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Metals and organochlorine pesticides in caudal scutes of crocodiles from Belize and Costa Rica

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Abstract

Despite high animal diversity in the Neotropics and the largely unregulated use and disposal of pesticides and industrial chemicals in Central America, few data exist regarding accumulation of environmental contaminants in Central American wildlife. In this study we examined accumulation of metals and organochlorine (OC) pesticides in caudal scutes of crocodiles from Belize and Costa Rica. Scutes from Morelet's crocodiles (*Crocodylus moreletii*) from two sites in northern Belize were analyzed for metals, and scutes from American crocodiles (*C. acutus*) from one site in Costa Rica were analyzed for metals and OC pesticides. All scutes ($n=25$; one scute from each of 25 individuals) contained multiple contaminants. Mercury was the predominant metal detected, occurring in all scutes examined from both species. Other metals detected include cadmium, copper, lead, and zinc. American crocodile scutes from Costa Rica contained multiple OC pesticides, including endrin, methoxychlor, *p,p′*-DDE, and *p,p′*-DDT, all of which occurred in 100% of scutes analyzed ($n=6$). Mean metal and OC concentrations varied in relation to those previously reported in crocodilian scutes from other localities in North, Central, and South America. OC concentrations in American crocodile scutes were generally higher than those previously reported for other Costa Rican wildlife. Currently, caudal scutes may serve as general, non-lethal indicators of contaminant accumulation in crocodilians and their areas of occurrence. However, a better understanding of the relationships between pollutant concentrations in scutes, internal tissues, and environmental matrices at sample collection sites are needed to improve the utility of scutes in future ecotoxicological investigations.

Keywords: American crocodile; Belize; Central America; Costa Rica; Metals; Morelet's crocodile; Non-lethal sampling; Organochlorine pesticides; Scutes

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1. Introduction

Regulations governing the production, distribution, and use of chemicals in Central America are often scant or inadequately enforced (Murray, 1994). As a result, large quantities of chemicals are routinely used in Central American countries for agriculture, mining, crop storage, and vector control at rates often comparable to or exceeding those in developed countries (Cawich and Roches, 1981; Castillo et al., 1997; Lacher and Goldstein, 1997; Alegria et al., 2000). In addition, many compounds banned in most industrialized countries continue to be used in Central America. For example, the persistent organochlorine (OC) pesticide DDT is still used for vector control in Belize (Grieco et al., 2000; Roberts et al., 2002; Alegria et al., 2000), and continued agricultural use is suspected (Rainwater, 2003). Moreover, inadequate chemical storage conditions have also been reported in Central America, further increasing the potential for environmental contamination (Alegria, 1999). Numerous chemical pollutants including heavy metals, polychlorinated biphenyls (PCBs), and OCs have been found in sediments in most Central American countries (Devevey et al., 1993; Gibbs and Guerra, 1997; Michel and Zengel, 1998; Carvalho et al., 2002; Wu et al., 2000a; Spongberg, 2004; Defew et al., 2005), and multiple OC pesticides have been detected in ambient air samples (Alegria et al., 2000; Shen et al., 2005). The wide use and environmental occurrence of these chemicals in Central America and the rich biodiversity of the Neotropics (Wilson, 1992) suggest a high potential for contaminant exposure and accumulation in wildlife inhabiting this region. However, few studies on contaminant concentrations in Central American wildlife have been conducted (Castillo et al., 1997; Klemens et al., 2003).

Crocodilians (crocodiles, alligators, caimans, gharials) are large predatory reptiles that inhabit tropical and subtropical regions worldwide (Thorbjarnarson, 1992; Ross, 1998). Due to their high trophic status and long life span, these animals are susceptible to exposure and accumulation of environmental contaminants released (e.g., chemical application, spill, faulty disposal) or atmospherically deposited in their habitats. Indeed, multiple xenobiotics, primarily metals and OC pesticides have been detected in crocodilians from numerous localities throughout their global range (Ding et al., 2001; Rumbold et al., 2002; Campbell, 2003 and references therein; Packett et al., 2004; Rauschenberger et al., 2004; Roe et al., 2004; Pepper et al., 2004; Sepúlveda et al., 2004; Almli et al., 2005; Jeffree et al., 2005; Presley et al., 2006; Wu et al., 2006; Xu et al., 2006; Yoshikane et al., 2006). In addition, developmental and reproductive abnormalities have been observed in an extensively studied crocodilian species, the American alligator (*Alligator mississippiensis*), inhabiting contaminated lakes in Florida, USA (Guillette et al., 2000 and references therein). While geographic and species-specific differences in sensitivity to contaminants may exist among crocodilians, the available data suggest that in general, exposure to environmental contaminants may present a subtle yet significant long-term risk to populations in contaminated areas (Thorbjarnarson, 1992; Gibbons et al., 2000).

