminant accumulation and potential impacts to the species, as well as if there is any correlation between sex, location of colony, or mass of Neotropic cormorant and contaminant burden. Cormorants were sampled in 2014 and 2015 from two sites on the Trinity River Watershed: Richland Creek Wildlife Management Area, and Lake Livingston. A liver section, spleen, kidneys, and gonads were sampled for histopathology, and a liver section was sampled for GC-MS analysis of OCPs, PCBs, and PBDEs, and breast feathers were sampled for Hg analysis by CVAAS. Results show total PCBs in liver sections of cormorants average 338±12 ng/g ww, total OCPs detected in liver sections average 235±9.5 ng/g ww with 4, 4' DDE as the most commonly detected at 184±4.2 ng/g ww, the average total PBDE content per liver is 10±0.8 ng/g ww and the average Hg detected in central breast feathers was 3±0.2 μg/g dw. None of the contaminants measured were present in concentrations indicative of adverse effects; however, altered structure, composition and function, or histopathology were detected in the livers and kidneys of most samples. A novel coccidian *Eimeria sp.* was also detected in the kidneys of several cormorants. There was no significant difference in contaminants between sexes or location. Neotropic cormorants along the Trinity River do not display contaminant levels indicative of hazardous conditions for PCBs, PBDEs, DDE, and Hg; however, liver and kidney lesions are present in more than half of the individuals. Lesions along the glomeruli, tubules, and interstitial tissues of the kidney were the main findings observed in kidneys, while in the liver, chronic granulomatous cholangiohepatitis with intralesional trematodes were the main histopathologic finding. Our results indicate that Neotropic cormorants roosting in two colonies along the Trinity River are not at risk for adverse effects due to OCPs, PCBs, PBDEs, and Hg.

DEDICATION

I dedicate this page to my wonderful partner, my mother and grandmother, as well as my extended family of native peoples throughout this continent and the world. It is with the support of these people that I have been able to accomplish anything. Thank you Caty, Cristy and Ana.

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CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Professor Miguel Mora [advisor] and Professor Daniel Roelke of the Department of Wildlife and Fisheries, and Thomas McDonald of the Department of Public Health.

The histopathology was analyzed and reported by Professor Raquel Rech of the Department of Veterinary Pathobiology. The contaminant groups OCPs, PCBs, and PBDEs were quantified by Dr. Jose Sericano of the Geochemical and Environmental Research Group. Mercury was quantified by Dr. Robert Taylor of the Trace Element Research Laboratory.

All other work was completed independently.

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INTRODUCTION

The Trinity River Basin in Texas was historically polluted, with the river being fed by municipal wastewater facilities from Dallas Fort Worth, and many feeder creeks, and accumulating contaminants through much of its watershed (Moring 1997, Perkin & Bonner 2014). The Trinity River Basin extends from northwest to southeast some 360 miles from near the Oklahoma-Texas State line, about 60 mi north of the DFW metropolitan area, to Trinity Bay at the mouth of the Trinity River, about 50 mi east of Houston, Texas (Moring 1997). The Trinity River Basin drainage area encompasses 18,570 square miles and has 38 Texas counties partially or entirely within the river basin boundary. Area land use is divided between primarily urban use around the DFW complex, to primarily agricultural immediately outside the metropolitan area, with a gradient going from completely agricultural to forested as the basin progresses, until it hits the Trinity Bay (Moring 1997, Perkin & Bonner 2014). These different land-use types, along with a variety of constructed reservoirs and wetlands, the ecoregion, and several coal burning power plants affect the persistent contaminant types that are found in representative wildlife at different portions of the river basin (Moring 1997).

Organochlorine pesticides, or OCPs, are pesticides composed of chlorinated hydrocarbons, which are not soluble in water, and accumulate in animal fat tissues. They are persistent in the environment and some are considered endocrine disruptors, examples including dichlorodiphenyltrichloroethane, or DDT, and aldrin. In many cases OCPs' metabolites cause endocrine disruption in wildlife as well as the parent compound, long after initial application (Dirksen et al. 1995, Custer et al. 2001). Dichlorodiphenyldichloroethylene, or DDE, a metabolite of DDT, and dieldrin, an OCP related to aldrin that can be created from aldrin further reacting in the environment, both cause calcium metabolism and neuroendocrine disruption (Dirksen et al. 1995, Kamata et al. 2010). OCPs are found in sediment, water and biota in areas where they have been used historically, which are almost ubiquitous, but they have for the most part been banned since the 1970s. Though OCPs are ubiquitous, some OCPs, such as the pesticide Chlordane's isomers, are found in certain areas more than others such as mainly in mainstem river sites rather than at agricultural or urban river sites (Moring 1997).

PCBs had a variety of uses for their thermally and electrically stable properties such as plasticizers, as dielectric fluids in electrical transformers, and as lubricants in heat transfer systems, but they have been shown to have biologic and toxic effects to both humans and wildlife such as death, birth defects, liver damage and tumors (Eisler 1986, Giesy & Kannan 1998). Their stability and insolubility in water, as well as a similarity to dioxins allows PCBs to be persistent in the environment despite being banned from manufacture in 1979 (Eisler & Belisle 1996).

PBDEs are fire retardants that were widely used in textiles, plastics, and foam furniture and automobile components from the 1970s to the 2000s, and that are prone to leaching out at every stage of the product's lifetime from manufacture to disposal (Birnbaum & Staskal 2004). They were shown to be on the rise in a monitored cormorant population from the 1980s until the 1990s, then decreasing but present when the study ceased in 2002 far from primary leaching sites and after their manufacturing restrictions in several states in USA, and in Canada in 1999 (Miller et al. 2015). PBDEs detection and accumulation in many species around the world, including *Phalacrocorax* spp. from Japan, USA, Canada, England, and its deleterious effect on offspring development, and retinoid, liver, and thyroid function, as studied in several species of birds, must be examined to determine the exposure risk for avian wildlife (Law et al. 2003, Watanabe et al. 2004, Birnbaum & Staskal 2004, Fernie et al. 2005, McKernan et al. 2009, Chen et al. 2010, Klosterhaus et al. 2012, Spears & Isanhart 2014, Dornbos et al. 2015). PBDEs are found in the highest amount near urban centers, but are ubiquitous in the environment (Miller et al. 2014, Miller et al. 2015).

Hg is a metal and natural byproduct of forest fires, volcanic eruptions, and is present in the earth. However, its main forms of deposition into the environment by quantity, up to 62% of the total emissions, is by electrical generation such as coal burning for power plants, and as a byproduct of gold mining (Pacyna et al. 2016). Deposition of airborne mercury onto wetlands or lakes where anaerobic conditions are present allows for the formation of methyl-mercury, or MeHg by anaerobic methylating bacteria (Lavoie et al. 2015). MeHg accumulates through the aquatic food web eventually reaching top level predators, such as Neotropic cormorants. MeHg is known to cause reproductive failures in birds as it accumulates, however, demethylating and excretion by feathers are mechanisms by which birds cope with MeHg toxicity (Henny et al. 2002). Feathers typically sequester a good proportion of mercury from internal tissues, with gull feathers sequestering 49% of orally administered mercury independent of the dose, though the exact percentage sequestered in feathers is likely to vary between species (Frederick et al. 2010). Because MeHg in feathers is proportional to overall Hg ingestion, feather MeHg concentrations at environmentally relevant levels, 5 µg/g dw total Hg in feathers, are associated with ingestion of a quantity of Hg causing reduced hatching success and sterility, and feathers with MeHg concentrations between 9-11 µg/g dw are associated with ingestion of an acutely toxic amount of Hg (Eisler 1987). Concentrations in feathers associated with acutely toxic effects are 1.5-fold those required to produce reproductive impairment in the same species, and are found in the highest quantities in areas with legacy Hg spills, such as the Carson River Superfund site in Nevada, and near coal burning power plants (Wolfe et al. 1998, Henny et al. 2002, Hall et al. 2014).

