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Regional variation and relationships between the contaminants DDE and selenium and stable isotopes in swallows nesting along the Rio Grande and one reference site, Texas, USA

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Cave swallows (*Petrochelidon fulva*) and cliff swallows (*P. pyrrhonota*) nest in numerous colonies throughout the Texas portion of the Rio Grande along the U.S. border with Mexico. We collected swallows during 1999 and 2000 from eight locations along the Rio Grande to determine if δ^{15}N and δ^{13}C values could be used to predict 1,1-di-(p-chlorophenyl-)2,2-dichloroethene (DDE) and selenium (Se) contaminant burdens in insectivorous birds nesting across a geographic gradient in the Texas–Mexico border and to discern if stable isotopes could help discriminate between local versus nonlocal acquisition of contaminants.

We analysed δ^{15}N and δ^{13}C in liver and muscle and DDE and Se in swallow carcasses. Within individuals, δ^{15}N was higher in liver than in muscle of both species by an average of 1.34‰, whereas δ^{13}C was 0.145‰ higher in muscle than in liver. Significant differences occurred among locations in δ^{15}N and δ^{13}C values in liver and muscle of both species. Cave swallows from three locations in the Lower Rio Grande Valley were more enriched in δ^{15}N than swallows from other sites. In general, swallows nesting in more northern latitudes along the Rio Grande had lower δ^{15}N and δ^{13}C values than those nesting farther south. Concentrations of DDE were significantly greater in swallows from El Paso, Llano Grande, and Pharr than in those from Brownsville, Falcon Lake, Laredo, Del Rio, and a reference site outside the Rio Grande. All swallows ($n = 21$) from El Paso, Llano Grande, and Pharr had DDE concentrations $\geq 3\,\mu g\,g^{-1}$ wet weight (ww), a value three times greater than the estimated threshold in avian prey that could cause potential reproductive failures in raptors. Concentrations of Se also were significantly greater in El Paso and Del Rio than at other locations. Most Se concentrations were not of concern for direct effects on birds or their predators. Principal component analysis indicated some positive correlations between δ^{15}N and δ^{13}C values in tissues and contaminant concentrations in carcass; however, analysis of covariance suggested a stronger effect of location on concentrations of DDE and Se. At the local level (Llano Grande and Pharr) there was a significant positive correlation between δ^{15}N in liver and DDE concentrations in swallow carcasses; however, Se concentrations were not significantly correlated with isotopes even at the local level. Our results provide a good database

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of δ^{15}N and δ^{13}C values for insectivorous birds nesting along the Rio Grande. Similar ecologies for cave and cliff swallows and their abundance and wide distribution along the Rio Grande make them ideal indicators of environmental pollution of this portion of the Texas–Mexico border.

Keywords: Birds; Carbon-13; Contaminants; DDE; Natural variations; Nitrogen-15; Rio Grande; Selenium; Swallows

1. Introduction

Cave swallows (*Petrochelidon fulva*) and cliff swallows (*P. pyrrhonota*) nest in numerous colonies throughout the Texas portion of the Rio Grande along the U.S. border with Mexico. Both species are insectivorous and feed mostly on aerial insects [1, 2], therefore, they are at considerable risk from the accumulation of pesticides and other agricultural chemicals through their diet. During the nesting season, cave and cliff swallows also are exposed to contaminants from sediments used for nest building and from chemical drift when feeding in or near agricultural fields sprayed with pesticides. Cave and cliff swallows migrate south of the border during the winter [1, 2], however, some cave swallows overwinter in southern Texas [3].

Agricultural pesticides are used extensively in the Rio Grande Basin, primarily in the Lower Rio Grande Valley (LRGV) (Texas Agricultural Extension Service, unpublished data). Consequently, heavy pesticide use along portions of the Rio Grande has caused mortality in birds [4] and other wildlife [5]. A recent assessment of trends of selected contaminants in wildlife of the Rio Grande revealed that during the period 1965–1995, 1,1-di-(p-chlorophenyl-)2,2-dichloroethene (DDE), a metabolite of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), was the most common persistent organochlorine (OC) reported in wildlife [5]. Mercury (Hg) and selenium (Se) were the most frequently reported inorganic contaminants. The presence of these contaminants along portions of the Rio Grande is of particular concern for several endangered species and for insectivorous birds such as cave and cliff swallows. In fact, DDE, Hg, and Se have been implicated in potential reproductive failures of the peregrine falcon (*Falco peregrinus*) in the Big Bend region of the Rio Grande [6].

Natural abundances of stable isotopes are powerful tracers and indicators of energy flows, material transfers, and environmental pollution [7–9]. Stable isotope ratios of nitrogen ($^{15}N/^{14}N$, expressed as δ^{15}N) and carbon ($^{13}C/^{12}C$, expressed as δ^{13}C) in consumer tissues have been shown to reflect those in the diet [10, 11] and have been used to determine trophic relationships within food webs [12, 13]. In terrestrial environments, δ^{13}C values generally indicate whether a food chain or food web is based on C_3 plants ($\delta^{13}C \approx -23$ to -32‰), C_4 plants ($\delta^{13}C \approx -16$ to -9‰) or a mixture of the two groups. Consumers can be up to 1‰ more enriched in ^{13}C (*i.e.*, larger δ^{13}C value) than their diet [14], resulting in modest trophic enrichment as carbon progresses along a food chain. δ^{15}N values have been utilized to identify sources of nitrogen available to primary producers (*e.g.*, soil mineral N, biologically fixed N, fertilizers, animal waste, atmospheric pollutants), and have also been used as indicators of the trophic position of consumers [15, 16]. In general, there is a progressive enrichment of δ^{15}N along a food chain by approximately 3‰ with each trophic transfer [14–16]. These fundamental attributes of carbon and nitrogen isotopes in food chains afford powerful methods for quantifying trophic structure and dynamics and for quantifying the human impact on the environment. In avian ecology, δ^{15}N and δ^{13}C values have successfully documented food habits [16, 17], discriminated between breeding and wintering areas of migratory birds [17–19], and characterized breeding and nonbreeding locations with respect to exposure and accumulation of environmental contaminants [20, 21]. Industrial and agricultural land use activities that contribute to eutrophication are also correlated with increased $\delta^{15}N$ values in food webs [21, 22].

