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BIOMARKERS OF EXPOSURE AND EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON SWALLOWS NESTING ALONG THE RIO GRANDE, TEXAS, USA

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Abstract—We collected adult cave swallows (*Petrochelidon fulva*) and cliff swallows (*P. pyrrhonota*) during the breeding seasons in 1999 and 2000 from eight locations along the Rio Grande from Brownsville to El Paso (unless otherwise specified, all locations are Texas, USA) and an out-of-basin reference location. Body mass, spleen mass, hepatosomatic index (HSI), gonadosomatic index (GSI), thyroxine (T_4) in plasma, DNA damage measured as the half-peak coefficient of variation of DNA content (HPCV) in blood cells, as well as acetylcholinesterase and butyrylcholinesterase in brain were compared with concentrations of organochlorines, metals, and metalloids in carcasses to determine potential effects of contaminants on swallows during the breeding season. Concentrations of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p′*-DDE) were significantly greater in swallows from El Paso than in those from most locations, except for Pharr and Llano Grande. All swallows from these three locations had *p,p′*-DDE concentrations of 3 μg/g wet weight or greater. Swallows from El Paso either had or shared the highest concentrations of *p,p′*-DDE, polychlorinated biphenyls, and 13 inorganic elements. Swallows from El Paso exhibited greater spleen mass and HPCV values as well as lower T_4 values compared with those from other locations. Thyroxine was a potential biomarker of contaminant exposure in swallows of the Rio Grande, because it was negatively correlated with *p,p′*-DDE and Se. Spleen mass was positively correlated with selenium and HSI and negatively correlated with body mass, GSI, Mn, and Ni. Overall, the present study suggests that insectivorous birds living in areas of high agricultural and industrial activity along the Rio Grande bioaccumulate environmental contaminants. These contaminants, particularly *p,p′*-DDE, may be among multiple factors that impact endocrine and hematopoietic function in Rio Grande swallows.

Keywords—Biomarkers 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene Inorganic elements Rio Grande Swallows

INTRODUCTION

During 1993, the Rio Grande was considered to be the most polluted river in America, primarily because of sewage and industrial pollution (http://www.americanrivers.org). Concern regarding widespread pollution and its potential impacts on humans and wildlife prompted several studies and monitoring programs for the Rio Grande [1,2]. However, most contaminant studies were focused on water, sediments, and fish [1,3]. Until recently, no attempt has been made to assess the degree of pollution of the Rio Grande in relation with the impacts of contaminants on terrestrial wildlife.

A large section of the Rio Grande constitutes the border between the United States and Mexico. Water from the Elephant Butte Reservoir in the upper Rio Grande Basin is used primarily for irrigation and municipal purposes; thus, the Rio Grande below El Paso (unless otherwise specified, all locations are Texas, USA) receives water mainly from storm runoff, treated municipal wastewater, and irrigation return flows [4]. The Rio Conchos flows from Mexico into the Rio Grande near Presidio/Ojinaga and provides more than three-quarters of the Rio Grande flow. Throughout the lower part of the basin, from Falcon Reservoir to the Gulf of Mexico, water withdrawn for irrigation does not return to the Rio Grande but, rather, returns to other irrigation canals or evaporates. The river discharges directly into the Gulf of Mexico, whereas floodways and irrigation canals empty into the Laguna Madre.

The Lower Rio Grande Valley (LRGV) comprises approximately 420,240 ha of cropland and is one of the primary agricultural regions of the Texas–Mexico border (http://www.nass.usda.gov/census/census97/volume1/tx-43/toc97.htm); however, other, smaller agricultural regions occur along the Rio Grande from El Paso to Brownsville. Potential contaminant sources along the Rio Grande include pesticides and fertilizers used in agriculture, contaminants in untreated municipal wastewater, and contaminants from electronic, textile, and other assembly plants, most commonly known as maquiladoras. In 1998, approximately 3,000 permits to operate maquiladora plants existed in Mexico, 80% of which were located along the border [5].

Organochlorines (OCs), organophosphates (OPs), carbamate pesticides, polychlorinated biphenyls (PCBs), metals and metalloids, polycyclic aromatic hydrocarbons (PAHs), and a few other environmental pollutants have been reported in water, sediments, and biota of the Texas portion of the Rio Grande for several years [3,4]. An analysis of contaminant data from 1965 to 1995 revealed that the LRGV had the most locations with the highest concentrations of contaminants in biological samples [3]. However, two other regions (Big Bend and El Paso) also had high concentrations of contaminants, particularly 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p′*-DDE,

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Fig. 1. Map with locations of the collection sites along the Rio Grande, Texas, USA: Brownsville (25°57′N, 97°25′W), Llano Grande (26°07′N, 97°57′W), Pharr (26°09′N, 98°10′W), Mission (26°11′N, 98°19′W), Falcon Lake (26°50′N, 99°15′W), Laredo (27°24′N, 99°28′W), Del Rio (29°19′N, 100°50′W), and El Paso (31°39′N, 106°19′W). The reference location in Somerville (30°20′N, 96°28′W) is not shown on the map.

hereafter DDE), Se, and Hg. Some of these chemicals could have negative effects on wildlife, including endocrine disruption, altered behavior, and reduced reproductive success [6].

Cave swallows (*Petrochelidon fulva*) and cliff swallows (*Petrochelidon pyrrhonota*) nest throughout the Rio Grande in culverts and under bridges over standing water [7]. Both species are insectivorous and feed mostly on aerial insects [8,9]. Exposure of swallows to agricultural pesticides, particularly OPs and carbamates, has been widely documented [10,11]. Swallows can be useful avian models for monitoring environmental contaminants because of their wide distribution, association with a variety of aquatic and terrestrial habitats, foraging areas near their nests, and insectivorous diets. The objectives of the present study were to test the feasibility of using cave and cliff swallows as indicators of exposure and effects of environmental contaminants in the Rio Grande from El Paso to the Gulf of Mexico. We tested the hypothesis that persistent OC pollutants and inorganic contaminants accumulate in insectivorous birds in the Rio Grande at levels that could be associated with thyroid hormone alterations and potential DNA damage. We also determined acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities in swallow brains as indicators of potential exposure to OP and carbamate insecticides.

MATERIALS AND METHODS

Sampling locations and tissue collection

We collected a total of 137 adult cave and 55 adult cliff swallows during the breeding season in the years 1999 (n = 129) and 2000 (n = 63) from eight locations along the Rio Grande from Brownsville to El Paso and from one reference location near Somerville (Fig. 1 and Table 1). The locations near the Rio Grande were selected based on proximity to urban, industrial, and agricultural settings and potential for significant contaminant contribution to the environment. The location in Somerville (500 km north of the Rio Grande) was selected as a reference site to compare and contrast biomarker and contaminant concentrations with locations in the Rio Grande. Cave swallows were collected at most locations in the Rio Grande (except for Falcon Lake); however, cliff swallows were collected only from Brownsville, Falcon Lake, El Paso, and Somerville. Most cave swallows were collected from Llano Grande (n = 31) and Pharr (n = 36), and most cliff swallows were collected from Falcon Lake (n = 20) and El Paso (n = 13). During 2000, swallows were collected only from Brownsville, Llano Grande, Pharr, and El Paso (Table 1).