Several of these contaminants including OCPs, PCBs, and MeHg have been detected in contaminant studies of the Trinity River or in its watershed (Van Metre & Callender 1996, Moring et al. 1997, Perkin & Bonner 2014). Sediment cores exploring the pollution of Lake Livingston, a reservoir lake on the mainstem of the Trinity River, from 1969-1992 found DDT and its metabolites to be greatest near 1970 at 18 µg/kg dw and lower in 1992 when it decreased to 6 µg/kg dw, while dieldrin and chlordane increased in concentrations in sediments from 1980s until 1992 when concentrations were 4.0 µg/kg dw (Van Metre & Callender 1996). The distribution of persistent contaminants along the Trinity River watershed is not uniform, land use and ecoregion in the river basin affect the contaminants that leach into the aquatic food web along different points. A study of the Trinity River watershed among multiple sites with various land use from Dallas/Fort Worth down the Trinity River and into the Trinity Bay detected more DDT and metabolites in biota in agricultural sites, 58 µg/kg ww, and along mainstem Trinity River sites, 158 µg/kg ww, than in urban use areas, 35 µg/kg ww, while Chlordane and Nonachlor isomers, were found more often and in in higher concentration at mainstem river sites, 131 µg/kg ww, in river biota including Asiatic clams and fish, than in agricultural use or urban use river sites, 35 µg/kg ww and 45 µg/kg ww respectively (Moring 1997).

In order to monitor the status of these persistent contaminants in aquatic systems, a variety of sources must be sampled such as sediment, biota and water Neotropic cormorants are able to accumulate persistent contaminants at far higher concentrations than those that are present in the sediment or water. The Neotropic cormorant range is Pan- American, extending from the Northern Gulf Coast of Mexico along the United States, down to the southern tip of South America. Populations of Neotropic cormorants are abundant, they are non-migratory over most of their range, and feed relatively close to the breeding colony more than 90% of the time, which allow us to make inferences about residues in their tissues representing local sources (Quintana et al. 2004). The objectives of this study were to determine accumulation of persistent organic pollutants, POPs, particularly OCPs, PBDEs, and Hg in Neotropic cormorants from the Trinity River and to evaluate the potential negative impacts of POPs on aquatic birds nesting along the Trinity River.

METHODS

Study Sites and Sample Collection

Richland Creek Wildlife Management Area (RCWMA) is adjacent to Richland Chambers Reservoir near Corsicana, Texas, and adjacent to the mainstem Trinity River. The area is a constructed wetland which multiple species use for foraging and nesting including Neotropic cormorants, Snowy egrets (*Egretta thula*), Great egrets (*Ardea alba)*, Wood storks (*Mycteria americana)*, American white pelicans (*Pelecanus erythrorhynchos)*, Roseate spoonbills (*Platalea ajaja)*, and other waterbirds. Neotropic cormorants roost at the northeast corner of the management area adjacent to the mainstem river, and feed at the r eservoir, in the management area, and in the mainstem river. Lake Livingston (LALIV) is a reservoir lake in Livingston, Texas, on the mainstem Trinity River with a cove on the northeast side of the lake that contains a regular Neotropic cormorant roost which feeds primarily in the lacustrine and riverine portions of the lake adjacent to the roost. The sites are shown in Figure 1.

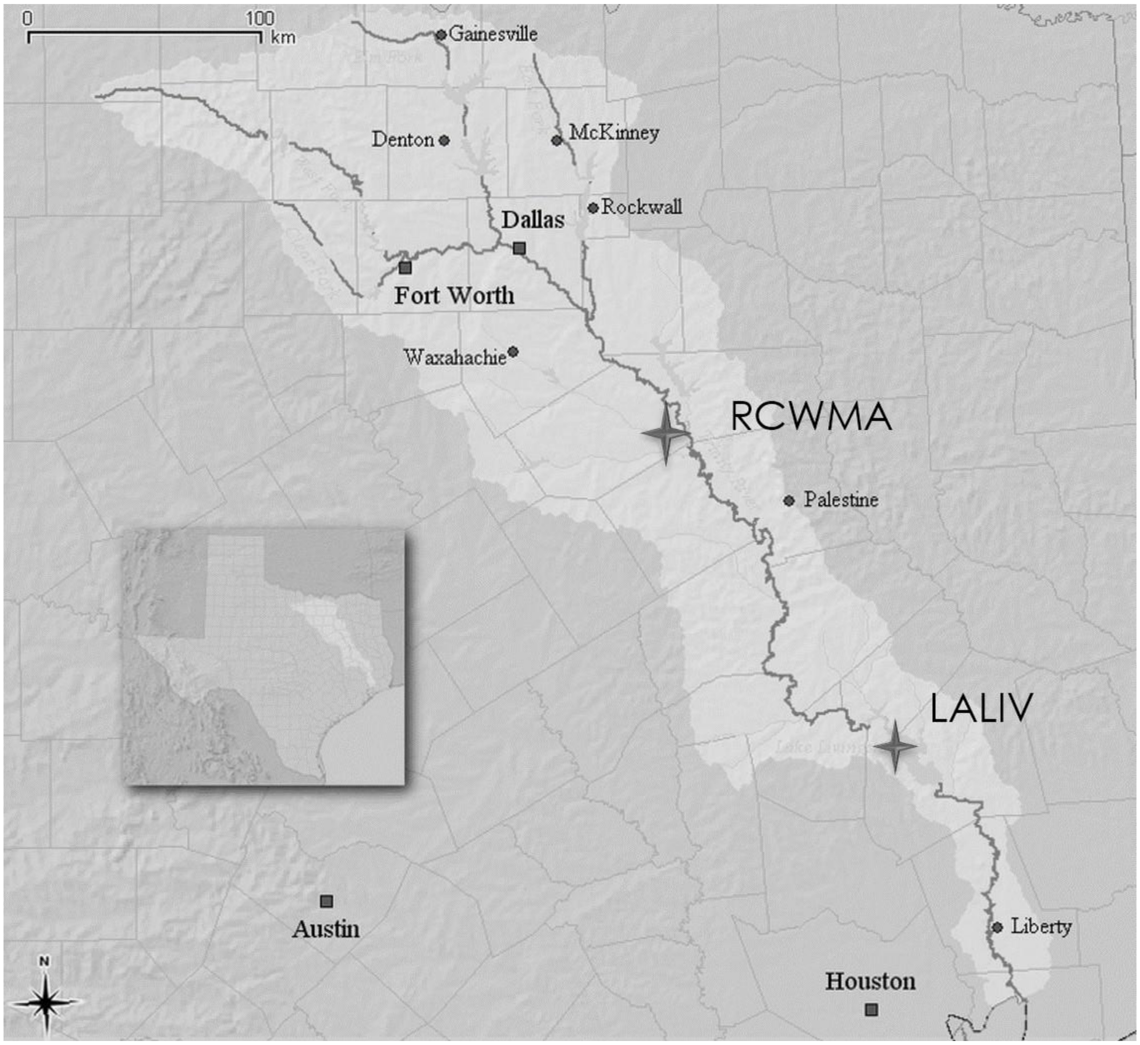

Figure 1 Location of Neotropic cormorant colonies. Colonies are marked as stars, in Richland Creek Wildlife Management area, RCWMA, and Lake Livingston, LALIV. Adapted from Kuru / CC-BY-SA-3.0 and USGS data.

