Arch. Environ. Contam. Toxicol. 42, 319–324 (2002)
DOI: 10.1007/s00244-001-0020-7

ARCHIVES OF
Environmental
Contamination
and Toxicology

Mercury in Morelet's Crocodile Eggs from Northern Belize

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Received: 2 August 2001/Accepted: 24 October 2001

Abstract. Recent studies have examined mercury accumulation in crocodilians. However, though most researchers have focused on tissue concentrations, few have examined mercury levels in crocodilian eggs. In July 1995, we analyzed mercury in 31 nonviable Morelet's crocodile (*Crocodylus moreletii*) eggs collected from eight nests across three localities in northern Belize. All eggs were found to contain mercury. Based on an individual egg basis, mean concentration of mercury for all three localities was among the lowest reported for any crocodilian species. When localities were examined separately, mean concentrations for Laguna Seca and Gold Button Lagoon were comparable to those observed in other studies, and the mean for Sapote Lagoon was the lowest ever reported. Based on mean nest concentrations, mercury in eggs from Laguna Seca was approximately two- and tenfold higher than for Gold Button Lagoon and Sapote Lagoon, respectively. Variability in mercury concentrations among localities is likely the result of site-specific differences in mercury input, bioavailabilty, and bioaccumulation. Mercury concentrations were relatively uniform in eggs from the same nest and among nests from the same localities. The presence of mercury in Morelet's crocodile eggs suggests exposure in adult females, developing embryos, and neonates. However, crocodiles in these areas show no overt signs of mercury toxicity, and no indication of population decline is evident. A paucity of data on the effects of mercury on crocodilians precludes meaningful speculation as to the biological significance of tissue and egg concentrations. Controlled laboratory studies and long-term population monitoring are needed to address these questions.

Over the last decade, studies describing the exposure and response of crocodilians to environmental contaminants have increased substantially. This is largely the result of extensive research showing population declines and reproductive impairment in American alligators (*Alligator mississippiensis*) inhabiting contaminated lakes in Florida, USA (Jennings *et al.* 1988; Woodward *et al.* 1993; Guillette *et al.* 1994, 1996; Crain *et al.* 1998), as well as numerous reports of pesticide and polychlorinated biphenyl exposure in various crocodilian species worldwide (Best 1973; Ogden *et al.* 1974; Vermeer *et al.* 1974; Wheeler *et al.* 1977; Hall *et al.* 1979; Wessels *et al.* 1980; Matthiessen *et al.* 1982; Phelps *et al.* 1986, 1989; Delany *et al.* 1988; Heinz *et al.* 1991; Skaare *et al.* 1991; Cobb *et al.* 1997, 2002; Bargar *et al.* 1999; Guillette *et al.* 1999; Wu *et al.* 2000a, 2000b). Most of these studies have focused on organochlorine chemicals, but additional concern has been raised concerning the exposure and impacts of heavy metals, particularly mercury, on crocodilians (Brazaitis *et al.* 1996, 1998; Brisbin *et al.* 1998).

Mercury contamination is a global concern (Eisler 1987; Rudd 1995; Brisbin *et al.* 1998; Hanisch 1998) and is currently considered the most serious environmental threat to wildlife in the southeastern United States (Facemire *et al.* 1995). Mercury is introduced into the environment through a variety of natural and anthropogenic sources (Facemire *et al.* 1995; Eisler 1987; Hanisch 1998) and readily accumulates in wildlife (Wolfe *et al.* 1998). Conditions including low pH and low dissolved oxygen (Miskimmin *et al.* 1992; Driscoll *et al.* 1994) enhance the formation of methylmercury (the most bioavailable form of mercury; Surma-Aho *et al.* 1986; Grieb *et al.* 1990) in lowland swamps, rivers, lakes, and other wetlands typically inhabited by crocodilians (Ruckel 1993; Jagoe *et al.* 1998), and mercury is believed to be the heavy metal of greatest concern to these animals (Brisbin *et al.* 1998; Burger *et al.* 2000). Several researchers have reported mercury levels in crocodilian tissues (Vermeer *et al.* 1974; Delany *et al.* 1988; Hord *et al.* 1990; Ware *et al.* 1990; Yoshinaga *et al.* 1992; Ruckel 1993; Facemire *et al.* 1995; Heaton-Jones *et al.* 1994, 1997; Yanochko *et al.* 1997; Jagoe *et al.* 1998; Brisbin *et al.* 1998; Rhodes 1998; Elsey *et al.* 1999; Burger *et al.* 2000), but fewer have examined mercury concentrations in crocodilian eggs (Ogden *et al.* 1974; Stoneburner and Kushlan 1984; Phelps *et al.* 1986; Heinz *et al.* 1991; Bowles 1996). Because wildlife species are highly sensitive to mercury toxicity during embryonic development and neonatal life (Eisler 1987; Wolfe *et al.* 1998), information on mercury concentrations in eggs is critical when attempting to ascertain the effects of mercury exposure on developing oviparous organisms.