In Central America, contaminant accumulation in crocodilians has been reported only in Belize. Two crocodilian species occur in Belize, Morelet's crocodile (*Crocodylus moreletii*) and the American crocodile (*C. acutus*) (Platt and Thorbjarnarson, 2000a,b). Both species are currently considered endangered by the World Conservation Union, International Union for the Conservation of Nature and Natural Resources (IUCN) and are listed on Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Groombridge, 1987; Platt and Thorbjarnarson, 2000a,b). Because of their endangered status, contaminant accumulation in these crocodiles can only be determined using non-lethal sampling procedures (Burger et al., 2000). As a result, contaminant studies on Belizean crocodiles have focused primarily on residues in non-viable eggs. OC pesticides have been detected in eggs of both species from multiple localities throughout the country (Wu et al., 2000a,b, 2006; Pepper et al., 2004), and mercury accumulation in Morelet's crocodile eggs has also been reported (Rainwater et al., 2002). OC pesticides have also been detected in caudal scutes of Morelet's crocodiles from northern Belize (DeBusk, 2001). Crocodilian caudal scutes are triangular, vertically extended, laterally flattened dermal scales located on the dorsal surface of the tail (Richardson et al., 2002). Caudal scute removal is a common marking technique used to identify crocodilians in both the field and in captivity (Bustard and Choudhury, 1981; Webb et al., 1989; Jennings et al., 1991; Soberon et al., 1996; Platt et al., 2002; Richardson et al., 2002; Elsey et al., 2004), and this technique can also provide non-lethal tissue samples for analysis of environmental contaminant accumulation in marked individuals (Odierna, 1995; Yanochko et al., 1997; Jagoe et al., 1998).

The primary objective of this study was to examine accumulation of persistent environmental pollutants in caudal scutes of Neotropical crocodilians living in contaminated habitats. Specifically, we examined 1) metal concentrations in caudal scutes of Morelet's crocodiles from two sites in Belize and 2) metal and OC pesticide concentrations in caudal scutes of American crocodiles from one site in Costa Rica. Based on data indicating sediment contamination at these sites, we hypothesized that caudal scutes from crocodiles living in these areas would contain multiple pollutants.

2. Materials and methods

2.1. Study areas and sample collection

Crocodiles were captured and sampled at two sites in Belize and one site in Costa Rica (Fig. 1). In Belize, Morelet's crocodiles were sampled from Gold Button Lagoon and the New River Watershed. Gold Button Lagoon (17°55′N, 88°45′W) is a man-made lagoon (ca. 142 ha) located on Gold Button Ranch, a private cattle ranch approximately 25 km southwest of Orange Walk Town in the Orange Walk District. New River Watershed is comprised of the New River, New River Lagoon, and associated tributaries in the Orange Walk and Corozal Districts. Crocodiles sampled from this area were captured in New River Lagoon (17°42′N, 88°38′W; ca. 23 km long) and the southern-most 18 km of New River. Multiple OC pesticides have been detected in sediments and crocodile eggs from both Gold Button Lagoon and New River Watershed (Wu et al., 2000a, 2006; Pepper et al., 2004) at concentrations similar to those found in sediments and alligator eggs at sites considered to be of low to intermediate contamination in Florida, USA (Gunderson et al., 2003; Rauschenberger et al., 2004). Sediments from Gold Button Lagoon and New River Watershed have not been examined for metals, but mercury has been detected in crocodile eggs from Gold Button Lagoon (Rainwater et al., 2002), suggesting contamination of the lagoon with mercury and possibly other metals. In Costa Rica, American crocodiles were captured from the Río Grande de Tárcoles (09°47′N, 84°37′W) in Puntarenas Province, just west of Carara National Park and approximately 5 km inland from the Gulf of Nicoya. The Río Grande de Tárcoles contains a breeding population of American crocodiles (Sasa and Chaves, 1992) and has been contaminated by a variety of agricultural and industrial inputs (Fuller et al., 1990). Sediments from various localities along the Tárcoles have been shown to contain multiple metals at concentrations ranging from background to high (Fuller et al., 1990), but contamination by OCs has not been reported.