Twenty-four Neotropic cormorants were collected during the summer and fall of 2014 and 2015 under United States Fish and Wildlife Service permit MB027977-2 and Texas Parks and Wildlife Department permit SPR-0493-605. All animal collection and handling procedures followed Texas A&M University approved animal use protocol, IACUC 2014-0183. Ten cormorants were harvested with a shotgun approximately half a mile from the colony at feeding sites at RCWMA in the summer, and nine from the feeding area immediately adjacent the colony at Lake Livingston in the fall of 2014. An additional five specimens were collected from RCWMA in the summer of 2015 a similar distance from the colony on the feeding ponds. Immediately after collection, the specimens were weighed, livers were dissected from the carcass, and an approximately 3g section was stored in an amber jar on ice until return to the lab, after which, sections were placed in a -80C freezer until organic contaminant analysis. The kidneys, spleen, gonads and a section of liver were immediately fixed in 10% neutral buffered formalin for histopathological analysis. Carcasses were returned to the lab wrapped in foil and on dry ice. In the laboratory, ten breast feathers from each specimen were plucked from over the keel, washed, and placed in polyethylene bags until mercury analysis.

Chemical and Histopathology Analysis

Livers were analyzed for PBDEs, PCBs, and OCPs at the Texas A&M Geochemical and Environmental Research Group, College Station, Texas. Approximately 0.5 g of liver sections were mixed with anhydrous Na_2SO_4 and the tissue macerated with a Tissumizer (Pro Scientific, Model PRO250) for three minutes. Contaminants were extracted with methylene chloride. The extracts were concentrated and purified via silica gel/alumina column and then run through high performance liquid chromatography (HPLC) to reduce matrix interference. PCBs and PBDEs were detected using a gas chromatograph, Agilent 5890A (Agilent Technologies, USA) coupled to a low-resolution 5975C inert mass selective detector in the selected ion monitoring (GC/MSDSIM) and a 30 m 9 0.25-mm i.d. fused silica capillary column with DB-5MS (J&W Scientific Co., USA).. Helium was used as the carrier gas. For PCBs, injection temperature was 270°C, initial oven temperature was 75°C, held for 3 minutes, and raised at 15°C/min until 150°C, then raised 2°C/min until 260°C, then raised 20°C/min until 300°C and held for 1 minute. PBDEs injection temperature was 270°C and initial oven temperatures was 130°C, held for 1 minute then raised 12°C/min until 154°C, then raised 2°C/min until 210°C, then 3°C/min until 300°C and held for 3 minutes. OCPs were detected using an Agilent 6890 N Gas Chromatograph (Agilent Technologies, USA) coupled to a low-resolution 5975C inert mass selective detector in the selected ion monitoring (GC/MSDSIM) and a 30 m 9 0.25-mm i.d. fused silica capillary column with DB-5MS (J&W Scientific Co., USA). Injector temperature was 300°C and initial oven temperature was 100°C, raised 10°C/min until 200°C, then 5°C/min until 300°C and held for 3 minutes. Helium was used as the carrier gas. Limits of detection were determined for 22 individual PCB congeners and ranged from 0.02 ng/g ww to 0.11 ng/g ww with an average for mono- through tri- chlorinated congeners of 0.04 ng/g ww and an average for tetra- through deca- chlorinated congeners of 0.05 ng/g ww based on a 20 g wet tissue sample. Limits of detection for OCPs and PBDEs were less than 1 ng/g ww each. Accuracy and precision were determined using duplicates and internal surrogates, relative percent difference for samples was less than 0.1 and percentage recoveries ranged from 73% to 99%.

Mercury was analyzed at the Texas A&M Trace Element Research Lab, College

Station, Texas, by cold vapor atomic absorption spectroscopy (CVAAS). Samples were digested with nitric acid, hydrochloric acid, and hydrogen peroxide and then made to volume with deionized water. Mercury was determined in the digestate by CVAAS on a Cetac QuickTrace M-7600 instrument. (Cetac Technologies, Omaha, NE, USA), SRM 2976 was used as a reference material to evaluate accuracy.

Histopathology analysis was conducted on samples collected in 2014 and were prepared by placing excised organs including one kidney, one gonad, a liver section and the spleen of each sample when possible into prefilled containers of neutral buffered formalin filled to 60 mL. Incisions were made into the organs to ensure deep fixation in tissues that were more than 5 mm thick. Tissues were analyzed by histopathology at the Texas A&M University Veterinary Pathobiology Lab, School of Veterinary Medicine.

Statistical Analysis

A Shapiro-Wilks test for normality was performed on Neotropic cormorant contaminant values in liver for PCBs, OCPs, and PBDEs, and for Hg in feathers. If the data were normal for contaminant values for both locations or for both sexes, they were compared by Student's T-test, and if they were not, they were compared by the non- parametric Mann-Whitney U-test ($p < 0.05$). R version 3.3.0 was used for statistical analysis (R Core Team 2016, Vienna, Austria).

RESULTS

Concentrations of total PCBs were not significantly different between RCWMA in 2014 and 2015 and LALIV in 2014. There was also no significant difference detected between sexes regarding total PCBs concentrations. Of 164 congener or congener groups tested for, 65 were detected, however, only 34 were detected in at least half of all samples and reported (Figures 2 and 3). RCWMA in 2014 had an average total PCBs concentration of 162.2 ng/g ww and the highest concentrations of individual congeners or groups were from PCB 132/153/168, PCB 160/163/164, PCB 177, PCB 180/193, and PCB182/187 (Table 1, Figure 2). LALIV in 2014 had an average total PCBs concentration of 418.6 ng/g ww with the most concentrated congeners or groups being PCB 182/187, PCB 132/153/168, PCB 138/158, PCB 160/163/164, and PCB 180/193 (Figure 3). RCWMA in 2015 had an average total PCBs concentration of 494.1 ng/g ww and the same 5 congeners and groups of the highest concentration as LALIV in 2014. Average total PCBs for all cormorants was 338 ng/g ww in liver. Hepta chlorinated PCB congeners were detected in the highest amounts, followed by hexa and penta chlorinated congeners.

4,4’ DDE was the most concentrated OCP at any site and for every individual, however, there was no significant differences between sites, years, or sexes for any OCP. At RCWMA in 2014, the 4,4’ DDE concentrations was 82 ng/g ww. In LALIV, the 4,4’ DDE concentration was 213 ng/g ww. In 2015 at RCWMA, the 4,4’ DDE concentration was 311 ng/g ww. 4, 4’ DDE comprised between 73 and 89% of DDT and its metabolites (sum DDT) detected for all samples, averaging 184±4.2 ng/g ww in liver (Figure 4). Of the 22 OCPs tested for not including sum DDT, 20 were detected including tetrachlorobenzene 1,2,4,5, tetrachlorobenzene 1,2,3,4, pentachlorobenzene, hexachlorobenzene, alpha hexachlorocyclohexane (HCH), beta HCH, gamma HCH, heptachlor, heptachlor epoxide, oxychlordane, alpha chlordane, gamma chlordane, cis-nonachlor, trans-nonachlor, aldrin, dieldrin, endrin, pentachloroanisole, chloropyrifos, mirex, and endosulfan II. Of these, only 4,4 DDT and 4,4 DDE, Alpha HCH, Heptachlor Epoxide, and Dieldrin were detected in liver at concentrations on average greater than 3 ng/g ww (Figure 5). Alpha HCH and heptachlor epoxide averaged higher concentrations in LALIV, 21 and 16 ng/g ww, than in RCWMA where the concentrations for 2014 were 0.5 and 1.9 ng/g ww and for 2015 were 0.4 and 2.1 ng/g ww respectively. The dieldrin concentration present at RCWMA in 2014 was 5.2 ng/g ww, 3.7 ng/g ww at LALIV and 3.3 ng/g ww at RCWMA in 2015.