Swallows were captured from late May to early June with mist nets dropped under bridges over canals and drainages from the Rio Grande where the nesting sites were located. Most swallows were collected between 7:30 and 10:00 AM from a single nesting site at each location except for El Paso and Brownsville, where two nearby locations within the same drainage system were sampled. At the time of collection, most colonies were in the late-incubation and hatching stages. The swallows were taken out of the net and placed in nylon bags for up to 15 min until blood collection. Between 0.5 and 1 ml of blood was collected from the jugular vein with 1-ml tuberculin syringes and 25-gauge needles. Immediately after blood collection, the birds were killed by cervical dislocation. Carcasses were weighed, wrapped in aluminum foil, placed in plastic bags, and stored in dry ice until taken to the laboratory

Table 1. Collection locations and number of swallows collected in the Rio Grande and a reference site (TX, USA)[a]

Location	Coordinates		1999				2000			
			CASW		CLSW		CASW		CLSW	
	Latitude (N)	Longitude (W)	M	F	M	F	M	F	M	F
Brownsville	25°57′	97°25′	0	4	2	4	2	1	2	1
Llano Grande	26°07′	97°57′	10	8			6	7		
Pharr	26°09′	98°10′	11	10			5	10		
Mission	26°11′	98°19′	3	9						
Falcon	26°50′	99°15′			15	5				
Laredo	27°24′	99°28′	8	12						
Del Rio	29°19′	100°50′	6	7						
El Paso	31°39′	106°19′	2	0	7	6	6	10	1	1
Somerville[b]	30°20′	96°28′							7	4

[a] CASW = cave swallows; CLSW = cliff swallows; F = female; M = male.
[b] Reference location, approximately 500 km north of the Rio Grande.

for further analyses. Approximately three to five drops of whole blood were preserved in 500 μl of freezing media (Ham's F-10 media with 18% fetal calf serum and 10% glycerin) and placed in cryogenic vials. Blood and media were thoroughly mixed, allowed to saturate, then stored in dry ice and, ultimately, in an ultracold freezer (−80°C) until further analysis. The remaining blood was placed in 3-ml blood-collection tubes containing sodium heparin, and the tubes were placed immediately on ice until all samples were collected. Blood tubes were centrifuged for 15 min at 1,500 rpm approximately 2 h after field collection. Plasma was extracted and immediately stored on dry ice.

In the laboratory, the spleen, liver, gonads, brains, and a small portion (0.5 g) of breast muscle were removed and stored in cryovials at −80°C until further analyses. The rest of the carcass was used for chemical analysis after removing the remaining head, legs, beak, stomach contents, and feathers. All tissues were weighed with an Ohaus® microbalance (Ohaus, Florham Park, NJ, USA) to the nearest 0.01 g. Gonadosomatic index (GSI) and hepatosomatic index (HSI), with liver and gonad mass expressed as a percentage of body mass, were obtained to determine reproductive status, liver enlargement, and their relationships with contaminant loads in carcasses.

Flow cytometry

Blood samples (whole blood stored in freezing media) were used to determine cell-to-cell variation in DNA content following the methods described by Vindelov and Christensen [12]. Samples were randomized before processing to avoid experimental bias. The samples were quickly thawed, and 50 μl of whole blood were added to a trypsin/detergent solution for digestion. After 10 min, trypsin inhibitor and RNase were added to stop the reaction and to degrade RNA, which also could bind with the dye. The solution was filtered through a 30-μm nylon mesh, and propidium iodide dye was added. After a minimum of 15 min on ice, the samples were analyzed on a Coulter Epics Elite flow cytometer (Beckman Coulter, Fullerton, CA, USA). Cells were illuminated with a laser (Coherent, Santa Clara, CA, USA) at 514 nm and 500 mW of power to excite the propidium iodide. Fluorescent emission was then measured. Cells were gated on side scatter, forward scatter, and the ratio of peak to integrated fluorescence. Ten-thousand nuclei, which satisfied all gating parameters, were measured from each sample, and the intercellular variation in DNA content was reported as the half-peak coefficient of variation (HPCV). Greater variation in DNA content results in broader distribution peaks, and the coefficient of variation (CV) is used to describe the width of the peak: $CV = 100 \cdot (\text{standard deviation/mean})$ [13]. The HPCV was calculated by taking the area under the distribution curve starting from the point that is half the height of the distribution curve. Samples that did not have 10,000 nuclei counted by the end of the 4-min run were excluded from statistical analyses.

Thyroid hormone radioimmunoassays

Thyroid hormones were measured by radioimmunoassay (RIA) using procedures described by Leiner et al. [14]. Standard curves ranged from 15.6 to 125 pg/tube. Samples were prepared by diluting plasma with RIA buffer to 1:20 (thyroxine [T_4]) and 1:5 (triiodothyronine [T_3]). Plasma that was dark red (~10% of samples) was considered to be hemolyzed and was not analyzed. Incubation volumes were 50 μl of diluted plasma, 50 μl of antibody (anti-T_4 diluted 1:2400, anti-T_3 diluted 1:12,000; both from ICN Biomedical, Costa Mesa, CA, USA), and 150 μl of RIA buffer containing 25,000 cpm of high-specific-activity, radioiodinated T_3 or T_4 (both from Perkin-Elmer, Billerica, MA, USA). Radioimmunoassay data were analyzed using the RIAMENU program developed by Dr. Paul Licht (University of California, Berkeley, CA, USA).

Initial analysis indicated that most of the T_3 values in swallows were below the sensitivity of the T_3 assay (<0.5 ng/ml). Because sufficient blood for measurement in a more sensitive RIA was not available, T_3 was not further analyzed. The T_4 RIA was validated for swallows using four pools of swallow plasma: A cave swallow pool comprising 150 μl of plasma randomly selected from three samples collected from Pharr, three samples from El Paso, and two samples from Del Rio; a cave swallow pool comprising 150 μl of plasma randomly taken from five samples from Pharr, two samples from Del Rio, and one sample from El Paso; an El Paso pool comprising 120 μl each from three randomly selected 1999 El Paso birds; and a Del Rio pool comprising 275 μl from two 1999 birds. To confirm that cave swallow plasma diluted parallel to the standard curve, all four pools were serially diluted from 1:2 to 1:16 in RIA buffer for comparison to the standard curve. The minimum detectable limit for T_4 was 0.2 ng/ml. All pools ran parallel to the T_4 standard curve. To test for hormone recovery from plasma, T_4 measured in a 250-μl aliquot of each pool supplemented with 5 μl of 10 ng/ml of T_4 in MeOH was compared to T_4 in the same pool supplemented with 5 μl of MeOH. Mean hormone recovery for the four pools was 104.5%. The intra-assay CV for the T_4 assay was 10.27 ($n = 7$), and the interassay CV was 8.84 ($n = 5$).