A total of 25 caudal scutes (one from each of 25 crocodiles) was analyzed for environmental contaminants in this study (Table 1). Scutes from Morelet's crocodiles were collected from Gold Button Lagoon

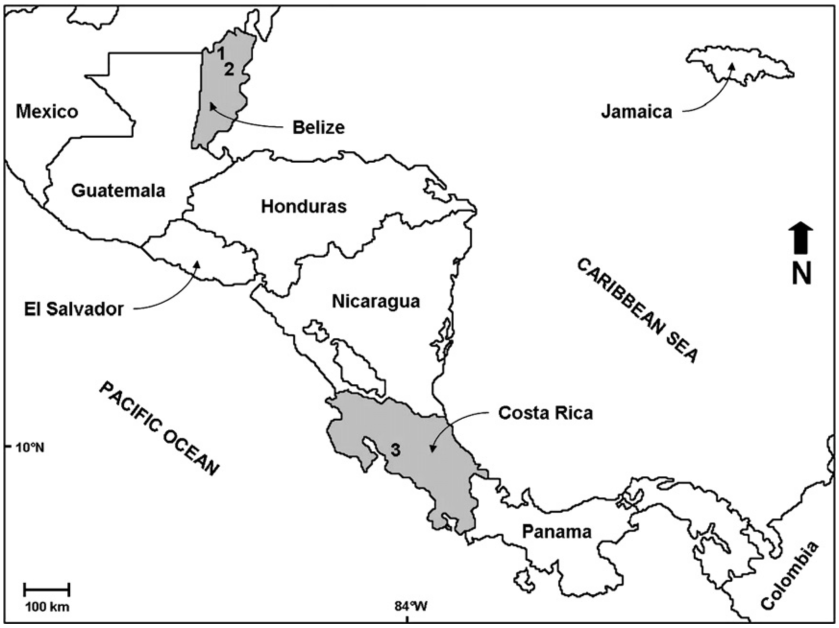

Fig. 1. Map of Central America showing locations (numbers) of crocodile caudal scute collections during this study. Morelet's crocodiles were sampled from Gold Button Lagoon (1) and the New River Watershed (2) in Belize (shaded), and American crocodiles were sampled from the Río Grande de Tárcoles (3) in Costa Rica (shaded).

Table 1
Number, sex, and body size of Morelet's (Belize, 1997–2001) and American (Costa Rica, 2003) crocodiles sampled in this study

Location	*n*	Female: male	Snout-vent length (cm)	
			Mean (±SE)	Range
Gold Button Lagoon, Belize	9	5:4	89.8±6.7	65.0–129.5
New River Watershed, Belize	10	4:6	104.4±9.6	59.5–156.7
Río Grande de Tárcoles, Costa Rica	6	1:5	155.7±5.5	134.0–172.0

($n=9$) and New River Watershed ($n=10$) between March and October, 1997–2001 as part of a study on the ecotoxicology of this species in northern Belize (Rainwater, 2003). American crocodile scutes ($n=6$) were collected from Río Grande de Tárcoles in March 2003 as part of a separate study on the ecology, ecotoxicology, and conservation status of this species. Crocodiles were noosed-captured, measured, sampled, and released under permit from the Belize Ministry of Natural Resources (Morelet's crocodiles) and the Costa Rica Ministry of Atmosphere and Energy (American crocodiles). The sex of each animal was determined by cloacal examination of the genitalia (Allsteadt and Lang, 1995), and snout-vent length (SVL; measured ventrally from the tip of the snout to the anterior margin of the cloaca) was determined using a tape measure. Each crocodile was permanently marked for future identification by removing a unique series of caudal scutes using a sterile scalpel (Bustard and Choudhury, 1981; Jennings et al., 1991; Richardson et al., 2002), and each removed scute was retained for contaminant analysis. Each scute was placed in a sterile, labeled Whirl Pak™ bag (NASCO, Fort Wilkinson, WI), and frozen at −20 °C until shipment to Texas Tech University where it was then stored at −80 °C until analysis. Following measurements and sample collections, each crocodile was released at its site of capture. Caudal scutes from both crocodile species were analyzed for metals, and American crocodile scutes were also analyzed for OC pesticides.

2.2. Metals analysis

One whole scute from each crocodile sampled was analyzed for the presence of the metals cadmium, copper, lead, mercury, and zinc and the metalloid arsenic following the methods of Adair and Cobb (1999) and Cobb et al. (2000). Briefly, scutes were cut into 1 cm^2 pieces and predigested for a minimum of 8 h in acid-rinsed 250-mL glass beakers using concentrated nitric acid in a 2:1 acid:sample mass ratio. Samples were heated to 120 °C using standard hotplates to digest the remaining tissue and evaporated to approximately 20 mL before addition of 30% hydrogen peroxide. Digested samples were then filtered through glass fiber filter paper, quantitatively transferred to appropriately-sized volumetric flasks (25 or 50 mL depending on quantity of sample), and brought to volume using ultra-pure water. Samples were analyzed within 72 h on a Leeman DRE inductively coupled plasma spectrophotometer with auto-sampler (Leeman Labs, Inc. Hudson, NH). Accuracy and precision of sample digestion and analysis procedures were determined using National Institute of Standards and Technology standards (NIST, Gaithersburg, MD) and method blanks. This validation demonstrated the following percent recoveries: arsenic: 49.6%, cadmium: 93.8%, copper: 74.2%, lead: >124%, zinc: 110.2%. The detection limit for arsenic, cadmium, copper, lead, and zinc in water was 0.05 μg/g. Mercury samples were analyzed using a cold vapor-continuous flow injection-mercury analysis system (FIM-400, Perkin Elmer, Boston, MA), and a 61.9% recovery was achieved. The detection limit for mercury was 0.5 ng/g in water samples.