Morelet's crocodile (*Crocodylus moreletii*) is a medium-

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sized freshwater crocodile found in the Atlantic and Caribbean lowlands of Mexico, Guatemala, and Belize (Ross 1998). It is currently recognized as endangered under the U.S. Endangered Species Act (Code of Federal Regulations 1977) and is listed on Appendix I of the Convention on International Trade in Endangered Species of Flora and Fauna (CITES) (Ross 1998). By the late 1960s, Morelet's crocodile was nearly extirpated in Belize as the result of overharvesting for the commercial skin trade (Charnock-Wilson 1970; Frost 1974). However, following legal protection under the Wildlife Protection Act of 1981, populations in northern Belize have made a substantial recovery (Platt 1996; Platt and Thorbjarnarson 2000).

Although Morelet's crocodile populations in northern Belize seemingly face no immediate threats (Platt and Thorbjarnarson 2000), exposure to environmental pollutants may present a subtle yet significant long-term threat to populations in contaminated areas (Gibbons *et al.* 2000). Wu *et al.* (2000a, 2000b) found numerous organochlorine pesticides in Morelet's crocodile eggs from Belize, many of which are believed to be linked to reproductive failure in Florida alligators (Guillette *et al.* 1994, 1996; Crain *et al.* 1998). The purpose of this study was to examine levels of mercury in Morelet's crocodile eggs from different localities in northern Belize. We hypothesized that crocodile eggs from remote areas of Belize would contain mercury, based on the propensity of mercury to accumulate in crocodilian habitats and the occurrence of mercury in alligator eggs collected from remote areas of the southeastern United States (Ogden *et al.* 1974; Stoneburner and Kushlan 1984; Bowles 1996).

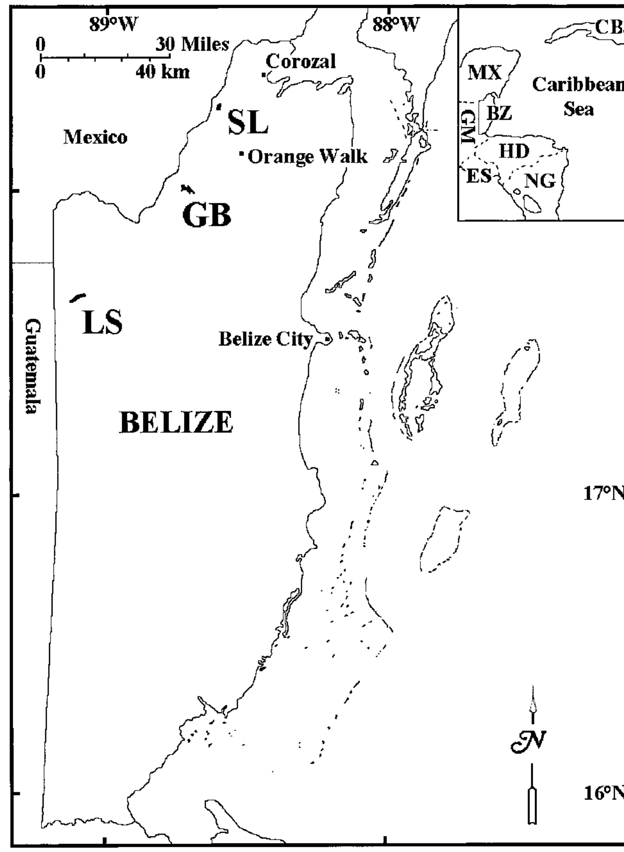

Fig. 1. Map of Belize showing localities from which Morelet's crocodile eggs were collected in July 1995 (GB = Gold Button Lagoon, LS = Laguna Seca, SL = Sapote Lagoon)