Cholinesterase analysis

We analyzed AChE and BChE activities in swallow brains according to the method described by Ellman et al. [15] as modified by Gard and Hooper [16]. Briefly, brain tissue was excised from carcasses, mixed with 0.05 M Tris buffer, and then homogenized. The homogenate was further diluted with the same buffer before cholinesterase analysis. Diluted brain was assayed with the use of a 96-well microplate reader. All samples were run in triplicate at 25°C. Acetylthiocholine iodide, 5,5′-dithiobis(2-nitrobenzoic acid), Tris 8.0 buffer, and diluted enzyme were added to microplate wells. Cholinesterase activities were expressed as micromoles of acetylthiocholine hydrolyzed per minute per gram of brain tissue. Butyrylcholinesterase activity was determined using a selective BChE inhibitor, tetraisopropylpyrophosphoramide (iso-OMPA), and incubating for 5 min. Activity remaining after incubation for this period was considered to be from AChE; thus, BChE activity was estimated as the difference between measured total cholinesterase and AChE activity.

Chemical analysis

We analyzed 27 cave and 15 cliff swallows (three to eight individuals from each location) for OC contaminants and inorganic elements. The methodology for OCs and inorganic elements has been described elsewhere [7]. The analyzed OCs included hexachlorobenzene, hexachlorocyclohexane (α, β, γ, and δ isomers), chlordane (α and γ isomers), *cis*-nonachlor, *trans*-nonachlor, dieldrin, endrin, heptachlor epoxide, mirex, oxychlordane, toxaphene, DDT, DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), and PCBs.

The inorganic elements analyzed included Al, As, Be, B,

Table 2. Body and spleen mass (g), hepatosomatic index (HSI), and gonadosomatic index (GSI) from swallows nesting along the Rio Grande and a reference site (TX, USA)[a]

Location	Body mass		HSI		GSI		Spleen mass[b]	
	M	F	M	F	M	F	M	F
Cave swallows								
Brownville	19.5 ± 1.0 (2) A	20.8 ± 1.0 (5) AB	3.08 (1) A	4.0 ± 0.56 (5) AB	1.85 A	0.40 ± 0.6 A	0.019 A	0.041 ± 0.03 AB
Llano Grande	19.9 ± 1.3 (16) A	20.1 ± 1.9 (15) B	3.08 ± 0.38 (16) A	3.36 ± 0.29 (15) B	1.71 ± 0.33 A	0.32 ± 0.64 A	0.020 ± 0.02 A	0.030 ± 0.02 B
Pharr	20.1 ± 1.4 (16) A	21.1 ± 1.3 (20) AB	3.14 ± 0.26 (12) A	3.29 ± 0.52 (11) B	1.64 ± 0.44 A	0.61 ± 0.86 A	0.034 ± 0.02 A	0.032 ± 0.02 B
Mission	19.7 ± 0.3 (3) A	20.4 ± 1.9 (9) AB	2.03 ± 0.3 (3) A	3.46 ± 0.72 (9) AB	2.03 ± 0.3 A	0.46 ± 0.72 A	0.015 ± 0.01 A	0.022 ± 0.01 B
Laredo	20.9 ± 1.0 (8) A	22.1 ± 1.4 (12) A	3.08 ± 0.25 (8) A	4.05 ± 0.74 (12) A	1.81 ± 0.54 A	0.36 ± 0.47 A	0.016 ± 0.01 A	0.035 ± 0.03 B
Del Rio	20.3 ± 0.3 (6) A	19.4 ± 0.7 (7) B	3.31 ± 0.21 (6) A	3.8 ± 0.44 (7) AB	1.72 ± 0.3 A	0.10 ± 0.04 A	0.036 ± 0.02 A	0.037 ± 0.01 B
El Paso	19.6 ± 1.4 (8) A	20.4 ± 1.3 (10) AB	3.53 ± 0.46 (4) A	3.7 ± 0.25 (4) AB	1.62 ± 0.4 A	0.30 ± 0.15 A	0.026 ± 0.01 A	0.094 ± 0.03 A
Cliff swallows								
Brownville	19.4 ± 0.4 (4) A	19.6 ± 1.5 (5) A	3.53 ± 0.10 (4) A	4.04 ± 0.42 (5) A	1.41 ± 0.5 A	0.26 ± 0.28 A	0.024 ± 0.01 AB	0.039 ± 0.01 A
Falcon Lake	18.4 ± 1.0 (15) A	18.1 ± 0.8 (5) A	3.31 ± 0.28 (15) A	3.75 ± 0.28 (5) A	1.45 ± 0.6 A	0.16 ± 0.01 A	0.032 ± 0.02 B	0.051 ± 0.02 A
El Paso	19.2 ± 1.0 (8) A	19.7 ± 2.1 (7) A	3.66 ± 0.50 (8) A	3.58 ± 0.28 (7) A	1.33 ± 0.4 A	0.28 ± 0.24 A	0.072 ± 0.06 A	0.049 ± 0.03 A
Somerville[c]	18.6 ± 1.0 (7) A	18.9 ± 0.7 (4) A	ND	ND	ND	ND	ND	ND

[a] Values are presented as the arithmetic mean ± standard deviation. Sample sizes are given in parentheses. Within columns, for each species, values not sharing the same letter were significantly different. F = female; M = male; ND = not determined.
[b] Sample sizes for spleen mass and GSI were the same as those for HSI (in parentheses).
[c] Reference site.

Ba, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Se, Sr, V, and Zn. Approximately 0.2 to 0.5 g of sample was digested in screw-cap Teflon® bombs with concentrated, high-purity nitric acid. Bombs were heated from 2 to 8 h and opened three times to release CO_2 buildup. This procedure resulted in total digestion, and all trace elements in the tissue were solubilized. The digest was analyzed for most elements with a model ELAN 2DRC inductively coupled–plasma mass spectrometer (Perkin-Elmer, Norwalk, CT, USA). Arsenic and Se were analyzed by a graphite furnace mounted to an atomic absorption spectrophotometer (model 4100 ZL; Perkin-Elmer). Mercury was analyzed by the standard cold-vapor atomic absorption method based on the U.S. Environmental Protection Agency (U.S. EPA) method 7471 with an update based on U.S. EPA method 1631 [17]. The lowest detection limits for trace elements ranged from 0.005 to 2 μg/g dry weight. Percentage recoveries of spiked samples and certified reference materials were greater than 90%. Mean relative percentage differences between duplicates were less than 10%.