Concentrations of average total PBDEs was not significantly different between RCWMA in 2014 and 2015 and LALIV in 2014. Total PBDEs concentration was not significantly different between sexes either. In 2014, 4 congeners were detected at RCWMA and LALIV, they were PBDEs 47, 99, 100, and 153, however, only PBDEs 47 and 99 were present in at least half of the samples. Total PBDEs concentration at RCWMA in 2014 was 2.9 ng/g ww. At LALIV, total PBDEs concentration was 1.2 ng/g ww. At RCWMA in 2015, 8 congeners were detected, PBDEs 28, 47, 66, 99, 100, 153, 154 and 155, but only PBDEs 47, 99, 100, and 153 were detected in at least half of the samples (Figure 6). The total PBDEs concentration at RCWMA in 2015 was 1.11 ng/g ww. The average total PBDEs for all samples was 10 ng/g ww in liver (Table 1).

Hg was analyzed in feather samples of cormorants collected at both RCWMA in 2014 and LALIV in 2014and there was no significant difference detected between sites or sexes. Average Hg concentration at RCWMA in 2014 was 3.434 µg/g dw, and at LALIV it was 1.896 µg/g dw. The average Hg detected in all central breast feathers was 3 µg/g dw.

Notable results of the histopathological examination include underlying infections or inflammation present in either the kidneys or liver of almost every individual, 17 out of 19 cormorants from 2014, as well as the presence of internal parasites in 8 of the 19 specimens (Table 2). Degree of inflammation was denoted in table 2 on a scale of 0 to 3 where 0 is no inflammation, incrementally increasing from some, to moderate, to heavy inflammation. In the kidneys, the main histologic finding is proliferative and lymphoplasmacytic ureteritis with occasional multifocal interstitial nephritis and intralesional *Eimeria sp*. Several life stages of *Eimeria sp*. are observed in the kidneys, especially meronts and microgametes, with rare oocysts. One cormorant had focally extensive renal atrophy secondary to ureterolith. In the liver, the main histologic finding is multifocal chronic granulomatous cholangiohepatitis with intralesional trematodes, containing numerous eggs. At this time we have not been able to identify species of trematode. 3 cormorants were immature, with one, a female from LALIV, displaying a reversed internal morphology with a heart on the right side of the thoracic cavity, and functional ovary on the right side.

Location	RCWMA	LALIV	RCWMA
Year	2014	2014	2015
n	9	10	5
Sex Ratio (F:M)	4:5	3:7	2:3
Mass (kg)	1.09±0.16	1.2±0.23	1.11±0.15
ppDDE (ng/g ww)	81.7 ±16.5	213 ±20.7	311.5 ±46.8
ppDDT (ng/g ww)	3.8 ±0.5	12.3 ±1.1	15.9 ±1.9
Alpha HCH (ng/g ww)	0.5 ±0.04	20.7 ±6.4	0.4 ±0.1
Heptachlor Epoxide (ng/g ww)	1.9 ±0.2	16.1 ±4.5	2.1 ±0.3
Dieldrin (ng/g ww)	5.2 ±0.6	3.7 ±0.2	3.3 ±0.5
Total PCBs (ng/g ww)	162.2 ±0.7	418.6 ±0.6	494.1 ±1.1
Total PBDEs (ng/g ww)	2.9 ±0.2	3.5 ±0.2	36.5 ±5.7
Hg (µg/g dw)	3.434 ±0.569	1.896 ±0.076	

Table 1 Average contaminant values for Neotropic cormorant livers

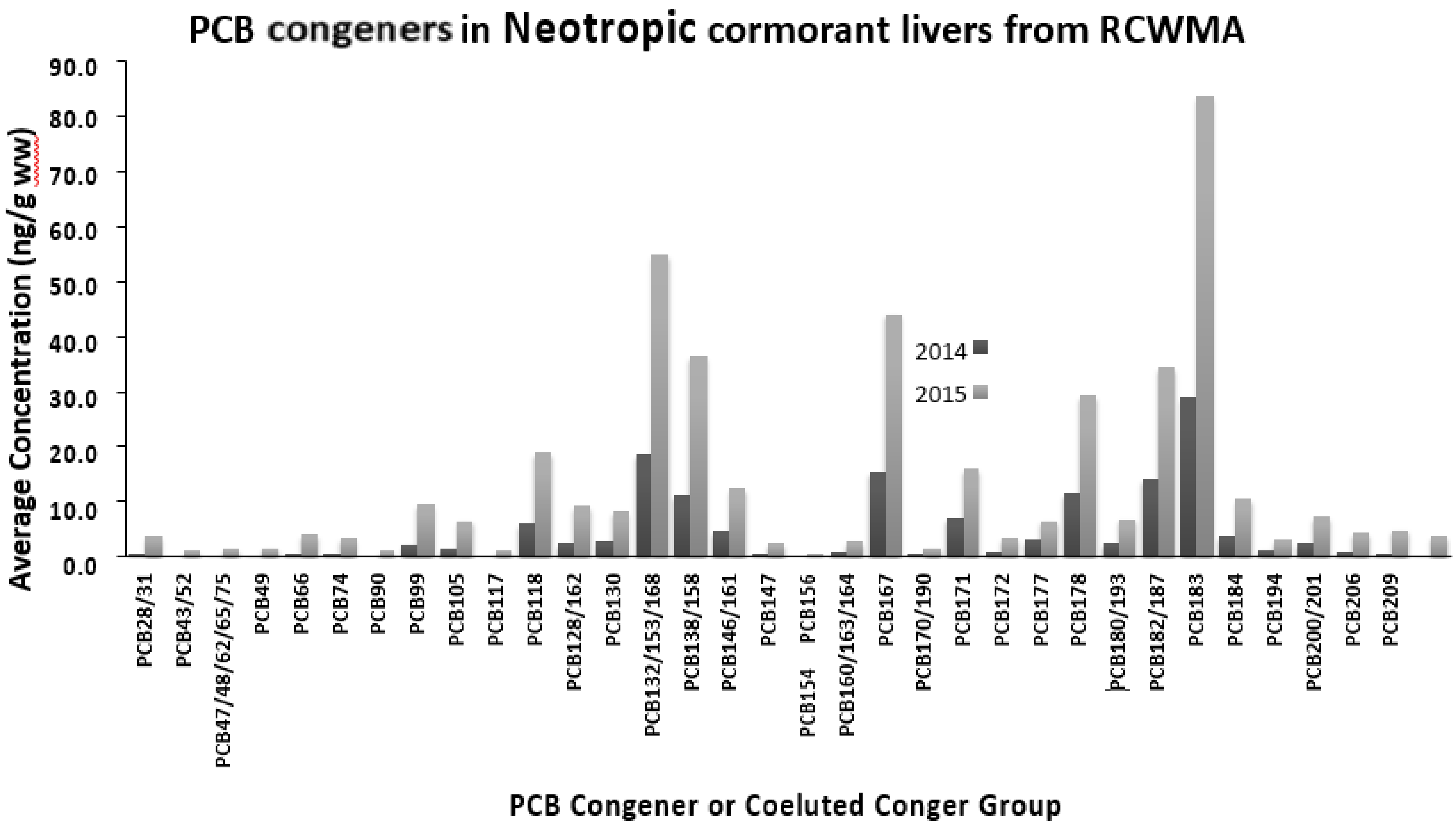

Figure 2 PCB congeners detected in Neotropic cormorant livers from Richland Creek Wildlife Management area over two years