Materials and Methods

Study Sites and Sample Collection

In July 1995, 31 nonviable Morelet's crocodile eggs were collected from eight undisturbed, naturally incubating nests found at three nonalluvial lagoons (Platt and Thorbjarnarson 2000) in northern Belize (Figure 1). Sixteen eggs were collected from three nests at Sapote Lagoon (18°19′N, 88°33′W), located approximately 20 km southwest of Corozal Town and part of the Ramonal and Sapote Agricultural Reserve. Twelve eggs were collected from four nests at Gold Button Lagoon (17°55′N, 88°45′W), located on a private cattle ranch approximately 25 km southwest of Orange Walk Town. Three eggs were collected from one nest from Laguna Seca (17°37′N, 89°05′W), located on Gallon Jug Farm within the protected Rio Bravo Conservation and Management Area, approximately 90 km west of Belize City. Nonviable eggs were identified by the absence of an opaque patch or band on the eggshell (Ferguson 1985), assigned unique identification numbers, placed on ice for shipment to the United States, and stored at –20°C until analysis.

Mercury Analysis

Content (shell not included) of each egg was homogenized, and three aliquots digested for total mercury analysis using the method of Adair and Cobb (1999). Samples (approximately 0.15 to 0.2 g) were placed into acid-washed, 8-ml glass vials (loosely capped) and predigested overnight at room temperature in 1 ml of concentrated acid (1:1 metals grade HNO_3/H_2SO_4). Next, samples were digested for 2 h in an 80°C water bath and then placed into a freezer (–20°C) to cool for 10 min. A total of 200 μl of hydrogen peroxide was added to each vial in 25-μl increments over a period of 2 h, after which vials were placed back into the cooled water bath until the water temperature reached 80°C (approximately 1 h). Digested samples and three vial rinsates were filtered through glass fiber filter paper into 10-ml volumetric flasks. The samples were brought to volume with Milli-Q water and stored in 15-ml centrifuge tubes until analysis. Samples were analyzed by cold vapor atomic absorption for total mercury (method limit of detection = 0.05 μg/g in a 200 mg egg sample; qualitative range = 0.02–0.05 μg/g). Mean mercury concentrations were expressed as μg/g wet mass.

Results

Mercury was detected in all 31 crocodile eggs examined in this study. Mean (± SE) concentrations (μg mercury/g egg, wet mass) were based on nest means rather than individual eggs. Mean concentration of mercury for all localities (n = 8 nests) was 0.11 ± 0.05 (range = 0.02–0.21). Mean concentrations for Gold Button Lagoon and Sapote Lagoon were 0.11 ± 0.02 (range = 0.06–0.14, n = 4) and 0.02 ± 0.01 (range = <0.02–0.03, n = 3), respectively, and the concentration for the single nest at Laguna Seca was 0.21. Although small sample sizes precluded statistical analysis, some trends in mercury concentrations among nests and sites were noted. Mercury levels appeared to be relatively uniform among eggs from the same nest (although variability increased as concentrations approached the limit of detection) and, with the possible exception of a single nest at Gold Button Lagoon (GB3), among nests within each site (Figure 2). Among sites, however, the mercury concentration for Laguna Seca was approximately two- and tenfold higher than means for Gold Button Lagoon and Sapote Lagoon, respectively.

Most reports of mercury levels in crocodilian eggs present concentrations in individual eggs or means based on individual eggs. For comparative purposes, we calculated means for Morelet's crocodile eggs examined in this study based on individual eggs as well. Mean concentration of mercury for all localities (0.07 ± 0.01, range = <0.02–0.23) was among the lowest reported for any crocodilian (Table 1), while the levels for Laguna Seca (0.21) and Gold Button Lagoon (0.11 ± 0.01) were comparable to those reported in other studies. Mean mercury concentration for Sapote Lagoon (0.02 ± 0.002) was the lowest reported for eggs in any species, excluding those examined by Heinz *et al.* (1991), in which mercury was not detected (Table 1).