Statistical analysis

All statistical analyses were performed with the use of SAS® software (Ver 8.2; SAS Institute, Cary, NC, USA). At only two locations (Llano Grande and Pharr) were sufficient numbers of males (n = 32) and females (n = 35) collected during 1999 (n = 39) and 2000 (n = 28); thus, comparisons of annual differences in body mass, GSI, HSI, spleen mass, HPCV, and T_4 were determined only for these locations. If no significant differences between years were observed, then the data for both years were combined, by sex, for the statistical analysis. The data for body mass, spleen mass, GSI, and HSI were normally distributed; thus, we used a t test to determine differences between years and between males and females. We used a t test applied to ranks (data were not normally distributed) to test for differences between years in HPCV and T_4 content of males and females at Llano Grande, Pharr, and El Paso. If no significant differences in HPCV and T_4 values were observed between years and between sexes, then the data were combined for other statistical analyses. Body mass, spleen mass, GSI, HSI, HPCV, and T_4 were compared by sex among locations with the general linear model (GLM) method.

For the cholinesterase data, at only two locations (Llano Grande and Pharr) were sufficient samples available for comparisons between years. If no significant differences between years were observed, then the data for both years were combined, by sex, for statistical analysis. Differences between years could not be determined statistically in cliff swallows from Brownsville and El Paso because of small numbers; however, the data were numerically similar between years. The cholinesterase data were normally distributed as determined by the univariate and Shapiro-Wilks methods. We used t tests to compare both brain cholinesterase activities between male and female cave swallows from locations in Llano Grande, Pharr, Mission, Laredo, and Del Rio and in cliff swallows from Brownsville, Falcon Lake, El Paso, and Somerville. Differences between species also were compared by t tests. To determine if AChE and BChE differences among locations were influenced by body mass, we performed analysis of covariance (ANCOVA) by species and sex with the use of GLM methods using location as a class variable and body mass as a covariate. Type III sum of squares and F statistics were used to determine the significance of each variable. If the effect of body mass was not significant, differences in cholinesterase activities

Table 3. Median half-peak coefficient of variation (HPCV) and thyroxine (T_4; ng/ml) values in swallows from the Rio Grande and a reference site (TX, USA)[a]

Species	Location	HPCV (DNA)	T_4
Cave swallow	Brownsville	3.0 [2.4–7.3] (4) ABC	8.5 [3.8–12.3] A
	Llano Grande (1999)	2.2 [1.7–5.2] (18) C	11.2 [3.8–24.6] A
	Llano Grande (2000)	4.1 [2.4–8.2] (13) A	12.7 [7.7–20.6] A
	Pharr (1999)	2.3 [1.4–5.4] (21) BC	12.3 [1.7–22.8] A
	Pharr (2000)	2.5 [2.1–4.8] (15) BC	8.9 [4.9–18.9] A
	Mission	2.2 [1.8–3.8] (12) BC	10.5 [2.2–21.4] A
	Laredo	2.2 [1.6–3.2] (20) BC	13.7 [5.4–38.8] A
	Del Rio	3.2 [1.9–6.1] (13) AB	11.7 [5.0–46.8] A
	El Paso	2.3 [1.7–4.1] (18) C	12.3 [3.0–23.6] A
Cliff swallow	Brownsville	3.1 [2.3–4.5] (9) AB	16.8 [8.9–19.4] A
	Falcon Lake	2.9 [2.1–4.0] (20) B	13.7 [6.8–34.7] A
	El Paso	4.0 [1.9–5.7] (15) A	7.9 [2.1–21.0] B
	Somerville	2.1 [1.6–3.1] (11) C	15.8 [3.4–25.7] A

[a] Ranges are provided in brackets and sample sizes in parentheses. Within columns, for each species, values not sharing a letter were significantly different.

among locations were determined by one-way analysis of variance with the GLM method. The Tukey multiple-comparisons procedure was used to determine which means were significantly different.

We used *t* tests to determine differences in OC and inorganic element concentrations between species at two locations (Brownsville and El Paso) and between years at four locations (Brownsville, Llano Grande, Pharr, and El Paso). The contaminant data were not significantly different between species or between years for each location; thus, the data for both species and both years were combined for each location. Seventy-nine percent ($n = 33$) of the carcasses analyzed were females, and no differences were found in contaminant concentrations between sexes (Llano Grande, Pharr, and El Paso). Therefore, we also combined data from both sexes for the statistical analysis. The data were log-transformed to meet the assumptions of normality. One-half of the limit-of-detection values was used to substitute for the nondetect values in all inorganic elements and OCs that were detected in more than 50% of samples. Differences in concentrations of contaminants among locations were determined by the GLM method.

Spearman rank correlation procedures were used to determine relationships between ranks of the biomarkers T_4, HPCV, AChE, and BChE and morphometric values, such as body mass, HSI, GSI, and spleen mass, with contaminant concentrations in swallow carcasses from all locations. For all statistical analyses, the level of significance was set at $p < 0.05$.

RESULTS

Body mass, spleen mass, HSI, and GSI

No significant differences were found between collection years in body mass of male and female cave swallows at any location, and no significant differences were observed between sexes in body mass of cave swallows from Brownsville, El Paso, Llano Grande, and Mission. However, body mass was significantly greater in males than in females from Del Rio ($p < 0.05$), and body mass was greater in females than in males from Laredo and Pharr ($p < 0.05$). No significant differences were found among locations in body mass of male cave swallows from the Rio Grande; however, females from Laredo were significantly heavier ($F_{6,71} = 3.3$, $p < 0.01$) than those from Llano Grande and Del Rio but were similar to those from other locations (Table 2). In general, female cave swallows were slightly heavier than males except for those from Del Rio. No significant differences were found in body mass of cliff swallows either between sexes and among locations, including the reference location in Somerville (Table 2).

Hepatosomatic index was significantly greater in female than in male cave swallows from Brownsville, Laredo, and Llano Grande, and it also was greater in female than in male cliff swallows from Brownsville and Falcon Lake ($p < 0.01$). Gonadosomatic index was significantly greater in males than in females of both species at all locations ($p < 0.01$). Spleen mass was similar between sexes at all locations except for El Paso, where female cave swallows had significantly greater spleen mass compared with male cave swallows ($p < 0.01$) (Table 2). Gonadosomatic index, spleen mass, and HSI were not significantly different among male cave swallows from all locations. The GSI also was similar among females from all locations. However, HSI was significantly greater in females from Laredo ($F_{6,56} = 3.92$, $p < 0.01$) than in those from Llano Grande and Pharr, and spleen mass was significantly greater in females from El Paso ($F_{6,56} = 5.9$, $p < 0.0001$) than in those from all other locations except for Brownsville.

In cliff swallows, HSI and GSI were not significantly different among males or females from three locations. Spleen mass also was similar in females from all locations; however, it was significantly greater in males from El Paso ($F_{2,24} = 4.2$, $p < 0.05$) than in those from Falcon Lake.