2.3. Organochlorine pesticides analysis

Caudal scutes of adult crocodilians are composed of a tough outer keratinized epidermis and a dense inner dermal matrix containing a fat core (Maderson, 1985; Richardson et al., 2002). In this study, fat from American crocodile scutes was analyzed for the presence of OCs. All visible fat was removed from each scute, weighed, minced, and allowed to dry for 24 h. Each fat sample was then mixed with 15 g anhydrous sodium sulfate, transferred to 33-mL extraction cells, and fortified with two internal standards, tetrachloro-*m*-xylene (TCMX) and decachlorobiphenyl (DCBP). Samples were then extracted with hexane using a Dionex 200 Accelerated Solvent Extractor (ASE) under the following parameters: heat=5 min, static=5 min, flush %=60%, purge=60 s, cycles=2, pressure=1500 psi, and temperature=100 °C. Extracts were collected in 60-mL glass vials with Teflon caps. Extract volumes were reduced using rotary evaporation, transferred and diluted to 5 mL, and filtered (0.45 μm Acrodisc®) into autosampler vials.

Lipids were removed from scute extracts using florisil solid phase extraction columns. Columns were conditioned with hexane, sample (2 mL) was loaded onto the column, and the eluent was collected. The sample was then eluted three times with petroleum ether:ethyl ether (95:5), and the eluent was collected. The cleaned extract was reduced to near dryness with nitrogen evaporation, diluted to 2 mL, and transferred to autosampler vials.

A certified OC pesticide spiking solution consisting of heptachlor, lindane (γ-BHC), dieldrin, endrin, aldrin, and *p,p′*-DDT was obtained from Accustandard, Inc. (New Haven, CT) as were individual standards of methoxychlor, *p,p′*-DDE, TCMX, and DCBP. Samples were spiked with TCMX and DCBP in order to monitor the efficiency of the extraction and analysis. Organic solvents were pesticide or GC/MS grade.

A Hewlett-Packard 6890 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) and a 30 m × 0.32 mm DB-5 column was used to separate and quantify the OCs. Inlet and detector temperatures were 250 °C and 315 °C, respectively. The temperature program was as follows: initial temperature was 100 °C; increased from 100° to 180 °C at 25 °C/min; increased from 180° to 220 °C at 3 °C/min with a 3-min hold; increased from 220 °C to a final temperature of 300 °C at 11 °C/min with a 8-min hold. OCs were identified using congruence of standard and unknown retention times and were quantified using integration of peak areas. Average percent recovery was 77%; however, sample concentrations were not adjusted for extraction efficiency. The limit of detection for OCs (based on detector response for *p,p′*-DDE) in scute fat was 3.0 ng/g. All pesticide concentrations are reported on a wet weight basis (ng/g).

2.4. Statistical analyses

Statistical analyses were performed using program JMPin statistical software (Version 3.2, SAS Institute, Cary, NC, USA). Due to small samples sizes and failure of non-transformed and transformed data to achieve normality and homogeneity of variance, non-parametric procedures were used to examine differences in contaminant concentrations in scutes between sexes and sampling sites in Belize (Wilcoxon rank sums test) and among Gold Button Lagoon, New River Watershed, and Río Grande de Tárcoles (Kruskal–Wallis test). Relationships between body size (SVL) and scute contaminant concentrations were examined using linear regression analysis. Sample concentrations falling below detection limits were assigned values of one-half the detection limit for that particular analyte. All statistical tests were considered significant when $p \leq 0.05$. Both arithmetic and geometric means were calculated to allow for comparisons with other studies.

3. Results

3.1. Metals

Four metals were detected in Morelet's crocodile scutes from Belize: cadmium, copper, lead, and mercury (Table 2). Zinc and arsenic were not detected. Mercury was the most frequently detected metal, occurring in 100% of scutes from both New River Watershed ($n = 10$) and Gold Button Lagoon ($n = 9$). Copper was also detected in all scutes from New River Watershed and in seven (78%) scutes from Gold Button Lagoon. Scutes from New River Watershed also contained cadmium (frequency of occurrence = 10%), and lead (20%), while no other metals were detected in scutes from Gold Button Lagoon. No significant differences in mean mercury and copper concentrations in crocodile scutes were observed between Gold Button Lagoon and New River Watershed or between males and females within each site.