$\delta^{15}N$ values have been correlated with organochlorines and Hg biomagnification through the food chain [23–26]. Significant correlations between $\delta^{15}N$ values and OCs in lake trout (*Salvelinus namaycush*) and other freshwater organisms have resulted in the use of $\delta^{15}N$ as a good predictor of concentrations of OCs in freshwater biota [23, 27]. Several other significant correlations between $\delta^{15}N$ and selected contaminants in fish and wildlife have been reported [26, 28, 29].

There are temporal and geographic components that influence isotopic and contaminant concentrations. Understanding differences in assimilation and depletion of $\delta^{15}N$ and $\delta^{13}C$ in animal tissues is important because $\delta^{15}N$ and $\delta^{13}C$ values in liver are likely to reflect short-term diets, whereas $\delta^{15}N$ and $\delta^{13}C$ values in muscle are likely to reflect longer term diets [30, 31]. In some birds, liver represents a short-term dietary integration of about a week and muscle over a few months [32, 33]. Therefore, differences in $\delta^{15}N$ and $\delta^{13}C$ values in liver and muscle of some migratory birds may suggest differences in contaminant sources. Stable isotope studies correlating contaminant loads with $\delta^{15}N$ and $\delta^{13}C$ in migratory birds across latitudinal gradients have been limited. The purpose of this study was to obtain stable isotope baseline data and to determine if $\delta^{15}N$ and $\delta^{13}C$ values could be used to predict DDE and Se contaminant burdens in insectivorous birds nesting across a geographic gradient in the Texas–Mexico border. A second objective was to determine if $\delta^{15}N$ and $\delta^{13}C$ values could be helpful in discriminating between local versus nonlocal acquisition of contaminants.

2. Material and methods

2.1 *Sampling locations and tissue collection*

We collected adult cave and cliff swallows during the breeding season in May and June in 1999 and 2000 from eight locations along the Rio Grande from Brownsville to El Paso and from one location near Somerville, Texas (table 1, figure 1). The location in Somerville (500 km north of the Rio Grande) was selected as a reference site to compare and contrast isotope and contaminant patterns with locations in the Rio Grande. We collected specimens from only one species at most locations, except in Brownsville and El Paso, where we collected cave and cliff swallows during both years. We captured swallows with mist nets, which were set for about 10–30 minutes depending on the rate of capture. The birds were removed from the mist net and were euthanized immediately by cervical dislocation. Carcasses were weighed, wrapped in aluminum foil, placed in plastic bags, and stored in dry ice until taken to the lab for dissection and carcass preparation. In the laboratory, the carcasses were stored at $-80\,°C$ until further processing. For the stable isotope analyses, we removed a portion of the liver and breast muscle with scissors and surgical blades that were previously rinsed with acetone.

2.2 *Stable isotope analyses*

We analysed stable isotopes of nitrogen and carbon in liver and muscle of most swallows collected during 1999 and 2000 (table 1). Approximately 2 mg of tissue sample was dried at 60 °C for 24 h, pulverized, and weighed into tin capsules. Samples were then analysed for $\delta^{13}C$ and $\delta^{15}N$ using a Carlo Erba EA-1108 elemental analyser (CE Elantech, Lakewood, NJ) that was interfaced with a Finnigan Delta Plus isotope ratio mass spectrometer (ThermoFinnigan, San Jose, CA) operating in continuous flow mode. Precision for $\delta^{13}C$ was $< 0.1‰$ whereas

Table 1. Collection locations and number of swallows collected during 1999 and 2000 in the Rio Grande and a reference site, Texas.

Location	Coordinates		Nesting culvert conditions	Habitat type	1999				2000			
	Latitude N	Longitude W			CASW		CLSW		CASW		CLSW	
					M	F	M	F	M	F	M	F
Brownsville	25° 57′	97° 25′	Running water	Urban, industrial	0	4	2	4	2	1	2	1
Llano Grande	26° 07′	97° 57′	Running water	All agriculture	10	8			6	7		
Pharr	26° 09′	98° 10′	Running water	Agriculture, urban, wastewater treatment plant	11	10			5	10		
Mission	26° 11′	98° 19′	Running water	All agriculture	3	9						
Falcon	26° 50′	99° 15′	Running water	Edge of lake, no agriculture			15	5				
Laredo	27° 24′	99° 28′	Dry creek	Mostly agriculture	8	12						
Del Rio	29° 19′	100° 50′	Mostly dry	Little agriculture	6	7						
El Paso	31° 39′	106° 19′	Running water	Urban, wastewater treatment plant	2	0	7	6	6	10	1	1
Somerville[a]	30° 20′	96° 28′	Running water	Agriculture, pastures							7	4

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

that for δ^{15}N was $< 0.2‰$. δ^{13}C values were expressed relative to the VPDB standard [34], and δ^{15}N values were expressed relative to atmospheric N_2 [35].

2.3 *Chemical analysis*

We analysed three to eight carcasses from each site (27 cave and 15 cliff swallows) for organochlorine contaminants and inorganic elements. The carcasses were prepared by removing the head, legs, beak, stomach contents, feathers, and a portion of the liver and breast

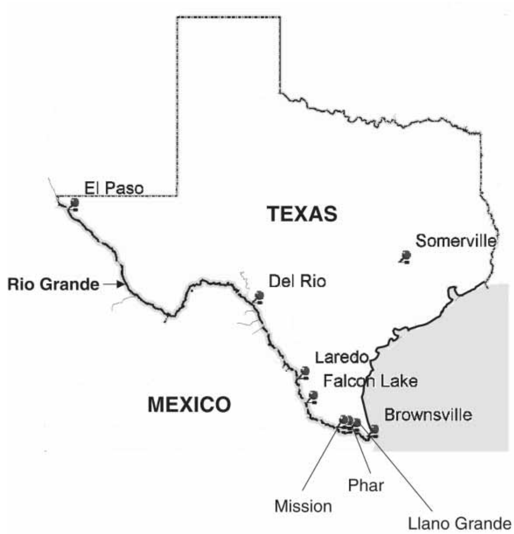

Figure 1. Map of the Rio Grande border region with locations of the collection sites.