Variation in DNA content

No sex differences in HPCV values were found in cave swallows. The HPCV of DNA content was significantly greater in cave swallows (sexes combined) collected during 2000 ($p < 0.001$) than in those collected during 1999 at Llano Grande; however, HPCV values were not significantly different between years at other locations. The HPCV values of cave swallows were significantly different among locations ($F_{8,125} = 7.3$, $p < 0.0001$) and were greater in cave swallows from Llano Grande during 2000 than in those from Pharr, Mission, and El Paso from both years combined (Table 3). The HPCV values also were significantly greater in cave swallows from Del Rio than in those from El Paso and Llano Grande during 1999.

In cliff swallows, no significant differences in HPCV values were found between males and females from any location. However, HPCV values of males and females combined were significantly greater in cliff swallows from El Paso than in those from Falcon Lake and Somerville, and these values also

Table 4. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity (μm acetyl thiocholine iodide hydrolyzed/min/g tissue) in brain of cave and cliff swallows from the Rio Grande and a reference site (TX, USA)[a]

Location	Cave swallow						Cliff swallow					
	n		AChE		BChE		*n*		AChE		BChE	
	M	F	M	F	M	F	M	F	M	F	M	F
Brownsville	1	5	14.9 AB	12.4 ± 0.6 BC	0.40 A	0.46 ± 0.2 A	4	5	13.7 ± 1.7 A	13.1 ± 2.4 A	0.78 ± 0.5 A	0.81 ± 0.4 A
Llano Grande	11	10	13.7 ± 1.6 B	14.0 ± 1.4 ABC	0.60 ± 0.3 A	0.70 ± 0.5 A						
Pharr[b]	16	20	13.8 ± 1.4 B	15.9 ± 2.2 A	0.84 ± 0.3 A	1.00 ± 0.7 A						
Mission	3	9	12.2 ± 0.9 B	14.0 ± 2.0 ABC	0.71 ± 0.3 A	1.12 ± 0.4 A						
Falcon Lake							14	5	13.9 ± 1.3 A	14.1 ± 1.4 A	0.75 ± 0.4 A	1.00 ± 0.5 A
Laredo[b]	8	12	14.7 ± 1.0 AB	11.7 ± 2.8 C	0.80 ± 0.1 A	0.80 ± 0.5 A						
Del Rio	6	7	12.8 ± 2.0 B	13.5 ± 1.5 ABC	0.71 ± 0.5 A	0.65 ± 0.5 A						
El Paso	8	10	16.4 ± 2.5 A	15.6 ± 1.2 AB	1.10 ± 0.6 A	0.84 ± 0.5 A	8	7	14.3 ± 0.8 A	14.6 ± 2.3 A	1.07 ± 0.4 A	1.11 ± 0.5 A
Somerville							7	4	15.2 ± 3.4 A	14.2 ± 1.5 A	0.8 ± 0.3 A	0.43 ± 0.5 A

[a] Values are presented as the mean ± standard deviation. Within columns, values not sharing the same letter are significantly different; M = male and F = female.
[b] AChE activity in brain of cave swallows from these locations was significantly different between males and females.

Table 5. Dichlorodiphenyldichloroethylene (DDE), oxychlordane, and polychlorinated biphenyls (PCBs; μg/g wet wt) in carcass of swallows from the Rio Grande and a reference location (TX, USA)[a]

Location	*n*	*p,p′*-DDE[b]	Oxychlordane	PCBs
Brownsville	6	0.8 B (0.2–2.0)	0.004 BC (0.001–0.008)	0.245 ABC (0.148–0.405)
Llano Grande	8	6.6 A (2.8–13.1)	0.021 A (0.011–0.035)	0.289 ABC (0.037–0.675)
Pharr	6	7.4 A (4.1–11.4)	0.026 A (0.017–0.048)	0.362 AB (0.194–0.554)
Falcon Lake	4	0.5 B (0.3–0.8)	0.003 C (0.002–0.005)	0.068 D (0.047–0.119)
Laredo	4	0.9 B (0.5–1.5)	0.003 C (0.002–0.005)	0.134 BCD (0.086–0.228)
Del Rio	4	0.7 B (0.5–1.4)	0.004 BC (0.002–0.006)	0.069 D (0.047–0.108)
El Paso	7	12.4 A (6.6–24.7)	0.008 B (0.006–0.011)	0.499 A (0.316–0.670)
Somerville	3	0.3 B (0.2–0.6)	0.005 BC (0.004–0.006)	0.101 CD (0.086–0.122)

[a] Values are presented as the geometric mean, with ranges in parentheses. Means that do not share the same letter within each column are significantly different.
[b] DDE concentrations have been discussed separately in relation with stable isotopes [7].

were greater in those from Falcon Lake and Brownsville than in those from Somerville ($F_{3,51} = 15.5$, $p < 0.0001$) (Table 3).

Thyroid hormones

Thyroxine concentrations were similar between males and females at each location; however, T_4 concentrations were significantly greater in female cave swallows ($p < 0.05$) from Pharr during 1999 than in those collected during 2000. Thus, the data were kept separate for each year for statistical comparisons (Table 3). No significant differences were found in T_4 concentrations among locations in cave swallows (males and females combined). In cliff swallows, no significant differences were found in T_4 concentrations between males and females at any location; however, T_4 concentrations were significantly greater in cliff swallows from Brownsville, Falcon Lake, and Somerville ($F_{3,51} = 8.2$, $p < 0.0001$) than in those from El Paso (Table 3).

Cholinesterase activity

No significant differences in AChE and BChE activities were found between male cave swallows collected during 1999 and those collected during 2000 at El Paso and Pharr and between female cave swallows collected during 1999 and 2000 at Llano Grande and Pharr. Only these locations had sufficient data for both years. The BChE activities also were similar between male and female cave and cliff swallows (Table 4). In contrast, AChE activity was significantly greater ($p < 0.001$) in male than in female cave swallows from Laredo, whereas female cave swallows from Pharr had significantly greater activity compared with male cave swallows ($p < 0.01$) (Table 4).

Body mass did not have a significant effect on the variation of AChE and BChE activities in male and female cave or cliff swallows (ANCOVA). Mean AChE activity was significantly different among male cave swallows ($F_{6,46} = 4.24$, $p < 0.001$) from various locations in the Rio Grande and was greater in male cave swallows from El Paso than in those from Pharr,

Table 6. Inorganic elements (μg/g dry wt) in swallows from the Rio Grande and a reference location (TX, USA)[a]