Table 2
Mean (±SE) concentrations (ng/g wet mass) of metals detected in caudal scutes (one per animal) of Morelet's crocodiles from Belize and American crocodiles from Costa Rica during this study

Metal/metalloid	Morelet's crocodile		American crocodile
	Gold Button Lagoon (9)	New River Watershed (10)	Río Grande de Tárcoles (6)
Arsenic	ND	ND	ND
Cadmium	ND	70.7 ± 45.7^{a} (33.6)	337.5 ± 312.5^{a} (51.5)
Copper	$346.0 \pm 89.8^{a,b}$ (215.1)	451.8 ± 38.0^{a} (436.7)	125.0 ± 67.8^{b} (57.9)
Lead	ND	109.7 ± 77.8^{a} (40.2)	491.5 ± 420.4^{a} (96.0)
Mercury	98.7 ± 21.6^{a} (84.0)	72.7 ± 20.4^{a} (46.2)	93.5 ± 27.0^{a} (80.6)
Zinc	ND	ND	4140.2 ± 544.7 (3942.1)

Numbers in parentheses to the right of site names indicate the number of animals sampled at that site.
Values in parentheses to the right of arithmetic means are geometric means. Detection limits: arsenic, cadmium, copper, lead, and zinc (in water) = 0.05 µg/g; mercury (in water) = 0.5 ng/g.
ND = non-detect.
For each metal, mean concentrations with different superscripts are significantly different ($p \leq 0.05$).

Table 3
Summary statistics for organochlorine pesticide concentrations (ng/g wet mass) detected in fat from caudal scutes (one per animal) of American crocodiles ($n=6$) from the Río Grande de Tárcoles, Costa Rica

	Organochlorine pesticide							
	Lindane	Heptachlor	Aldrin	*p,p′*-DDE	*p,p′*-DDT	Dieldrin	Endrin	Methoxychlor
Mean (±SE)	ND	ND	ND	340.2±81.9	254.8±50.5	8.0±5.6	229.8±40.0	533.8±456.6
Range	ND	ND	ND	72–601	120–454	ND–36	101–368	207–1103
Geometric mean	ND	ND	ND	277.9	230.6	3.2	210.9	462

Detection limit for OCs (based on detector response for *p,p′*-DDE)=3.0 ng/g. ND = non-detect.

Five metals were detected in American crocodile scutes from Costa Rica: cadmium, copper, lead, mercury, and zinc. Again, arsenic was not detected. Mercury and zinc were the predominant metals detected, both occurring in 100% of scutes examined ($n=6$) (Table 2). Cadmium, copper, and lead were detected less frequently, occurring in 33%, 50%, and 17% of scutes, respectively. The small sample size of American crocodile scutes and the male-biased sex ratio (5:1) of animals sampled (Table 1) precluded statistical comparison of scute metal concentration between sexes.

Comparisons among the three sites revealed that the mean copper concentration in American crocodile scutes from Río Grande de Tárcoles was significantly lower than that observed in Morelet's crocodile scutes from New River Watershed ($p=0.0140$). No other differences in mean metal concentrations among the three sampling sites were observed.

Linear regression analysis revealed a significant negative relationship between Morelet's crocodile body size (SVL) and scute copper concentrations ($R^2=0.38$; $p<0.0087$; $n=17$). This relationship appeared to be driven by male crocodiles, as sex-specific analyses indicated that the SVL-scute copper relationship was significant in males ($R^2=0.68$; $p<0.0065$; $n=9$) but not females ($R^2=0.12$; $p<0.3957$; $n=8$). No other significant relationships existed between scute contaminant concentrations and crocodile body size or sex in either species.

The most highly metal-contaminated Morelet's crocodile was an adult female from New River Watershed which contained 482 ng/g cadmium, 650 ng/g copper, 808 ng/g lead, and 146 ng/g mercury in its caudal scute. The most highly metal-contaminated American crocodile was an adult male which contained 1900 ng/g cadmium, 420 ng/g copper, 2590 ng/g lead, 50 ng/g mercury, and 2468 ng/g zinc in its caudal scute. No other crocodile scutes contained cadmium concentrations detectable by the analytical methods employed in this study, and only three other scutes contained detectable concentrations of lead.