muscle (< 0.5 g) used for the stable isotope analyses. Prior to chemical analysis, the carcasses were homogenized with a meat grinder. For the DDE analysis, approximately 2 g of sample homogenate was mixed with anhydrous sodium sulfate and extracted with hexane. The samples were then extracted and analysed following the National Oceanic and Atmospheric Administration (NOAA) status and trends method [36] with some modifications [37]. The extracts were purified by silica/alumina column chromatography and with high-performance liquid chromatography (HPLC). Residues were quantitated by gas chromatography and electron capture detection (GC-ECD) (^{63}Ni) in splitless mode with a DB-5 (30×0.25 mm ID) fused-silica capillary column [38]. Approximately 10% of the samples were confirmed by second injection on a DB-17 capillary column or by gas chromatography–mass spectrometry (GC–MS). Spike recoveries were above 80% in all cases; variation among duplicates was less than 10%. The lowest detection limit for DDE was approximately 1 ng,g^{-1} wet mass (ww).

Selenium was analysed by graphite furnace atomic absorption spectrophotometry. Approximately 0.2–0.5 g of the 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 lowest detection limit for Se was 0.5 μg g^{-1} dw. Percent recoveries of spiked samples and certified reference materials were above 90%. Mean relative percent differences among duplicates were $<$10%. Percent moisture was obtained separately by drying approximately 1 g of sample in a forced air oven at 75 °C for 24 h or until constant weight.

2.4 *Statistical analysis*

We used Wilcoxon rank-sum test procedures [39] to compare stable isotope ratios between male and female cave swallows from locations in Llano Grande, Pharr, Mission, Laredo, and Del Rio both in 1999 and 2000 and in cliff swallows from Brownsville, Falcon, and El Paso in 1999 and Somerville in 2000. The same procedure was used to compare differences in stable isotopes between species and differences between years and between tissues for each species. Because δ^{15}N and δ^{13}C values for each species were mostly similar between years, the data for both years were combined for each location and for each species for other statistical analyses. Only one species was collected at most locations hence the stable isotope data for each species was treated separately for comparisons among locations. Differences among locations were determined by analysis of variance with the general linear model (GLM) method for ranked data. These methods were used because the data were not normally distributed as verified by the Shapiro-Wilks test and the univariate procedure [39]. For the GLM analysis, the Tukey-Kramer method was used to determine significant differences among means.

A smaller set of samples than those analysed for stable isotopes was selected for the chemical analysis. A total of 33 of 42 samples analysed were females; thus, for some locations male and female contaminant data were combined for the statistical analysis. Also, contaminant data for cliff and cave swallows were combined for Brownsville and El Paso. Concentrations of DDE and Se were log-transformed to meet the assumptions of normality. DDE and Se differences among locations were compared by analysis of variance with the GLM method [39]. The Tukey-Kramer multiple comparisons procedure was used to determine which means were significantly different. Further, principal component analysis [39] was used to help with the interpretation of the relationships among contaminant burdens and δ^{15}N and δ^{13}C values in liver, muscle, and location. To better determine if the accumulation of DDE and Se in carcass was a function of δ^{15}N and δ^{13}C values in liver or muscle or a function of location or both, we performed analysis of covariance with the use of GLM methods [39] by using location as a class variable and δ^{15}N and δ^{13}C values as covariates. Type III sum of squares and F statistics were used to determine the significance of each variable. DDE and Se concentrations in swallows from two close locations in the Lower Rio Grande Valley (Pharr and Llano Grande are approximately 25 km apart) were combined (DDE, Se, and stable isotope values were similar between the two locations) for a linear regression analysis of contaminants with stable isotopes with the GLM method. For all the statistical analyses, the level of significance was set at $p \leq 0.05$.

3. Results

3.1 *Sex, species, and annual variation in stable isotopes*

Median $\delta^{13}C$ and $\delta^{15}N$ values in liver and muscle of cave swallows were for the most part not significantly different between males and females at all locations during 1999 and 2000. However, in 1999 male cave swallows from Laredo had significantly greater $\delta^{15}N$ values ($p < 0.01$) in liver and muscle than females.

Similarly, median $\delta^{13}C$ and $\delta^{15}N$ values in liver and muscle of cliff swallows were not significantly different between male and females from most locations; however, in 1999 cliff swallow males from El Paso and Falcon had significantly greater ($p < 0.01$) $\delta^{15}N$ values in liver and muscle than females.

At the species level, $\delta^{15}N$ values in liver and muscle were not significantly different between cave and cliff swallows from Brownsville and El Paso. However, $\delta^{13}C$ in muscle was significantly greater in cave than in cliff swallows from both locations.

There were no significant differences between years in $\delta^{15}N$ and $\delta^{13}C$ values in liver and muscle of cave swallows at each location, except at Llano Grande Lake, where $\delta^{13}C$ was significantly greater in swallows collected in 2000 than those collected in 1999 ($p < 0.05$). In cliff swallows, there were no significant differences between years in $\delta^{15}N$ and $\delta^{13}C$ values in liver and muscle at any of the locations. $\delta^{15}N$ and $\delta^{13}C$ values combined for each location and for each species are shown in table 2.

3.2 *Tissue variations in stable isotopes*

$\delta^{15}N$ was enriched significantly ($p < 0.001$) in liver relative to muscle in both swallow species from all locations by an average of 1.34‰. In contrast, $\delta^{13}C$ values were similar in liver and muscle of cave and cliff swallows from most locations, except in those from Laredo, Pharr, and Brownsville. Cave swallows from Laredo had significantly greater $\delta^{13}C$ values in muscle than in liver ($p < 0.001$), and those from Pharr had significantly greater $\delta^{13}C$ in liver than in muscle ($p < 0.05$). Cliff swallows from Brownsville had greater $\delta^{13}C$ in muscle than in liver ($p < 0.05$). Overall, $\delta^{13}C$ was 0.145‰ lower in muscle than in liver.