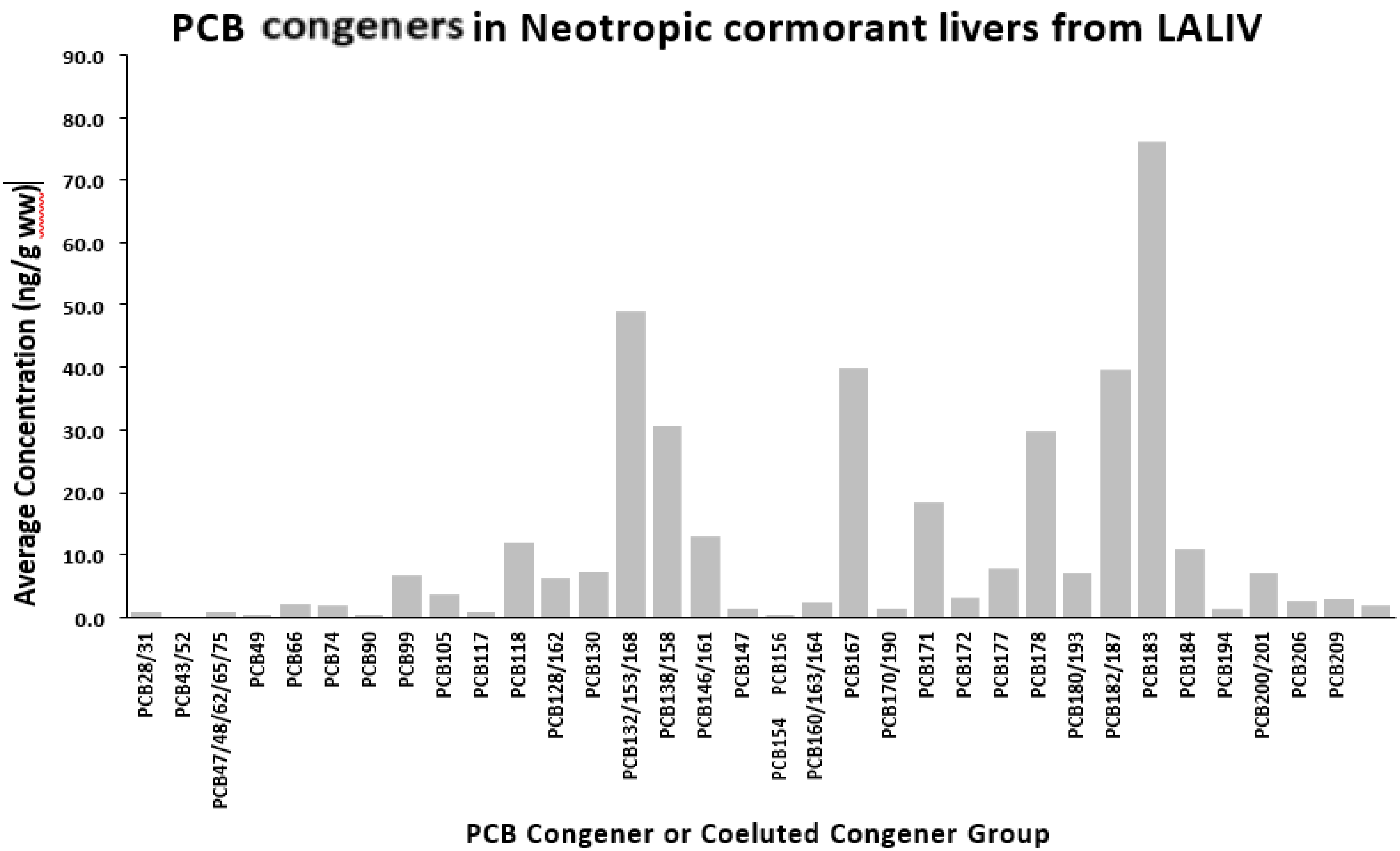

Figure 3 PCB congeners detected in Neotropic cormorant livers from Lake Livingston in 2014

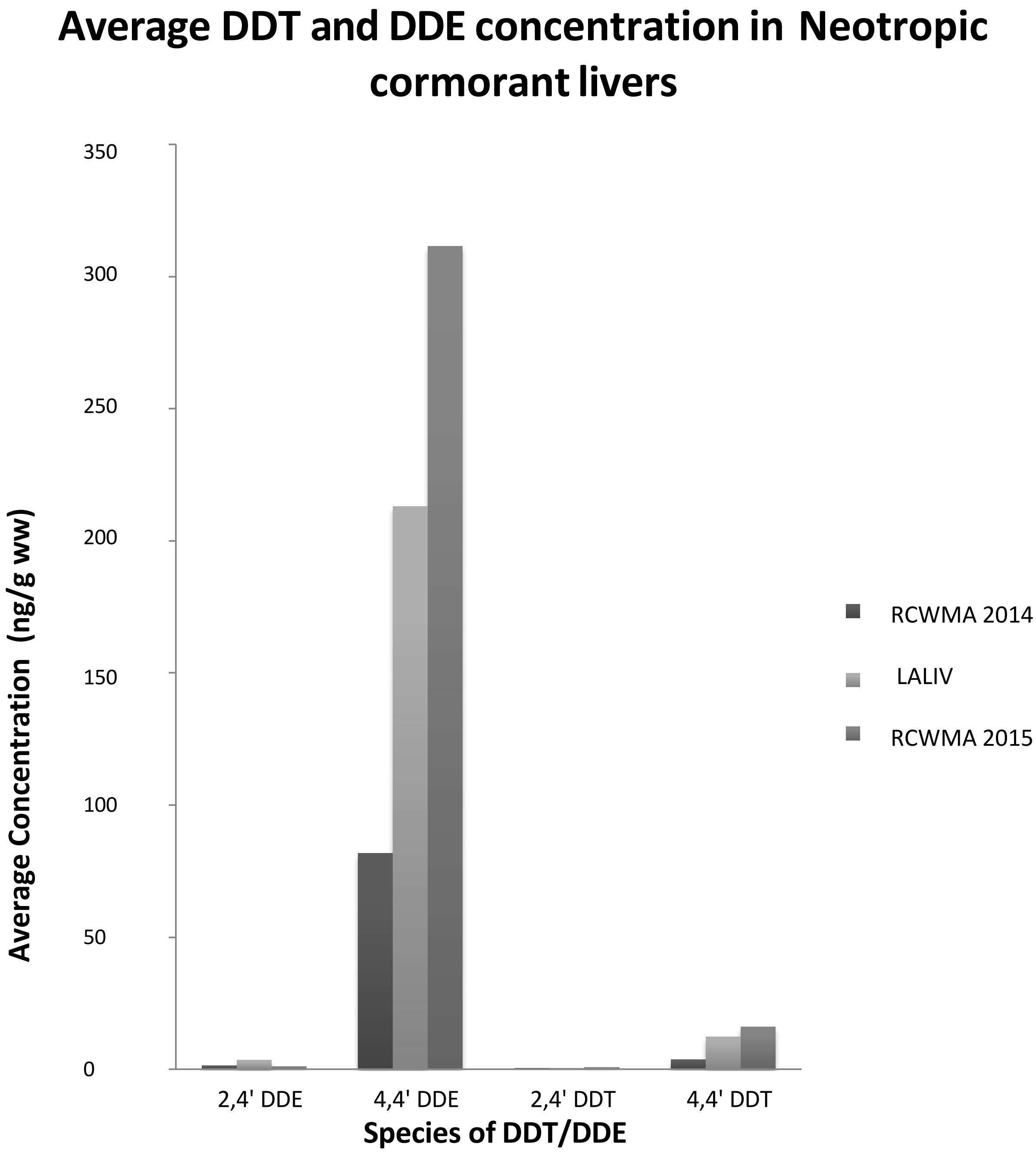

Figure 4 DDT and DDE isomers found in Neotropic cormorant livers. Samples from Richland Creek Wildlife Management Area in 2014 and 2015, and Lake Livingston in 2014.