3.2. OC pesticides

Five OC pesticides were detected in American crocodile scutes (fat cores only) from the Río Grande de Tárcoles: dieldrin, endrin, methoxychlor, *p,p′*-DDE, *p,p′*-DDT (Table 3). Aldrin, heptachlor, and lindane were not detected. Endrin, methoxychlor, *p,p′*-DDE, and *p,p′*-DDT were detected in all (100%) of the scutes examined ($n=6$), while dieldrin occurred in two (33%) scutes. Small sample size and the male-biased sex ratio precluded an inter-sex comparison of OC pesticide concentrations in scutes.

The lone female American crocodile from the Río Grande de Tárcoles was the most OC-contaminated individual sampled in this study, containing 601 ng/g *p,p′*-DDE, 36 ng/g dieldrin, 368 ng/g endrin, 454 ng/g *p,p′*-DDT, and 624 ng/g methoxychlor in its scute.

4. Discussion

Results of this study indicate that Morelet's crocodiles living in Gold Button Lagoon and New River Watershed in Belize and American crocodiles living in the Río Grande de Tárcoles in Costa Rica are exposed to and accumulate multiple environmental contaminants. Previous studies have reported accumulation of OC pesticides and mercury (Wu et al., 2000a,b, 2006; DeBusk, 2001; Rainwater et al., 2002; Pepper et al., 2004) in Morelet's crocodiles in northern Belize, and this study provides evidence that crocodiles in this region also accumulate cadmium, copper, and lead. In Costa Rica, the Río Grande de Tárcoles is known to receive pollutant inputs from a variety of sources, and multiple metals have been detected in the river's sediments (Fuller et al., 1990). This study demonstrates accumulation of cadmium, copper, lead, mercury, and zinc as well as several OC pesticides in the apex predator of this river system, suggesting the presence of these chemicals in river sediments and other associated biota.

The significant negative relationship between Morelet's crocodile body size (SVL) and scute copper concentrations in this study resembles the relationship previously observed between SVL and metals in osteoderms (dermal, bony scutes from the dorsal pelvic and neck region) of Australian freshwater crocodiles (*C. johnstoni*) (Jeffree et al., 2005). In the latter study, crocodile SVL was also negatively correlated ($R^2=0.52$; $p<0.001$) with scute copper concentrations, but similar significant relationships existed with several other metals as well (Jeffree et al., 2005). In contrast, other studies have reported significant positive relationships between crocodilian body size and scute metal concentrations (Yanochko et al., 1997; Jagoe et al., 1998; Jeffree et al., 2001). Together, these data suggest geographic or interspecific differences in the accumulation, distribution, metabolism, and elimination of metals in crocodilians. In the present study, the near absence of significant (positive or negative) relationships between crocodile body size and scute metal concentrations may be an artifact of small sample sizes at each site, particularly at Río Grande de Tárcoles.

Only three studies have previously examined metal concentrations in caudal scutes of wild crocodilians (Odierna, 1995; Yanochko et al., 1997; Jagoe et al., 1998). Odierna (1995) analyzed lead in caudal scutes of multiple caiman species (spectacled caiman, *Caiman crocodilus crocodilus*; yacaré caiman, *Caiman crocodilus yacare*; spectacled caiman-yacaré caiman hybrids, *C. crocodilus crocodilus* × *C. crocodilus yacare*; broad-snouted caiman, *Caiman latirostris*, black caiman, *Melanosuchus niger*, smooth-fronted caiman, *Paleosuchus trigonatus*) from numerous localities in Brazil. Most contained <500 ng/g lead, but some (37%) contained up to 2000 ng/g or greater, including one individual with 84,000 ng/g lead in its scute. Similarly, most crocodile scutes in the present study contained <200 ng/g lead. Only one scute (from an American crocodile) in this study contained lead (2590 ng/g) comparable to the intermediate concentrations observed in Brazilian caiman (Odierna, 1995).

Yanochko et al. (1997) and Jagoe et al. (1998) reported mercury concentrations in caudal scutes of American alligators from various localities in the southeastern USA. Mean mercury concentrations in American and Morelet's crocodile scutes examined in this study were similar to those detected in alligator scutes from central Florida and southern Georgia but notably lower (approximately 12- to 23-fold) than those detected in the Florida Everglades and in southwestern South Carolina (Table 4). Site-specific differences in metal concentrations have been previously observed in other studies examining crocodilian tissues and are believed to reflect the variability in contaminant profiles among animal collection sites (Delany et al., 1988; Heaton-Jones et al., 1997; Yanochko et al., 1997; Jagoe et al., 1998; Burger et al., 2000). Jeffree et al. (2001) and Markich et al. (2002) found that differences in elemental signatures (specific elements and their respective concentrations) in osteoderms of estuarine crocodiles (*C. porosus*) were sufficient to accurately distinguish individuals by their catchments of occurrence. Of the five metals detected in American crocodile scutes in the present study, only lead and zinc were previously reported in sediments from the same general reach of the Río Grande de Tárcoles (Fuller et al., 1990). However, because these sediments were collected approximately 17 years prior to the present study, the greater number of metals in crocodile scutes may reflect additional contaminant inputs into the river over the last several years, heterogeneity of sediment contamination, or both. Overall, these findings support the use of non-lethally collected crocodilian tissues as general indicators of environmental contamination; however more studies examining the specific relationships between metals (and other contaminants) in crocodilian tissues and those in associated sediment, soil, and water samples are needed to further discern the utility of these tissues for predicting actual pollutant concentrations in environmental matrices.