3.3 *Location differences in stable isotopes*

Both carbon and nitrogen isotope ratios tended to decrease with increasing nesting latitude (figure 2). Median $\delta^{15}N$ and $\delta^{13}C$ values in liver and muscle of cave and cliff swallows were significantly different among locations (table 2, figures 3 and 4). Among cave swallows, $\delta^{15}N$ in liver was significantly greater ($F_{6,102} = 104$, $p < 0.0001$) in swallows from Llano Grande (14.69‰) and Pharr (14.53‰) than in those from other locations and it was lower in swallows from Del Rio (10.32‰). $\delta^{15}N$ median values in muscle were significantly greater in swallows from Llano Grande ($F_{5,98} = 81$, $p < 0.0001$, 13.21‰) and were lower at Del Rio (8.89‰).

Table 2. Median stable isotope values and ranges (‰) in liver and muscle of cave and cliff swallows (both sexes and years combined for each species) from the Rio Grande and a reference site. Medians not sharing the same letter (within columns) were significantly different (comparisons based on ranks).

	Cave swallow				Cliff swallow			
	Liver		Muscle		Liver		Muscle	
Location	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Brownsville	12.41 C (11.58–12.67)	−18.38 BC (−18.8 to −17.06)	10.95 CDE (10.64–11.42)	−17.56 AB (−18.12 to −17.0)	12.13 C (11.94–12.36)	−18.71 A (−19.02 to −17.9)	10.85 C (10.23–11.33)	−17.96 A (−18.62 to −17.67)
Llano Grande	14.69 A (14.35–15.21)	−17.68 B (−18.78 to −15.91)	13.21 A (12.51–13.61)	−17.61 B (−18.46 to −15.92)	nd	nd	nd	nd
Pharr	14.53 A (14.29–14.95)	−16.30 A (−17.23 to −15.59)	12.82 B (11.16–13.3)	−16.69 A (−17.67 to −16.12)	nd	nd	nd	nd
Mission	14.05 B (13.66–14.27)	−19.65 D (−20.05 to −16.82)	nd	nd	nd	nd	nd	nd
Falcon	nd	nd	nd	nd	13.12 A (12.56–13.53)	−19.09 B (−19.86 to −18.46)	11.78 A (11.4–12.1)	−18.89 B (−19.86 to −18.38)
Laredo	12.03 C (11.09–12.41)	−19.65 D (−20.28 to −19.03)	10.99 D (10.5–11.33)	−19.03 C (−19.65 to −18.51)	nd	nd	nd	nd
Del Rio	10.32 D (9.94–11.23)	−21.05 E (−22.06 to −20.71)	8.89 E (8.65–9.54)	−20.96 D (−21.96 to −20.73)	nd	nd	nd	nd
El Paso	12.51 C (10.70–12.79)	−19.05 CD (−20.92 to −18.72)	11.46 C (11.03–11.9)	−19.08 C (−19.59 to −18.61)	12.49 B (11.82–13.34)	−19.53 C (−19.91 to −18.58)	11.33 B (11.01–11.91)	−19.78 C (−20.15 to −18.79)
Somerville[a]	nd	nd	nd	nd	nd	nd	8.36 D (7.91–8.98)	−22.67 D (−23.16 to −20.8)

[a]Reference site, nd = no data.

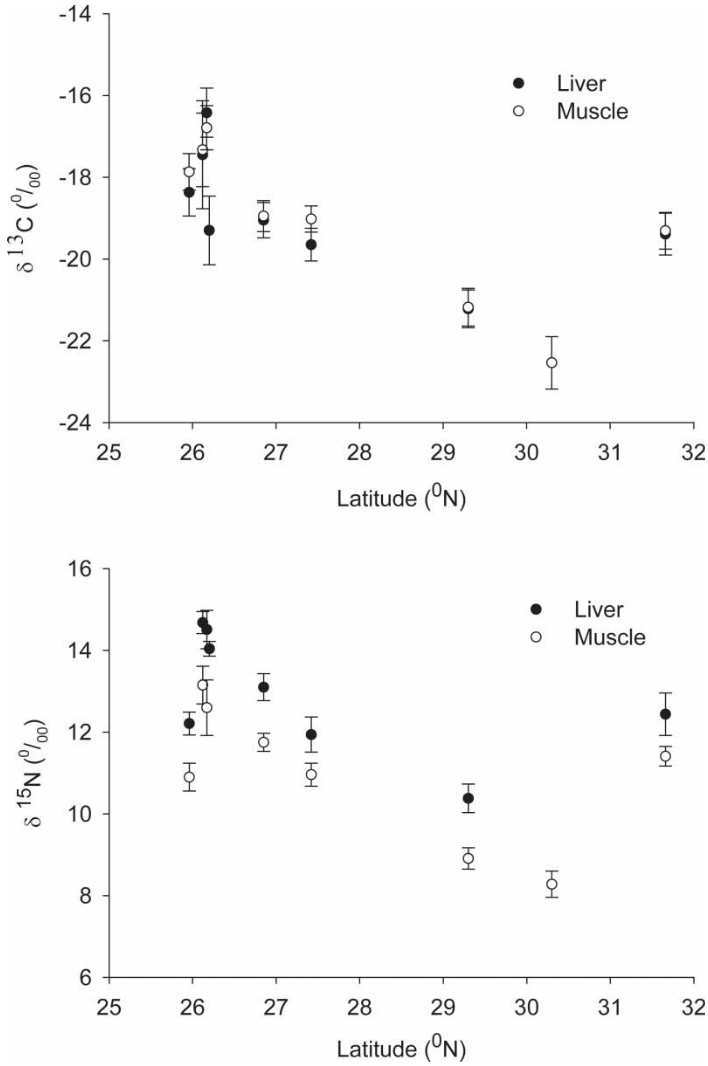

Figure 2. Relationships between nesting latitude and δ^{15}N and δ^{13}C (means ± standard deviations) in liver and muscle of cave and cliff swallows from the Rio Grande, 1999–2000.

Median δ^{13}C values were significantly greater in liver ($F_{6,102} = 61$, $p < 0.0001$) and muscle ($F_{5,98} = 74$, $p < 0.0001$) of swallows from Pharr (−16.30‰ and −16.69‰) and were lower in those from Del Rio (−21.05‰ and −20.96‰, for liver and muscle, respectively).