Location	*n*	Al	As	B	Ba	Cd	Cr
Brownsville	6	19.5 A (12.8–31.5)	0.24[b]	0.98 B (0.54–2.51)	4.4 ABC (2.7–7.5)	0.11 B (0.06–0.17)	1.09 A (0.6–3.1)
Llano Grande	8	13.2 A (7.8–21.3)	0.09 B (0.06–0.12)	0.98 B (0.6–1.43)	4.0 BC (2.3–6.8)	0.10 B (0.09–0.13)	0.88 A (0.71–0.98)
Pharr	6	19.9 A (5.1–45.8)	0.04 B (0.02–0.15)	0.84 B (0.2–1.4)	3.4 BC (1.9–7.2)	0.10 B (0.07–0.20)	0.68 A (0.46–1.52)
Falcon Lake	4	17.3 A (15.2–19.3)	ND	ND	4.6 ABC (4.0–5.7)	0.11[b]	1.01 A (0.76–1.25)
Laredo	4	16.3 A (11.1–29.9)	ND	ND	7.0 AB (4.0–12.7)	0.18 A (0.13–0.26)	0.94 A (0.77–1.13)
Del Rio	4	24.6 A (14.6–41.0)	ND	ND	4.8 ABC (3.9–5.5)	ND	1.13 A (1.04–1.24)
El Paso	7	22.3 A (18.9–25.0)	0.29 A (0.23–0.37)	1.58 A (1.21–2.48)	3.3 C (2.5–4.8)	0.18 A (0.13–0.22)	0.74 A (0.54–0.87)
Somerville	3	12.7 A (9.4–16.0)	ND	ND	9.0 A (8.3–10.2)	0.13 AB (0.12–0.14)	1.12 A (0.87–1.3)

[a] Values are presented as the geometric mean, with ranges in parentheses. Means that do not share the same letter within each column are significantly different. Selenium data were discussed previously in Mora et al. [7]. ND = not determined.
[b] Detected in one sample.

Llano Grande, Del Rio, and Mission, but it was similar at other locations (Table 4). In contrast, no significant differences were found among locations in BChE activity in males. In females, brain AChE activity was significantly greater in cave swallows from Pharr than in those from Laredo and Brownsville, but it was similar to that in those from the rest of the locations ($F_{6,64} = 7.02$, $p < 0.0001$). No significant differences were found among locations in BChE activities in females.

Mean AChE and BChE activities were not significantly different between male and female cliff swallows collected in 1999 in Brownsville, El Paso, or Falcon Lake and those collected in Somerville during 2000; the same was true for both years combined. Also, AChE and BChE activities were not significantly different among locations in male or female cliff swallows. Between species, AChE activity was not significantly different in male or female cave and cliff swallows from Brownsville; however, AChE activity was significantly greater ($p < 0.05$) in male cave swallows than in male cliff swallows from El Paso. The BChE activities were similar between sexes from both species at both locations.

Organochlorines

Only DDE, PCBs, and oxychlordane were detected in more than 50% of the samples (Table 5). No differences were found in contaminant concentrations between species or between years at each location. However, concentrations of DDE were significantly different among locations and were greater at El Paso ($F_{7,34} = 40$, $p < 0.0001$) than at most locations except for Pharr and Llano Grande, which were similar (DDE concentrations have been discussed previously in relation to $\delta^{15}N$ and $\delta^{13}C$ stable isotopes in tissues [7]). Concentrations of PCBs were significantly greater ($F_{7,34} = 10$, $p < 0.0001$) in swallows from El Paso than in those from Laredo, Del Rio, and Falcon Lake but were similar to those in swallows from Pharr, Llano Grande, and Brownsville. The PCBs in swallows from El Paso also were higher than in those from the reference site. Concentrations of oxychlordane, however, were significantly greater ($F_{7,34} = 19.8$, $p < 0.0001$) at Pharr and Llano Grande than at El Paso and the rest of the locations in the Rio Grande and the reference site.

Metals and metalloids

Beryllium and Mo concentrations were very low and near the limits of detection (mean range: Be, 0.02–0.26 μg/g dry wt; Mo, 0.05–0.18 μg/g dry wt) and were not significantly different among locations; therefore, the data were excluded from Table 6. Concentrations of Cd, Cu, Hg, Mn, V, and Se were significantly greater ($p < 0.01$) in swallows from El Paso than in those from at least one other location in the Rio Grande. Other elements, such as Al, Cr, Ni, Pb, and Zn, also were elevated in swallows from El Paso, although concentrations were not significantly different from those at other locations. Barium and Sr were the only elements detected at significantly lower concentrations in swallows from El Paso ($p < 0.005$) than in those from other locations (Table 6).

Relationships between morphometric and biomarker values and contaminant concentrations

Body mass, spleen mass, HSI, GSI, T_4, DNA damage (HPCV), AChE, and BChE were compared with concentrations of OCs, metals, and metalloids to determine relationships among them (Table 7). The following positive significant correlations were observed: Body mass with Mn, HSI with Pb, GSI with Mn and Zn, spleen mass with Se and HSI, AChE with DDE, and BChE with Hg (Table 7). Similarly, the following significant inverse correlations were detected: HSI with DDE and oxychlordane; GSI with HSI and Se; spleen mass with body mass, GSI, Mn, and Ni; HPCV with GSI and Ni; T_4 with DDE, Cu, and Se; and AChE with HSI (Table 7).

DISCUSSION

The results of the present study suggest that swallows (and likely other insectivorous species) nesting within some regions of the Rio Grande accumulate contaminants at levels shown to have physiological effects in other species. Concentrations of DDE were significantly greater in swallows from El Paso than in those from most locations except for Pharr and Llano Grande. All the swallows from these three locations had DDE concentrations of 3 μg/g wet weight or greater, which is approximately threefold greater than the estimated threshold value in the diet of some raptors at which reproductive failures

Table 6. Extended

Cu	Hg	Mn	Ni	Pb	Se	Sr	V	Zn
8.2 AB (5.6–11.4)	0.17 C (0.16–0.19)	3.2 AB (2.4–4.3)	0.23 B (0.10–0.84)	0.38 A (0.18–0.78)	1.1 BC (0.7–1.4)	26.6 A (18.3–64.3)	1.09 AB (0.8–2.5)	89.4 A (72.5–103)
9.5 AB (7.9–11.5)	0.15 D (0.14–0.16)	3.2 AB (2.4–4.9)	0.52 A (0.31–0.71)	0.19 A (0.05–0.57)	1.2 BC (0.8–1.8)	28.2 A (15.0–54.5)	0.46 B (0.13–1.15)	89.8 A (77.3–128)
8.7 AB (6.3–12.1)	0.20 B (0.12–0.28)	3.9 AB (2.7–5.5)	0.67 A (0.50–0.91)	0.19 A (0.09–0.29)	1.0 C (0.7–1.5)	20.6 AB (16.2–26.8)	0.65 AB (0.26–1.42)	101.1 A (71.7–141)
9.7 AB (7.8–10.8)	0.28[b]	2.7 B (2.3–3.1)	0.89 A (0.56–1.31)	0.25 A (0.05–2.18)	1.6 AB (1.3–1.9)	23.4 AB (16.2–29.7)	ND	92.2 A (73.7–107)
10.5 AB (9.1–11.2)	0.23 BC (0.21–0.27)	4.1 AB (3.1–5.4)	0.69 A (0.52–0.99)	0.89 A (0.53–1.83)	1.3 ABC (1.2–1.5)	16.8 AB (12.7–24.6)	ND	92.2 A (72.9–101)
9.6 AB (9.0–11.4)	ND	2.7 AB (2.0–3.6)	ND	ND	1.8 A (1.5–2.0)	15.0 AB (8.1–22.6)	1.24[b]	80.9 A (70.1–93.8)
11.2 A (10.7–11.9)	0.35 A (0.31–0.39)	4.1 AB (3.2–5.2)	0.45 AB (0.20–0.64)	0.35 A (0.19–0.58)	1.8 A (1.7–1.9)	14.6 B (13.7–15.1)	1.28 A (1.01–1.5)	99.3 A (85.1–112)
7.4 B (6.5–8.6)	ND	4.7 A (4.3–5.1)	1.42[b]	0.52 A (0.50–0.54)	0.9 C (0.8–1.0)	21.4 AB (20–22.2)	1.02 AB (0.82–1.3)	90.4 A (84.1–103)