Table 4
Mean (arithmetic) concentrations of mercury (wet mass) detected in crocodilian caudal scutes from North and Central America

Species	Location	*n*	Mercury (ng/g)	Reference
American alligator	Central Florida, USA	20	137	Jagoe et al. (1998)
	Everglades, Florida, USA	7	1347	Yanochko et al. (1997)
	Everglades, Florida, USA	10	1666	Yanochko et al. (1997)
	Okefenokee Swamp, Georgia, USA	9	76	Jagoe et al. (1998)
	Par Pond, South Carolina, USA	39	1205	Yanochko et al. (1997)
American crocodile	Río Grande de Tárcoles, Costa Rica	6	94	This study
Morelet's crocodile	Gold Button Lagoon, Belize	9	99	This study
	New River Watershed, Belize	10	73	This study

Original data on mercury concentrations in American alligator scutes, reported on a dry mass basis, were converted to a wet mass basis for comparison by dividing by a factor of 3.8 (wet/dry mass ratio in crocodile scutes calculated by Jeffree et al., 2001).

Only one study has previously examined OC pesticide concentrations in crocodilian caudal scutes. DeBusk (2001) found *p,p′*-DDE, *p,p′*-DDT, *p,p′*-DDD, and methoxychlor in scute fat from adult Morelet's crocodiles from Gold Button Lagoon and New River Lagoon, the same study sites in Belize investigated in the present study. The mean *p,p′*-DDE concentrations in scutes of American crocodiles from Río Grande de Tárcoles and Morelet's crocodiles from Belize were similar, but mean concentrations of *p,p′*-DDT and methoxychlor in American crocodiles were over 5-fold greater than those in Morelet's crocodiles from the two Belize study sites. As with metals, differences in OC concentrations among crocodile scutes from multiple localities likely reflect differences in contaminant profiles among sampling sites. Differences in crocodile gender, size, and body condition may also influence inter-site variability in OC concentrations. Elevated concentrations of *p,p′*-DDT in Tárcoles crocodile scutes relative to concentrations in sediments and Morelet's crocodile scutes, eggs, and nest media and from Belize (Wu et al., 2000a,b, 2006; DeBusk, 2001; Pepper et al., 2004) may reflect a more recent use of DDT in the Río Grande de Tárcoles area and subsequently a more recent exposure of American crocodiles to this parent compound.

Few reports of contaminant accumulation in Central American wildlife exist. However, three ecotoxicological studies on wildlife in northwestern Costa Rica provide data to which the results of the present study can be compared. Burger et al. (1993, 1994) detected multiple metals in wood stork (*Mycteria americana*) feathers and opossum (*Didelphis virginiana*) hair in Guanacaste Province, approximately 80 km northwest of the Río Grande de Tárcoles where crocodiles were sampled in the present study. Because tissue metal concentrations for storks and opossums were reported on a dry weight basis and those for crocodiles were reported on a wet weight basis, direct comparisons between metal concentrations observed in these studies cannot be made. In general, however, mean cadmium concentrations were similar among wood storks, opossums, and crocodiles, and mean lead and mercury concentrations in opossums and crocodiles were also similar (Burger et al., 1993, 1994). In contrast, mean lead and mercury concentrations in crocodile scutes were generally lower than those detected in wood stork feathers (Burger et al., 1993).