Among cliff swallows, δ^{15}N values in liver were significantly greater ($F_{2,40} = 32$, $p < 0.0001$) in swallows from Falcon Dam (13.12‰) than in those from El Paso (12.49‰) and Brownsville (12.13‰) (table 2, figure 4). Livers in cliff swallows from the reference site in Somerville were not analysed. δ^{15}N values in muscle were significantly higher ($F_{3,42} = 71$, $p < 0.0001$) in swallows from Falcon Dam (11.78‰) than in those from El Paso (11.33‰), Brownsville (10.85‰), and Somerville (8.36‰). Median δ^{13}C values were significantly greater in liver ($F_{2,40} = 13$, $p < 0.0001$) and muscle ($F_{3,42} = 73$, $p < 0.0001$) of swallows from Brownsville (−18.71‰ and −17.96‰) than in those from Falcon (−19.09‰ and −18.89‰), El Paso (−19.53‰ and −19.78‰ for liver and muscle, respectively), and Somerville (−22.67‰, muscle only).

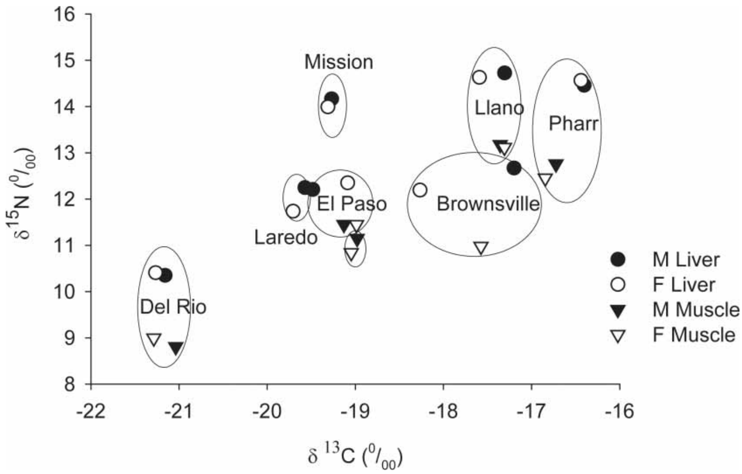

Figure 3. Relationships between δ^{15}N and δ^{13}C in liver and muscle (mean values) of cave swallows from the Rio Grande, 1999–2000.

3.4 *DDE and selenium location variations*

Concentrations of DDE ($\mu g\,g^{-1}$ ww) in carcasses were significantly different among locations ($F_{7,34} = 40$, $p < 0.0001$, Table 3), and were greater in swallows from El Paso ($12.4\,\mu g\,g^{-1}$) than in swallows from Brownsville ($0.8\,\mu g\,g^{-1}$), Falcon Lake ($0.5\,\mu g\,g^{-1}$), Laredo ($0.9\,\mu g\,g^{-1}$), Del Rio ($0.7\,\mu g\,g^{-1}$), and Somerville ($0.3\,\mu g\,g^{-1}$). DDE concentrations in swallows from El Paso were near two times greater than in swallows from Pharr ($7.4\,\mu g\,g^{-1}$) and Llano Grande ($6.6\,\mu g\,g^{-1}$) but were not significantly different. Five swallows from El Paso, three from Llano Grande, and one from Pharr had DDE residues above $10\,\mu g\,g^{-1}$ ww, with a maximum concentration of $24.7\,\mu g\,g^{-1}$ ww in one bird from El Paso. Most importantly, all swallows from El Paso, Llano Grande and Pharr ($n = 21$) had DDE concentrations $\geq 3\,\mu g\,g^{-1}$ ww.

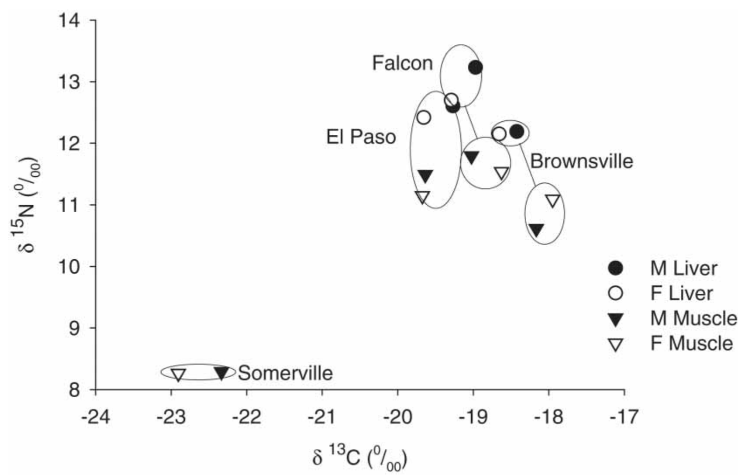

Figure 4. Relationships between δ^{15}N and δ^{13}C in liver and muscle (mean values) of cliff swallows from the Rio Grande and a reference site in Texas, 1999–2000.

Table 3. Percent moisture and percent lipid (arithmetic means ± SD) and geometric mean (range in parentheses) concentrations of DDE ($\mu g\,g^{-1}$ wet weight) and Se ($\mu g\,g^{-1}$ dry weight) in carcass of swallows from the Rio Grande and a reference site[a].

Location	N	% Moisture	% Lipid	DDE	Se
Brownsville	6	62.9 ± 4.0	10.8 ± 4.3	0.8 B (0.2–2.0)	1.1 BC (0.7–1.4)
Llano Grande	8	64.2 ± 3.3	8.9 ± 3.2	6.6 A (2.8–13.1)	1.2 BC (0.8–1.8)
Pharr	6	63.3 ± 2.5	9.5 ± 4.0	7.4 A (4.1–11.4)	1.0 C (0.7–1.5)
Falcon Lake	4	65.8 ± 1.9	17.5 ± 7.9	0.5 B (0.3–0.8)	1.6 AB (1.3–1.9)
Laredo	4	65.7 ± 1.9	10.2 ± 1.1	0.9 B (0.5–1.5)	1.3 ABC (1.2–1.5)
Del Rio	4	66.8 ± 1.7	10.1 ± 2.5	0.7 B (0.5–1.4)	1.8 A (1.5–2.0)
El Paso	7	65.0 ± 1.7	10.3 ± 4.3	12.4 A (6.6–24.7)	1.8 A (1.7–1.9)
Somerville[b]	3	63.3 ± 0.8	13.1 ± 5.1	0.3 B (0.2–0.6)	0.9 C (0.8–1.0)

[a]Means that do not share the same letter within each column are significantly different.
[b] Reference site.