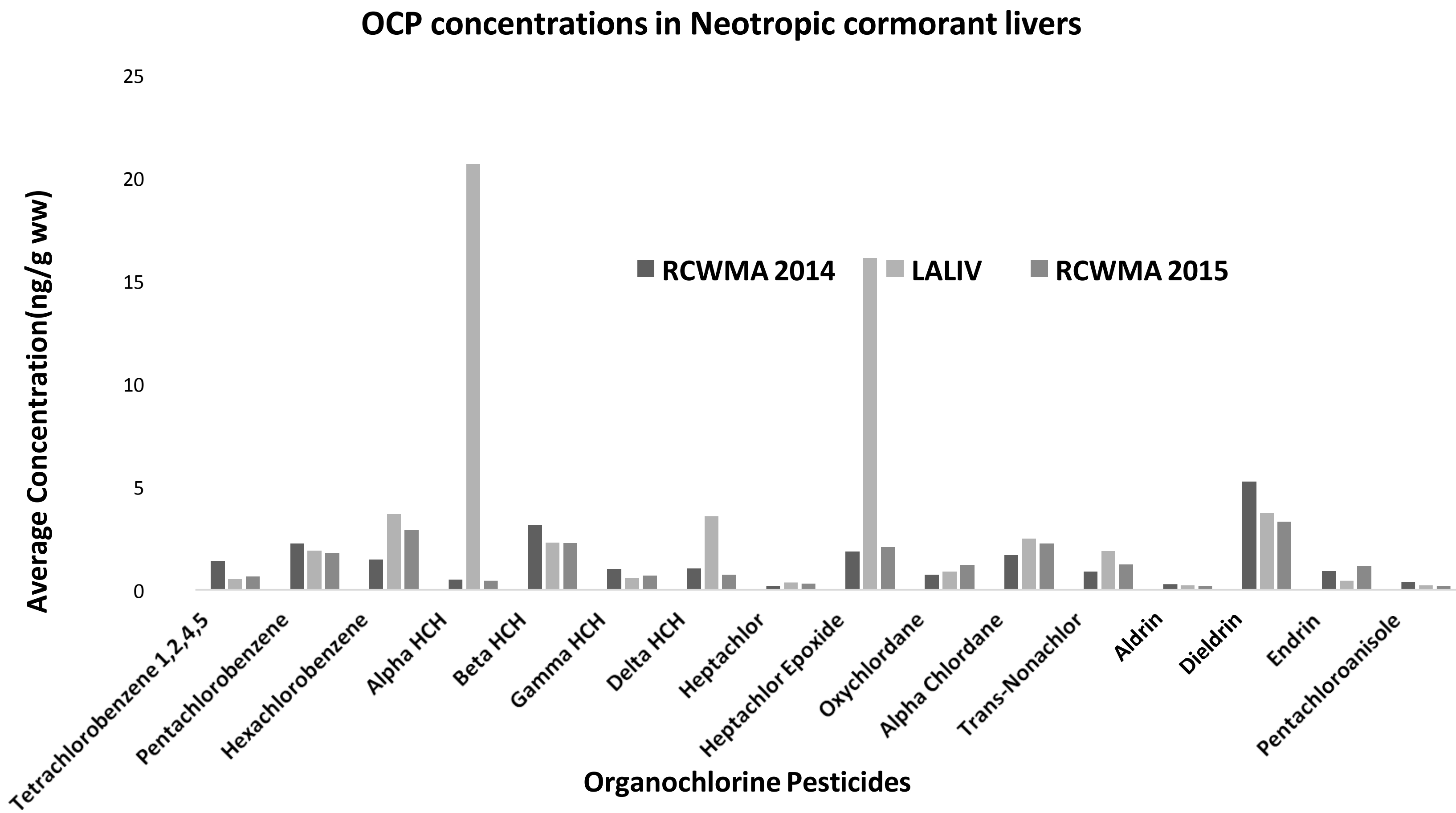

Figure 5 Organochlorine pesticides present in Neotropic cormorant livers. Samples from Richland Creek Wildlife Management Area in 2014 and 2015, and from Lake Livingston in 2014