could be observed [18]. Earlier analyses suggested that DDE concentrations were decreasing in biota in Texas [19,20]; however, results from more recent studies do not seem to conform to this pattern [21,22]. The DDE values reported in swallows from El Paso are among the highest reported in passerine birds from Texas during the last 20 years [3] and are above levels at which endocrine-disrupting effects have been observed in some wildlife [23].

The high concentrations of DDE in swallows from El Paso and LRGV could possibly be attributed to accumulation during the birds' winter migration to Latin America; however, stable isotope analysis of liver and muscle provided positive correlations between higher values of $\delta^{15}N$ in liver and DDE concentrations in carcasses [7]. Stable isotopes of $\delta^{15}N$ and $\delta^{13}C$ were linked to local sources; therefore, the major source of DDE contamination may be local, either from illegal use or from the high persistence of DDE in regions that experienced heavy use in the past. We collected birds during the middle of the breeding season, at least six weeks after the birds had returned to the breeding grounds and, most likely, after egg laying. Thus, females possibly had excreted some DDE through their eggs, and the contaminant values were more likely to reflect local sources. Nonetheless, DDE accumulation could be expected from regions with more recent use of DDT, such as Latin America. Oxychlordane and PCB concentrations were very low and likely not of concern for negative effects on swallow populations along the Rio Grande. These findings are not surprising, because previous studies also have reported low oxychlordane and PCB concentrations in biota of the Rio Grande [3].

Concentrations of metals and metalloids were all below levels that could be considered to be detrimental to the birds themselves or to the predators that feed on them. Greater concentrations of inorganic elements have been reported for other regions in the Rio Grande. Mercury concentrations in eggs of aplomado falcons (*Falco femoralis*) from the Brownsville region were as high as 4.1 μg/g during 1996 [24]. Carcasses of passerine birds (including swallows) from the Big Bend region had mean Hg and Se levels up to 2.5 and 14.6 μg/g dry weight, respectively, during 1997 [21].

Clear, significant differences were observed in concentrations of contaminants among locations in the Rio Grande. Interestingly, swallows from El Paso had the highest, or shared the highest, concentration of inorganic elements (13 of 15 [87%]) (Table 6) and OCs (two of three) (Table 5). These findings suggest that contaminants in the Rio Grande east of El Paso may pose a greater threat to wildlife, particularly predators that feed on swallows and other insectivorous birds.

Environmental contaminants were not correlated with body mass, but some were correlated with changes in spleen mass and HSI. Body mass was similar between male cave and cliff swallows from all locations; however, females tended to be heavier than males. Male and female cliff and cave swallows usually are similar in size [8,9]; however, increased weight in females should be expected early in the reproductive cycle, particularly during the egg-laying period. Spleen mass appears to be highly variable in birds; in willow tits (*Parus montanus*), it was larger during the molt period in late summer than during the rest of the year [25]. Nonetheless, an enlarged spleen mass during the breeding season also could result from an immune response to parasitic infestations and antigenic challenges [26]. Some of the swallows that we sampled were infested, to some degree, with endoparasites. However, we did not estimate parasite loads at each colony, and the colony at El Paso did not appear to be more infested than others. An enlarged spleen also could result from genetic damage of the blood cells (high HPCV values) as the spleen is stimulated to either produce or remove blood cells from the blood [26].

We did not observe any sex-related HPCV variations in cliff or cave swallows. Cave swallows from Llano Grande, Brownsville, and Del Rio and cliff swallows from El Paso and Brownsville had the greatest HPCV values among all swallow colonies. Swallows from our only out-of-basin reference site had lower HPCV values than those found at all sites within the Rio Grande Basin. The observed differences suggest that exposure of swallows to clastogenic compounds is more likely at some locations in the Rio Grande; however, cave swallows from El Paso also showed low HPCV values. Higher coefficients of variation in DNA content have been associated with DNA damage [27]. Chromium, PCBs, PAHs, and other compounds are known to induce DNA damage; however, our PCB values were low. Mean Cr concentrations were greater than 1 μg/g dry weight at two locations (Brownsville and Del Rio) that showed high HPCV values; however, the Somerville location away from the Rio Grande also had high Cr concentrations but low HPCV values. Chromium is very prevalent in samples of the Rio Grande and has been detected in 100% of sediment samples and in approximately 60% of tissue samples taken from 45 sites along the river [3,4]. Selenium also was highest in swallows from El Paso and Del Rio, but Se (as

Table 7. Spearman correlation coefficients of morphometric values and environmental contaminants in swallows (n = 42) from the Rio Grande (Texas, USA)[a]

	BM	HSI	GSI	SM	HPCV	T_4	AChE	BChE	DDE	Oxychlordane	Cu	Hg	Mn	Ni	Pb	Se	Zn
BM	1	−0.21	0.28	−0.31*	−0.07	−0.02	0.04	−0.28	0.27	0.29	0.01	−0.05	0.31*	−0.16	0.09	−0.23	0.03
HSI		1	−0.46**	0.40**	0.18	0.16	−0.43**	−0.04	−0.33*	−0.40**	0.11	0.03	−0.22	−0.25	0.33*	0.22	−0.13
GSI			1	−0.49**	−0.33*	0.02	0.10	−0.09	0.19	0.15	0.07	0.05	0.52**	0.28	−0.10	−0.35*	0.52***
SM				1	0.24	−0.17	−0.01	0.20	−0.13	−0.29	0.24	0.24	−0.39**	−0.38**	0.13	0.45**	−0.21
HPCV					1	−0.09	−0.24	0.04	0.04	−0.06	0.06	0.04	−0.21	−0.40**	−0.21	0.12	−0.22
T_4						1	−0.15	−0.04	−0.32*	−0.12	−0.34*	−0.24	0.01	0.02	0.15	−0.36*	0.09
AChE							1	0.31*	0.34*	0.27	0.16	0.17	−0.01	0.17	−0.21	0.11	0.10
BChE								1	−0.01	−0.10	−0.07	0.31*	−0.29	0.15	−0.06	0.19	0.04

[a] * $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$. BM = body mass; HSI = hepatosomatic index; GSI = gonadosomatic index; SM = spleen mass; HPCV = half-peak coefficient of variation; T_4 = thyroxine; AChE = acetylcholinesterase; BChE = butyrylcholinesterase; DDE = dichlorodiphenyldichloroethylene. Other organic and inorganic contaminants were not significantly correlated with any morphometric values. Correlations among organic and inorganic contaminants are not provided.

selenomethionine or as high-Se yeast) fed to beagle dogs showed a decrease rather than an increase in DNA damage [28]. Polycylic aromatic hydrocarbons were not analyzed in the present study, but they are known to cause chromosomal breakage and DNA adduct formation [29]. Swallow colonies in El Paso and Llano Grande were located close to and downstream from a sewage treatment discharge site, and the Llano Grande colony was under a bridge that sustained heavy truck and automobile traffic. Whether PAHs can be associated with the observed DNA damage at these two locations needs further investigation.