Concentrations of Se ($\mu g\,g^{-1}$ dw) in carcasses of cliff and cave swallows were significantly different ($F_{7,34} = 10$, $p < 0.001$, table 3) among locations and were greater in El Paso ($1.8\,\mu g\,g^{-1}$) and Del Rio ($1.8\,\mu g\,g^{-1}$) than in Brownsville ($1.1\,\mu g\,g^{-1}$), Llano Grande ($1.2\,\mu g\,g^{-1}$), Pharr ($1.0\,\mu g\,g^{-1}$), and Somerville ($0.9\,\mu g\,g^{-1}$). Concentrations of Se were also higher in swallows from Falcon Lake ($1.6\,\mu g\,g^{-1}$) than in those from Pharr and Somerville.

3.5 *Relationships between stable isotopes and DDE and Se*

Principal component analysis was helpful in the interpretation of the relationships among contaminant burdens and $\delta^{15}N$ and $\delta^{13}C$ values in liver, muscle, and location. Figure 5 shows the graphical display of the first two principal components resulting from the analysis of the relationship between isotopes in liver and DDE and Se. The first component explained 64% of the variance and the first two components explained 89% of the variance. Principal component 1 represented a strong effect of isotopes and DDE (as indicated by high loadings) contrasted by concentrations of Se. This effect was noticeable primarily by contributions from Llano Grande and Pharr that had high concentrations of DDE and $\delta^{15}N$ and $\delta^{13}C$ values in liver, contrasted

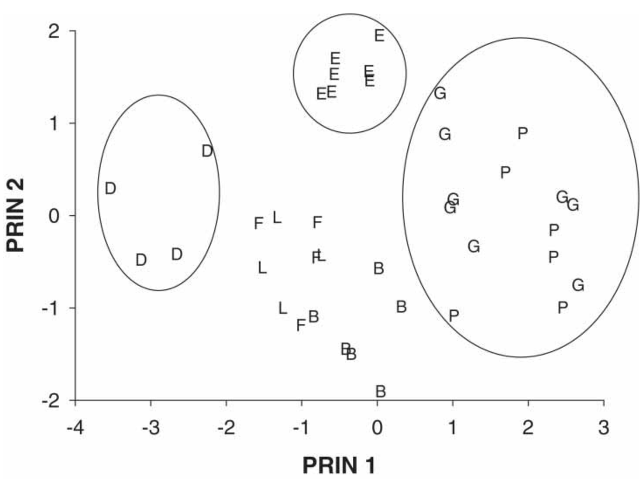

Figure 5. Plot of principal components 1 and 2 based on principal component analysis of $\delta^{15}N$ and $\delta^{13}C$ in liver and concentrations of DDE and Se in swallow carcasses. B = Brownsville, D = Del Rio, E = El Paso, F = Falcon, L = Laredo, G = Llano Grande, P = Pharr.

Table 4. Eigenvectors of the PCA analysis of DDE and Se in carcass and δ^{15}N and δ^{13}C values in liver of swallows from the Rio Grande.

	PRIN1	PRIN2	PRIN3
LogDDE	0.4114	0.6615	−0.6253
LogSe	−0.3811	0.7309	0.5036
δ^{15}N	0.5755	0.1259	0.5546
δ^{13}C	0.5952	−0.1110	0.2184

by Del Rio with high concentrations of Se but lower δ^{15}N and δ^{13}C values in liver (table 4, figure 5). Principal component 2 represented a strong contaminant (both DDE and Se) effect contrasted by lower isotope values. The location weighing more heavily on component 2 was El Paso (high concentrations of DDE and Se), contrasting with Brownsville (table 4, figure 5). Interestingly, the plot of the first two components resulted in data points clumped by locations, suggesting the significance of location in determining relationships between contaminants and stable isotopes. Principal component analysis of δ^{15}N and δ^{13}C values in muscle was not as powerful as that for liver. The first two principal components explained only 83% of the variance, however, the pattern was similar. Component 1 represented a strong isotope and DDE effect (higher loadings), whereas component 2 represented a strong effect of Se contrasted by low isotope values.

The GLM results for the effects of δ^{15}N and δ^{13}C values (liver and muscle) and location on concentrations of DDE and Se suggested that there was a significant effect of location on DDE concentrations in carcass ($F = 25$, $p < 0.0001$, and $F = 40$, $p < 0.0001$, for N and C, respectively); however, δ^{15}N and δ^{13}C (except δ^{13}C in liver) did not have a significant effect on DDE. Similarly, there was a significant effect of location on Se concentrations ($F = 7.2$, $p < 0.0001$ and $F = 3.7$, $p = 0.007$), but neither δ^{15}N nor δ^{13}C had a significant effect on Se. All the interactions were nonsignificant. Nonetheless, when DDE concentrations in swallows from two close locations (Pharr and Llano Grande are approximately 25 km apart) were combined (neither DDE or stable isotope values were different between the two locations), there was a positive significant linear relationship between DDE and δ^{15}N values in liver ($R^2 = 0.51$, $p < 0.05$); however, this relationship was not significant in muscle or with δ^{13}C, nor with Se.