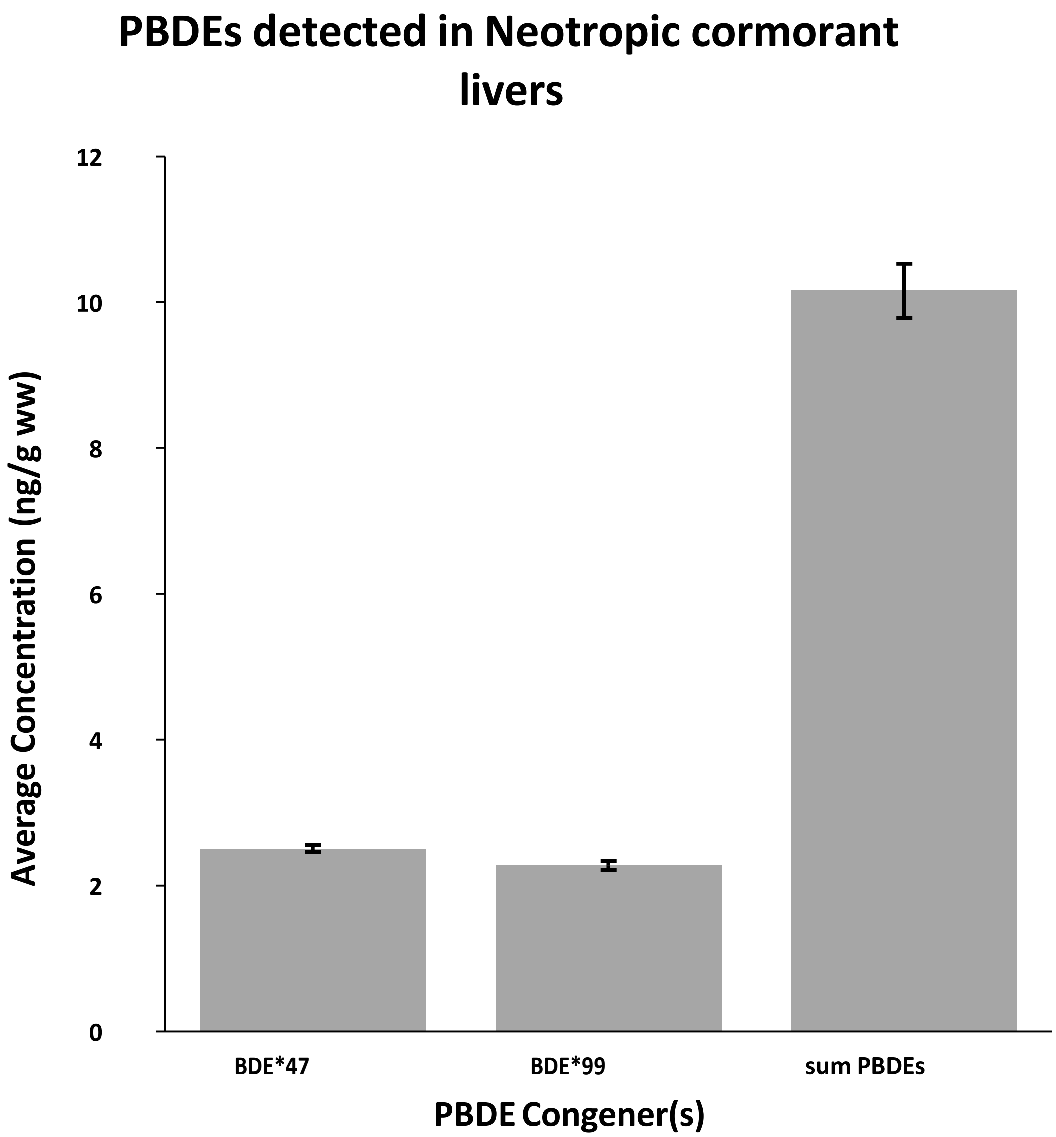

Figure 6 PBDE congeners and total PBDEs detected in Neotropic cormorant livers. PBDE congeners 47 and 99 were detected in more than half of all Neotropic cormorant livers (ng/g ww)

Location	ID	Sex	Liver	Parasites	Kidney	Parasites
RCWMA	1	m	1	T	0	*
RCWMA	2	f	2	T	1	*
RCWMA	3	f	2	*	*	*
RCWMA	4	f	2	*	*	*
RCWMA	5	m	1	*	2	c
RCWMA	6	m	0	*	1	*
RCWMA	7	m	0	*	0	*
RCWMA	8	f	1	*	0	*
RCWMA	9	m	0	*	1	*
LALIV	10	m	1	*	2	*
LALIV	11	m	1	*	2	*
LALIV	12	m	0	*	2	c
LALIV	13	m	0	*	2	c
LALIV	14	m	0	*	3	c
LALIV	15	f	0	*	*	*
LALIV	16	f	0	*	1	*
LALIV	17	m	2	*	1	*
LALIV	18	f	0	T	2	*
LALIV	19	m	0	*	2	c

Table 2 Histopathology overview. Liver and Kidney values of 0-3 are presented as degree of inflammation, T represents a trematode infection or detection in liver and C indicates an *Eimeria* infection or detection in kidney. No other significant lesions were detected in Spleen or Gonads of at least half of all Neotropic cormorants.

DISCUSSION

PCBs

Neotropic cormorants sampled in Galveston in 1980 and 1981 had total PCBs concentration in eggs of 6.36 and 7.61 µg/g ww respectively, values over 15 fold those found in livers from our study (King & Kyrnitsky 1986). Double-crested cormorant carcasses collected in 1982 and 1983 in the Houston Ship Channel had total PCB concentrations of 1.54 and 1.58 µg/g ww respectively (King et al. 1987). The PCB concentrations in carcasses are roughly 4 times greater than the concentrations we found in liver (King et al. 1987). Double-crested cormorant eggs sampled from different islands in Galveston Bay in 1996 had average total PCBs between 1640 ng/g ww and 5,720 ng/g ww (Frank et al. 2001). The lowest observed adverse effect level (LOAEL) for total PCBs in liver is 15 µg/g ww, based on sublethal effects on reproduction and immunosuppression (Eisler 1986; Scharenberg 1991). Comparing against this value, the Neotropic cormorants in our study had contaminant values far below those in which we would expect adverse effects and the lower concentrations seen here are likely the result of the phasing out and banning of manufacture of PCBs.

Past studies have detected mainly hexa chlorinated congeners PCB 153 and PCB 138 in the eggs of *Phalacrocorax spp.* in higher concentrations than other congeners in Galveston Bay (Frank et al. 2001). In our study, the highest concentration of PCB congener detected was for PCB 182/187, then PCB 132/153/168 which is likely primarily PCB 153, then PCB 160/163/164, a different pattern than what is typically found in water birds (Frank et al. 2001). The difference in the congener breakdown may be a result of a change in the congener fingerprint in the diet of the cormorants over time or a result of different locations' unique contamination profile.

OCPs

Other cormorants from adjacent the Trinity river watershed have been sampled for contaminants in the 1980s and in 1996, with carcass DDE values in Neotropic cormorants from 1980 and 1981 in Galveston Bay of 0.85 µg/g ww and 2.60 µg/g ww respectively, Double- crested cormorant, *Phalacrocorax auritus*, carcasses from the Houston Ship Channel in 1982 and 1983 with DDE values of 0.66 µg/g ww and 0.93 µg/g ww respectively, and eggs in 1996 from Neotropic cormorants which had mean DDE concentrations between 205 ng/g ww and 1,040 ng/g ww (King & Kyrnitsky 1986, King et al. 1987, Frank et al. 2001). The DDE detected in cormorant livers from our study are similar to concentrations that have been associated with the 5% eggshell thinning concentration, and not likely to have a significant effect on reproduction (Greichus & Hannon 1973, Dirksen et al. 1995).

There were decreases of 4,4' DDE detected in sediment and aquatic invertebrate concentrations from 1969 to 1992 in the Trinity River and Lake Livingston (Van Metre & Callender 1996, Moring 1997). While the decreases in concentration in sediment and biota from 1969 to 1992 are likely the result of restrictions placed on OCP use in the United States, research into trends of DDT and its metabolites over the last 25 years has shown the central zone of the continental United States, including the Trinity River basin, has had a decrease in sum DDT over time and a slight decreasing trend with increasing latitude (Mora et al. 2016)). The ratio of DDT to DDE, heptachlor to heptachlor epoxide, and aldrin to dieldrin, all above 5:1, likely indicate that contamination is majorly by exposure to persistent legacy contamination as degradation products and metabolites are present in higher concentrations than their parent compounds respectively.

PBDEs

Adverse effect levels of PBDEs have been difficult to determine as dosing studies have not been performed with *Phalacrocorax spp.*, however, studies in Osprey, *Pandion haliaetus*, and American kestrels, *Falco sparverius*, determined that a concentration of between 1-1.8 µg/g ww in ovo reduced pipping success and was correlated with decreased productivity (McKernan et al. 2009, Henny et al. 2009). Because PBDEs are present in egg lipids at about 45 times the concentration they are present in liver lipids in cormorants, and eggs have a 4.8 % fat content compared to a 4% liver fat content, an estimated value above 18.5 to 33.3 ng/g ww in liver would probably result in altered reproductive success in cormorants (Watanabe et al. 2004, Herzke et al. 2009). The concentrations of total PBDEs detected in Double-crested cormorant eggs from the West coast of Canada from 1990 until 2011 were between 26 and 385 ng/g ww, corresponding to liver values in adults of .5 to 7.1 ng/g ww, similar to our cormorant concentrations (Miller et al. 2015). In addition, total PBDE concentrations of San Francisco Double-crested cormorant eggs in 2008 measured 5,500 ng/g ww, corresponding to liver values in adults of 101.9 ng/g ww, ten times the level found in cormorants from this study (Klosterhaus et al. 2012). In our study mean concentrations of PBDEs in livers did not reach the level at which negative effects on reproduction are observed (18.5 ng/g ww in liver); thus cormorants nesting along the Trinity River are very likely not experiencing adverse effects due to PBDEs.