Klemens et al. (2003) found multiple OC pesticides in carcasses of numerous vertebrate species sampled in the Area de Conservación Guanacaste in northwestern Costa Rica, approximately 160 km north of the Río Grande de Tárcoles sampling location in the present study. With regard to the analytes screened for at both sites (aldrin, dieldrin, endrin, lindane, heptachlor, methoxychlor, *p,p′*-DDE, *p,p′*-DDT), OC concentrations in American crocodiles from Río Grande de Tárcoles were generally greater than those in wildlife from Guanacaste. The mean *p,p′*-DDE concentration detected in crocodile scutes from Río Grande de Tárcoles (340 ng/g) was 2- to 85-fold greater than mean *p,p′*-DDE concentrations detected in toads, frogs, turtles, mice, and birds from Guanacaste (Klemens et al., 2003). In addition, the mean dieldrin concentration in American crocodile scutes (8 ng/g) exceeded mean dieldrin concentrations in wildlife tissues from Guanacaste by 2- to 11-fold, with the exception of one bird species (yellow-bellied eleania, *Elaenia flavogaster*) (mean = 11 ng/g; Klemens et al., 2003). The concentration of endrin found in one specimen of Salvin's spiny pocket mouse (*Liomys salvni*) (13 ng/g) was 18-fold lower than the mean endrin concentration detected in caudal scutes of American crocodiles (Klemens et al., 2003). Moreover, methoxychlor was detected in all American crocodile scutes sampled (range = 207–1103 ng/g) but was not reported in wildlife from Guanacaste. Conversely, heptachlor (range = ND–32 ng/g) and lindane (<10 ng/g) were both found in wildlife at Guanacaste (Klemens et al., 2003) but not detected in crocodile scutes from Tárcoles. These data suggest greater overall OC contamination at Río Grande de Tárcoles compared to Area de Conservación Guanacaste. However, because of the differences in OC accumulation potential between crocodiles (high trophic level, long-lived) and the wildlife species examined at Guanacaste (lower trophic levels, shorter life spans), differences in the types and concentrations of OCs in tissues collected from these sites is not unexpected. When using contaminant concentrations in wildlife tissue as indicators of environmental pollution at respective sampling sites, collecting tissues from the same species at each site, or at least species with similar bioaccumulation potentials, will allow for more meaningful inter-site comparisons. In addition, using nonlethal samples such as feathers, hair, and scutes may increase sampling efficiency and reduce the impact of sampling on focal species (Burger et al., 1993, 1994, 2000).

The biological significance of the contaminant concentrations observed in crocodile caudal scutes in this study is unknown. Accumulation of pollutants is not necessarily hazardous to organisms (Hopkins, 2006), and few studies have specifically examined the effects of environmental contaminants on crocodilians. The available data suggest that in general, crocodilians can accumulate high concentrations of metals and OC pesticides (Brisbin et al., 1998; Yoshikane et al., 2006), exhibit a high degree of resistance to the acute toxic effects of multiple chemicals (Hammerton et al., 2003; Camus et al., 1998; Peters, 1983), but are susceptible to chronic effects of contaminants on reproduction and long-term health (Brisbin et al., 1998; Guillette et al., 2000; Lance et al., 2006). A combination of laboratory studies and long-term monitoring of a variety of carefully selected endpoints related to growth, behavior, reproduction, and survival are required to adequately examine the potential effects of metal and OC pesticide accumulation on crocodilians and other wildlife living in contaminated environments.

In summary, this study examined concentrations of persistent environmental pollutants in caudal scutes of crocodiles living in contaminated habitats in Central America. Multiple pollutants were detected in scutes of Morelet's crocodiles from Belize (metals) and American crocodile scutes from Costa Rica (metals and OC pesticides), indicating contaminant accumulation in these endangered species and suggesting the presence of contaminants in sediments and other biota associated with the three aquatic systems in which crocodiles were sampled. Mercury was detected in 100% of scutes collected, and endrin, methoxychlor, p, p'-DDE, and p, p'-DDT were detected in all American crocodile scutes examined. Contaminant concentrations varied with those previously reported in crocodilian tissues sampled elsewhere. OC concentrations in American crocodile scutes were generally higher than those previously reported in other Costa Rican wildlife, likely the result of contaminant biomagnification into higher trophic levels.

One of the primary advantages of caudal scutes as indicators of contaminant accumulation in crocodilians is their ease of collection with no permanent harm to the animal, a primary consideration when sampling animals in protected or declining populations, or those that are the subject of other studies. Because removal of caudal scutes is a routine method for marking crocodilians worldwide, retaining scutes for contaminant analysis further enhances the usefulness of this procedure. The utility of caudal scutes as predictors of contaminant burdens in internal tissues is less clear and based on the few data available may depend on the level of predictability desired (Yanochko et al., 1997; Jagoe et al., 1998; Burger et al., 2000). Additional laboratory and field studies involving multiple crocodilian species, size classes, and contaminant types (e.g., metals, OCs) are needed to adequately examine the predictive ability of caudal scutes in estimating xenobiotic concentrations in internal organs and, ultimately, the relationships between contaminant body burdens and health effects at the individual and population levels. Until such data are available, caudal scutes may best serve as general indicators of contaminant accumulation in crocodilians and their areas of occurrence (Yanochko et al., 1997; Jagoe et al., 1998; Jeffree et al., 2001, 2005; Markich et al., 2002).

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