4. Discussion

In this study we found significant differences among locations in δ^{15}N and δ^{13}C values in liver and muscle of cave and cliff swallows. Cave swallows from some locations in the Lower Rio Grande Valley (Mission, Pharr, and Llano Grande) were more enriched in δ^{15}N than swallows nesting in Brownsville and the rest of the sites along the Rio Grande. The differences in δ^{15}N content in liver of swallows from the various colonies could be explained perhaps by differences from feeding at different trophic levels; however, it is more likely that swallows from the different locations fed on similar insect prey that contained varying levels of δ^{15}N. Cave and cliff swallows feed on several families of flying insects, predominantly, Homoptera, Hemiptera, Diptera, and Hymenoptera [1, 2]. The higher δ^{15}N values in livers of swallows from the three LRGV sites could be explained because the colonies were established under bridges, over running water, and were surrounded by agricultural fields. The Pharr site also was about 100 m downstream of a discharge from a wastewater treatment plant. The Brownsville, El Paso, and Laredo nesting colonies had values of δ^{15}N that were more intermediate and also were established under bridges over running water; however, these colonies were located in more urban-like settings with little (Laredo), or no surrounding agriculture (Brownsville and El Paso). The colony at El Paso also was adjacent to a wastewater treatment plant on the Riverside Canal, East of the city of El Paso. The lowest $\delta^{15}N$ values in livers of swallows from the Del Rio site may also be explained by differences in nesting habitat. This colony was under a bridge over a dry creek and there was little surrounding agriculture.

Previous studies suggest that birds and other species feeding in agricultural areas or near wastewater treatment plants are more likely to be enriched in $\delta^{15}N$ than those feeding away from such sites [10, 21, 40]. Hebert and Wassenaar [21] found that higher ^{15}N values corresponded with areas that had greater proportion of agricultural land. Local variation in ^{15}N content in agricultural systems could be related to local nitrogen fertilizer inputs [10]. In addition, ^{15}N content downstream of sewage treatment plants is expected to be high; consequently, the amount of ^{15}N in emerging aquatic insects also would be expected to be high. Hansson and colleagues [41] found that discharges from a sewage treatment plant increased the $\delta^{15}N$ values in a food web significantly, and Hobson and colleagues [31] determined that nutritional stress in birds could increase tissue ratios of $\delta^{15}N$. We could not determine if any of the colonies that we sampled were under nutritional stress, although all of the birds collected were in good body condition (no emaciation was noticeable) and there was not a significant correlation between body mass and $\delta^{15}N$ in liver or muscle (data not shown). However, a few individuals were heavily infested with endoparasites; thus, it is possible that they may have been somewhat stressed.

$\delta^{15}N$ values in muscle followed a similar pattern than those in liver although $\delta^{15}N$ was less enriched in muscle. $\delta^{15}N$ was more enriched in liver than in muscle of both species by an average of 1.34‰, whereas $\delta^{13}C$ was 0.145‰ less depleted in muscle than in liver. It has been shown that isotopic turnover is faster in tissues with higher metabolic rate [30]. $\delta^{15}N$ in muscle reflects a diet that was taken earlier or over a few months, whereas the liver has a short turnover rate of about a week [33]. Turnover rates for ^{13}C in quail tissue were greater in liver than in muscle, with a half-life in liver of 2.6 days and 12.4 days for pectoral muscle [32]. Half-lives of dietary carbon were 6.4 days in liver and 27.6 days in muscle of gerbils [30]. By the time of our collection in mid-June, the swallow colonies were well-established along the Rio Grande; most were incubating and some nests had chicks. We estimate that swallows had been in the area for at least 6 weeks; therefore, it is likely that $\delta^{15}N$ and $\delta^{13}C$ in muscle reflected the diet in the breeding grounds rather than a diet from a wintering ground or a site different from the nesting site. In migrant species, the isotopic signature in tissues is influenced not only by the isotopic composition in the sampling area but also by conditions in areas visited during migration [41].

Cliff swallows from Falcon Dam had significantly greater $\delta^{15}N$ in liver and muscle than those from El Paso and Brownsville. The only possible explanation for these differences is that swallows from Falcon Dam were nesting under a bridge spanning a reservoir; therefore, they were likely feeding on more aquatic insects than cliff swallows from El Paso and Brownsville. De Niro and Epstein [10] suggested that $\delta^{15}N$ could be used to determine the relative amount of terrestrial and aquatic food sources; $\delta^{15}N$ of aquatic organisms should be more positive than that of terrestrial organisms. In contrast, $\delta^{13}C$ values in liver and muscle were significantly greater in swallows from Brownsville than in those from Falcon and El Paso. The differences in this case may result from differences in breeding latitude and longitude.

In general, swallows nesting in more northern latitudes along the Rio Grande had lower $\delta^{15}N$ and $\delta^{13}C$ values than those nesting farther south. Chamberlain and colleagues [42] observed a systematic decrease of $\delta^{13}C$ in feathers of black-throated blue warblers (*Dendroica caerulescens*) with increasing latitude. Rubenstein and colleagues [17] also demonstrated that

δ^{13}C in feathers decreased longitudinally from east (Puerto Rico) to west (Cuba). We also detected a longitudinal decrease in δ^{13}C values in liver and muscle of swallows from east (Brownsville) to west (El Paso); however, the δ^{13}C values in swallows from Del Rio were inconsistent with this pattern and were more depleted than those from El Paso. The variation of avian δ^{13}C with latitude can be explained by variations in the relative abundances of C_3 versus C_4 plants in the environment. In arid and semiarid regions, grasslands are generally dominated by C_4 plants enriched in ^{13}C [43]. In our study, colonies in the LRGV had greater δ^{13}C values. This can be explained because the LRGV sites were located primarily within agricultural sections of the valley where crops more enriched in ^{13}C, such as sorghum and sugarcane, are commonly grown. However, it is also possible that there were differences in δ^{13}C values in the aquatic prey of swallows at the different locations. Some aquatic larvae such as chironomids show seasonal variation in δ^{13}C values that are directly correlated with O_2 concentrations in lakes; lakes with low O_2 concentrations have depleted δ^{13}C values [44]. Thus, swallows from the Del Rio colony could have been feeding in an aquatic environment that was more oxygen depleted than the other sites.

Concentrations of DDE in carcasses were significantly greater in swallows from El Paso than in those from most locations, except for Pharr and Llano Grande. It is remarkable that all swallows ($n = 21$) from these three sites had DDE concentrations $\geq 3\ \mu g\ g^{-1}$ ww, with a maximum concentration of $24.7\ \mu g\ g^{-1}$ ww in one bird from El Paso. All these DDE concentrations were at least three times greater than the estimated threshold value in diet of peregrine falcons at which some reproductive failures have been observed. Enderson and colleagues [45] suggested that $1\ \mu g\ g^{-1}$ ww in the diet of peregrine falcons could potentially affect their reproduction.