The highest levels of PBDEs detected in cormorant tissues from North America was from Mandarte Island, BC, Canada, 7 µg/g ww in ovo, or 129.6 ng/g ww in liver, in the mid 1990s (Miller et al. 2015). The most current PBDE mean values for birds at the same location were 31.3 ng/g ww in ovo, or 0.6 ng/g ww in liver, and the colony is reportedly shrinking (Miller et al. 2015). It should be noted that because the effects PBDEs have on developing eggs and adult individuals is not well understood, the critical value we compare, 18.5 to 33.3 ng/g ww in liver is conservative and is used due to the lack of comparable tissue samples taken but should be amended as toxicological studies regarding the effect of PBDEs on reproductive success and adult survivability in cormorants are published (Miller et al. 2015).

Mercury

The values of mercury detected in current Neotropic cormorant feathers were higher than those detected in Neotropic cormorant livers in Galveston Bay in 1980 and 1981 which were measured to have 1.23 and 1.60 µg/g ww liver total Hg respectively, corresponding to 1.2 and 1.5 µg/g dw in feather with a liver to feather ratio of 1.2 : 1, considering a liver moisture content of 68% (King & Cromartie 1986, Saeki et al. 2000, Robinson et al. 2010). Mercury in feathers is almost 100% MeHg, it is physiologically isolated from the body, and the quantity of MeHg deposited in the feather is dependent on many processes (Schulwitz et al. 2015). Remobilized MeHg from fatty tissues, as well as circulating MeHg is incorporated into the forming feather, creating a snapshot of the Hg burden at the time of formation, however, the process of demethylating MeHg in the liver into inorganic Hg, the ingestion of MeHg, and the excretion of Hg via plumage muddies the relationship between feather Hg and Hg reaching target organs and inducing toxicity (Hall et al. 2014. Ackerman et al. 2016). A mercury LOAEL was recommended for a wide range of avian comparisons of 5 µg/g dw total Hg in breast feathers (Eisler 1987, Misztal-Szkudlin´ska et al. 2012). The feathers in cormorants from the Trinity River are under this threshold, thus, they are below levels known to cause adverse effects.

Though the samples do not indicate adverse effects, a possible reason for the higher concentrations of MeHg detected in the Trinity River feathers than in Galveston Bay livers in 1981 is the difference in the foraging areas of the two samplings as freshwater foraging habitat types typically have more bioavailable Hg than shallow saltwater systems, (Bryan et al. 2014, Torres et al. 2014). Estuarine biota typically has lower Hg concentrations due to geochemical sulfur dynamics within salt marshes, thus prey items from freshwater wetlands tend to result in higher Hg accumulation (Bryan et al. 2012). A synthesis of mercury trends in Northwestern North America found, however, that oceans, then salt marshes, then various freshwater sources had the highest mercury concentrations present among piscivorous birds, including Double-crested cormorants, a dynamic opposite the one reported for South East United States (Ackerman et al. 2016). The two colonies we tested were primarily exposed to Hg introduced via aerial deposition as point sources in Texas are found near the central coast at Lavaca Bay (Schulwitz et al. 2015).

The difference in land type use between our colony locations is that LALIV is a large reservoir surrounded primarily by coniferous forest in the South Central Plain (SCP) ecoregion, whereas RCWMA is adjacent a reservoir and divided between coniferous and deciduous forest covered areas and agricultural land in the East Texas Central Plain (ETCP) ecoregion (Van Metre & Callender 1996, Scudder et al. 2010). Several studies have shown that mercury ranges in North to North East Texas are dependent on ecotype in addition to aerial deposition, with the SCP having the highest mercury concentrations in fish followed by the ETCP (Drenner et al. 2013, Schulwitz et al. 2015). Ecoregions are areas of the environment with similar landscapes, soils and biota that are denoted to aid in conservation and management of ecosystems (Drenner et al. 2013). The lack of a statistical difference between locations for Hg is surprising as prior studies of South Eastern United States have demonstrated that largemouth bass, Micropterus salmoides, and equivalent fish contained Hg concentrations in their fillet that is related heavily with the ecoregion, specifically the conifer coverage and aerial deposition of Hg (Drenner et al. 2013).

The ecoregions with the highest Hg deposition, 32-34 $\mu g/m^2$ per year, were detected in East Texas from the Southern Coastal Plain with an average largemouth bass equivalent fillet Hg concentration of 601-700 ng/g ww (Scudder et al. 2010, Drenner et al. 2013). Other studies of Hg in piscivorous birds found higher levels of Hg in Egrets at Caddo Lake in the South Central Plain ecotype than those at Lewisville Lake near Dallas in the Texas Blackland Prairie ecotype (Schulwitz et al. 2015). Our results do not coincide with the fish Hg deposition map ranges, or these other studies of piscivorous birds in Texas as RCWMA had a higher mercury concentration than LALIV. A possible explanation for the result could be that the RCWMA colony feeds in a particularly low productivity area of the ETCP. Low productivity areas such as those with limited nutrients and high levels of organic carbon and nitrogen have been known to concentrate a higher amount of Hg in the base of piscivorous food chains whereas LALIV has a relatively large lacustrine area that provides ample productivity to initially dilute mercury before magnification even though the SCP ecoregion has a higher average fillet concentration than the ETCP (Bryan et al. 2014, Lavoie et al. 2015, Julian et al. 2016).

Histopathology

The histopathology results are of great interest, especially regarding the *Eimeria sp.* infection as only one other . cormorant infection has been described in North America in a Double-crested cormorant population suffering from a renal coccidiosis outbreak in Georgia in 2001, and two descriptions from European cormorants in 1933, and 1893 (Yabsley et al. 2002). The *Eimeria sp*. responsible for the 2001 outbreak was determined to be a novel species, *Eimeria auritus* and the detection of *Eimeria sp*. in LALIV Neotropic cormorants could represent both a novel species or another host species for the *Eimeria auritus* (Yabsley & Gibbs 2006). The proliferance of kidney disease and kidney lesions are strongly associated with elevated dietary Hg, a possible symptom that the Hg detected in our cormorants' is indicative of stress in conjunction with the cocktail effect of other OCPs, PCBs, and PBDEs (Wolfe et al. 1998, Bemis & Seegal 1999). Lesions along the glomeruli, tubules, and interstitial tissues of the kidney, including the interstitial nephritis seen in many of our birds are known to be associated with organochlorine concentration, particularly DDT and metabolites and total PCB levels (Sonne et al. 2013).

CONCLUSIONS

Our results indicate that Neotropic cormorants roosting in two colonies along the Trinity River are not at risk for adverse effects due to OCPs, PCBs, PBDEs, and Hg. However, the histopathology results indicate that there may be some damage associated with some of these chemicals and more likely from MeHg on the kidneys, as well as common parasitic infections of *Eimeria* sp. and trematodes. OCPs and PCBs were found in low quantities coinciding with past studies of the Trinity River and North East Texas noting a decline in these classes of contaminants. MeHg was found in a different concentration pattern than prior studies have shown for the region. The reason for this difference may be because of unique characteristics of the RCWMA colony feeding on fish in a low productivity area, concentrating the MeHg more than the much more productive LALIV colony's feeding area.

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