Previous studies have established the potential impact on reproduction of environmental contaminants measured in the present study. Kelce et al. [30] showed that DDE was a strong antiandrogenic compound in male rats. Concentrations of DDE less than 6 μg/g wet weight in eggs of alligators in Florida (USA) were associated with endocrine-disrupting effects that resulted in skewed sex ratios [31] and reduced embryonic viability [32]. Recently, it has become apparent that the thyroid may be an equally important target, and its disruption may impact animal growth and development [33]. In swallows, T_4 values provided interesting correlations with contaminant concentrations. Birds in the present study were sampled at approximately the same time during the breeding season to minimize the fluctuations of thyroid hormone associated with reproduction. Thyroxine levels were lower in cliff swallows from El Paso and were negatively correlated with concentrations of DDE, Cu, and Se (three of the contaminants that were detected at the highest concentrations in El Paso). In homing pigeons (*Columba livia*), Jefferies and French [34,35] found a significant increase in thyroid mass with increases in liver concentrations of *p,p′*-DDT and DDE. Those authors suggested that both chemicals at high dose rates (>3 mg/kg/d) produced hypothyroidism associated with reduced circulating levels of T_4. Negative correlations between OCs in blood and circulating levels of thyroid hormones also have been reported in glaucous gulls (*Larus hyperboreus*); gulls breeding at the most contaminated colony had lower plasma levels of T_4 [36]. However, elevated T_4 levels were reported in alligators (*Alligator mississippiensis*) from contaminated sites [37]. Mayne et al. [38] found a positive significant relationship between T_4 concentrations in the plasma of tree swallows and the number of pesticide mixtures applied to orchards but only a marginal correlation between T_4 and DDE. Species differences in thyroid hormone–transport proteins and thyroid hormone metabolism, coupled with the ability of the hypothalamo-pituitary-thyroid axis to compensate in response to disruption, likely contribute to these diverse responses [33]. Elevated OC concentrations also have been associated with enlarged liver and abnormal weights [39]. Cliff swallows from El Paso had increased spleen mass, lower T_4 concentrations, and higher HPCV values than swallows from other locations. However, T_4 values in cave swallows from El Paso were not different from those in swallows from other locations. Whether the high concentrations of DDE or the levels of Se and the few metals measured in swallows from El Paso are responsible for the observed effects remains undetermined.

Some differences were observed in brain cholinesterase activities between sexes and among locations in the Rio Grande. Acetylcholinesterase activity was greater in male cave swallows from El Paso than in those from LRGV locations; however, AChE activity in females was not different between El Paso and LRGV colonies. The swallow colony at El Paso was not adjacent to any agricultural fields; thus, potential exposure to OPs and carbamate insecticides was reduced, which could explain the observed differences in activities in males between the two regions. Nonetheless, the similarity in AChE activity in females from both regions suggests that proximity to agricultural fields is not sufficient to explain the AChE differences in males. It is possible that females in the LRGV could have been feeding more on aquatic than on terrestrial invertebrates in areas closer to their nests and, therefore, were less exposed to pesticides from agricultural fields. Stable-isotope studies suggested that cave and cliff swallows at each location were feeding on similar diets that included both aquatic and terrestrial food items [7]. Other studies have shown that cholinesterase activity may not necessarily be lower in birds from areas of suspected heavier use of pesticides than in birds from cleaner sites. Plasma AChE activity was greater in mountain plovers (*Charadrius montanus*) from the Central Valley (CA, USA), an agricultural region where pesticide use is elevated, than in those from the Carrizo Plains (CA, USA), where no agricultural chemical use was expected [40].

White-winged doves (*Zenaida asiatica*) collected during 1991 and 1992 in the vicinity of four of our sampling sites in the LRGV had brain AChE values that were lower than those in control birds, suggesting potential exposure to OPs; however, the average inhibition was less than 20% in all cases [41]. Those authors hypothesized that doves acquired these OPs primarily through ingestion of contaminated water from irrigated cotton fields. The mean brain AChE and BChE levels observed in swallows from the Rio Grande and the reference site were greater than those reported in apparently normal (nonexposed) barn swallows (*Hirundo rustica*) [42]. Also, mean brain AChE activity levels in 14- to 16-d-old nestling tree swallows were approximately 14 μmol substrate hydrolyzed/min/g tissue [11], which approached the mean values reported in the present study. Although lower AChE activity values were observed in male swallows from colonies located near agricultural fields, it is difficult from our results to discern whether swallows in the Rio Grande actually were exposed to any OPs or carbamates. Nonetheless, given that exposure to OPs has been reported in other birds of the LRGV [41], our data could be used as a baseline for comparisons in future monitoring studies or with other species.

CONCLUSION

Swallows from El Paso had the highest concentrations of DDE, PCBs, and inorganic elements (13 of 15) in carcasses. Swallows from El Paso exhibited elevated spleen mass (female cave swallows and male cliff swallows), high HPCV values (cliff swallows), and lower T_4 values in plasma (cliff swallows). Swallows from Llano Grande also exhibited significant changes in HPCV values from one year to another, and swallows from Del Rio also exhibited high HPCV values and elevated concentrations of Se. Thyroxine may serve as a useful biomarker of contaminant exposure in swallows of the Rio Grande; it was negatively correlated with DDE and Se, the contaminants detected at the greatest concentrations in the area and of most concern for sublethal and reproductive effects on wildlife. The spleen seemed to be the tissue most affected by contaminants, and spleen mass was the best indicator of exposure effects, because it was positively correlated with Se and HSI and negatively correlated with body mass, GSI, Mn, and Ni. Overall, the present study suggests that contaminants, particularly DDE, in selected portions of the Rio Grande, such as El Paso and the LRGV, could be associated with some potentially deleterious effects on wildlife. Further studies would be useful in elucidating whether factors other than contaminants are responsible for the increased spleen mass, increased variation in DNA content, and reduced T_4 levels in the blood of swallows from some locations. We suggest that cave and cliff swallows could be used for long-term contaminant monitoring studies of large hydrological basins, such as the Rio Grande.

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