Concentrations of Se in carcasses of cliff and cave swallows also were significantly greater in El Paso and Del Rio than in Brownsville, Pharr, and Somerville; however, most concentrations were below the threshold for direct effects on swallows themselves and below the threshold in the diet of predators at which some reproductive effects could be observed. Some aquatic birds ingesting Se as low as $5\ \mu g\ g^{-1}$ ww have shown to accumulate Se in eggs at high levels resulting in embryo deformities and mortality [46].

The high concentrations of DDE and moderate concentrations of Se in swallows from the Rio Grande could derive from several sources. Cave and cliff swallows nesting at different latitudes along the Rio Grande may be wintering in different areas in Mexico, Central America, and South America that could result in different patterns of acquisition of contaminants during the winter. Nonetheless, principal component analysis suggested that there was some correlation between higher values of δ^{15}N and δ^{13}C in liver and DDE and Se concentrations in carcass as shown by the eigenvectors in table 4 (see also figure 5). This relationship was primarily noticeable for the locations of Pharr, Llano Grande, and El Paso. However, analysis of covariance indicated that location had a significant effect on concentrations of DDE in carcass, but the effects of δ^{15}N and δ^{13}C were nonsignificant. The small sample size for the contaminant comparisons for each location may have contributed to the lack of effects of δ^{15}N and δ^{13}C on DDE concentrations; however, when the data from Pharr and Llano Grande were combined ($n = 14$) there was a significant relationship between δ^{15}N values and DDE concentrations. This last analysis confirms the results from the principal component analysis and suggests that at a much narrower geographic level there may be a tendency for DDE to increase with increasing δ^{15}N values in liver. The Llano Grande and Pharr sampling sites are situated along the Main Floodway in the LRGV and are only 25 km apart. The above suggests that DDE concentrations in swallows more likely reflect contamination from the local environment or breeding grounds. Hobson and colleagues [20] determined with the use of stable isotope analysis that migratory birds, such as double-crested cormorants (*Phalacrocorax auritus*), use more nutrients acquired in the breeding grounds for egg production and accumulate more contaminants from the breeding grounds in their eggs. This was also observed in migratory gulls and terns [47]. In this study, we collected birds at least 6 weeks after arrival to the breeding grounds; thus, not only the δ^{15}N and δ^{13}C values but also the contaminant values are more likely reflecting local sources. Nonetheless, the possibility of DDE accumulation farther south during migration cannot be discarded given that DDT was banned in the United States in 1972 and such high DDE accumulation could be expected mostly in heavily contaminated areas or regions where the use of DDT has been more recent. The DDE concentrations in swallows from El Paso, Llano Grande, and Pharr are among the highest reported in passerine birds from Texas in the last 20 years and should be of concern for potential negative effects on top avian predators that feed on insectivorous birds along the Rio Grande. The elevated concentrations of DDE in birds from these regions indicate that this highly persistent DDT metabolite is still a problem for many predators that feed on insectivorous birds, such as swallows. This is particularly of concern for sensitive endangered species such as the peregrine falcon whose reproductive failures in the Big Bend Region of the Rio Grande have been attributed to DDE, Se, and Hg [6].

Our comparisons of stable isotopes in liver and muscle with concentrations of DDE and Se in carcass are justified because it has been clearly demonstrated that concentrations of DDE and trace elements are strongly correlated among bird tissues and carcass. For example, Ohlendorf and colleagues [48] found a significant positive correlation between DDE in breast muscle and DDE in carcass of brown pelicans (*Pelecanus occidentalis*). Ohlendorf and colleagues [48] also found that concentrations of trace elements in most tissues of brown pelicans were most closely correlated with those in liver. Although contaminant concentrations would be expected to differ among tissues or compartments, the linear relationship of contaminant concentrations would be expected to be similar.

In the case of Se, the relationship with stable isotopes was not clear, although there was a slight tendency for Se to decrease as δ^{15}N and δ^{13}C values in tissue increased. A different pattern was reported in a recent study of metal accumulation in raccoons (*Procyon lotor)* in South Carolina; metal concentrations, including Se, tended to increase with increasing δ^{15}N levels in muscle [49]. However, it was unclear whether metal concentrations increased as trophic position increased in terrestrial environments, perhaps due to different foraging strategies or to the difficulties in assessing the trophic position of the study species in the ecosystem [49]. The swallows we sampled along the Rio Grande were feeding in both aquatic and terrestrial ecosystems and it is likely that the variations in Se and isotope values were largely dependent on which one of the two areas was the predominant feeding source. To more effectively assess the potential relationship between stable isotopes and Se concentrations, efforts should focus on obtaining more data from one single location with known high concentrations of Se, such as Del Rio and El Paso.

5. Conclusions

In this study we have demonstrated that there are clear significant differences in both δ^{15}N and δ^{13}C values in tissues and in DDE and Se concentrations among swallow populations nesting along the Rio Grande from Brownsville to El Paso. Principal component analysis suggested that there are some positive correlations between δ^{15}N and δ^{13}C values in tissues and contaminant concentrations in carcass and that such correlations become more clear at more contaminated sites. Further, results of the analysis of data from Llano Grande and Pharr suggest that δ^{15}N in liver could be a good predictor of DDE concentrations in swallows at a more local level; however, more analyses are necessary to clearly determine whether DDE or Se concentrations could be predicted from the analysis of stable isotopes alone. Once better correlations are established, it might be easier to discriminate between local versus nonlocal acquisition of contaminants such as DDE and Se. The timing of our sampling and the observed tendency for DDE to increase with increasing δ^{15}N and δ^{13}C levels suggest that the primary source of DDE in carcass may be from local sources rather than accumulation during migration or in wintering grounds. Our results provide a good database of δ^{15}N and δ^{13}C values for insectivorous birds nesting along the Rio Grande. Except for one case, δ^{15}N and δ^{13}C values were similar for cave and cliff swallows during both years at most locations, suggesting that the dietary sources remained unchanged. Similar ecologies for cave and cliff swallows and their abundance and wide distribution along the Rio Grande make them ideal indicators of environmental pollution of this portion of the Texas–Mexico border.

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