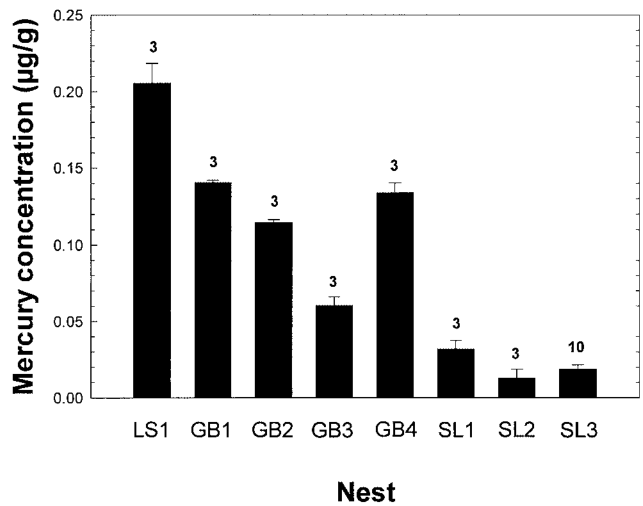

Fig. 2. Mean concentrations (± SE, µg/g wet mass) of mercury in Morelet's crocodile eggs from eight nests in northern Belize. Numbers above error bars indicate the number of eggs analyzed per nest. GB = Gold Button Lagoon, LS = Laguna Seca, SL = Sapote Lagoon

Discussion

Jagoe *et al.* (1998) reported considerable variation in mercury concentrations in alligator tissue among four localities in the southeastern United States and speculated this to be a reflection of differences in local and atmospheric mercury inputs as well as site-specific variations in mercury bioavailability. This is likely the case in Belize as well. Although the sources of mercury in crocodile eggs examined in this study are unknown, we suspect the occurrence of mercury in these wetlands results primarily from natural processes and atmospheric deposition (Miskimmin *et al.* 1992; Driscoll *et al.* 1994; Rudd 1995), given the remoteness of the collection sites and relatively few anthropogenic mercury sources in Belize (Hartshorn *et al.* 1984). The among-site variation observed in this study may be an artifact of small sample sizes, but we speculate that any true site-related differences in egg mercury levels can be attributed to site-specific differences in mercury input, bioavailability, and bioaccumulation. Mercury contamination in crocodilian eggs likely results from maternal transfer. Direct environmental contamination from nest media may be another possible route of exposure (Sahoo *et al.* 1996; Linder and Grillitsch 2000), but this process has not been specifically examined. Wu *et al.* (2000a) found multiple organochlorine pesticides in Morelet's crocodile nest material from one of the sites examined in this study (Gold Button Lagoon), but mercury levels were not determined.

Although the effects of mercury on mammals and birds are well documented (Eisler 1987; Wolfe *et al.* 1998), virtually nothing is known concerning the effects of mercury on reptiles (Hall 1980; Wolfe *et al.* 1998; Linder and Grillitsch 2000). Indeed, numerous reports of mercury exposure in crocodilians exist, but most focus primarily on human health hazards related to the consumption of crocodilian meat (Peters 1983; Delany *et al.* 1988; Hord *et al.* 1990; Yoshinaga *et al.* 1992; Ruckel 1993; Elsey *et al.* 1999). Peters (1983) dosed captive juvenile American alligators with methylmercury chloride (5.0 mg/kg) and concluded, "it does appear that the alligator is capable of carrying such levels without toxic manifestations." Likewise, no clinical signs of mercury toxicity were observed in 24 wild-caught alligators from Florida, despite the fact that each animal contained mercury in various tissues (ranges in ppm wet mass: tail muscle = 0.04–4.28; kidney = 0.15–65.33; liver = 0.14–99.48) (Heaton-Jones *et al.* 1997). Burger *et al.* (2000) found low concentrations of mercury (similar to those observed in the present study) in alligator tissues from multiple lakes in Florida and speculated these levels likely posed no threat to alligator health. Conversely, Heaton-Jones *et al.* (1994) found mercury in optic lobes, optic nerves, and retinas of Everglades alligators and concluded mercury accumulation in these tissues was impacting the alligator visual system. However, no clinical effects were examined. More recently, Brisbin *et al.* (1998) reported elevated mercury concentrations (ppm wet mass: muscle = 3.48, kidney = 33.55, liver = 158.85) in a large adult alligator found dead in a mercury-contaminated pond in South Carolina. Because these concentrations exceeded mercury levels shown to be lethal in various birds, mammals, and amphibians under laboratory conditions (Wolfe *et al.* 1998), and due to the animal's emaciated condition at the time of death, Brisbin *et al.* (1998) suggested this may be the first documented case of mercury-related mortality in a crocodilian.

Despite numerous reports of mercury in crocodilian habitats, tissues, and eggs, no clinical effects on crocodilians at the individual or population levels have been documented. Brisbin *et al.* (1998) emphasized the need for controlled laboratory studies to determine the toxicological impacts of mercury and other metals on crocodilians and cautioned against attributing crocodilian mortality or morbidity to these compounds until such information is available. Similarly, Linder and Grillitsch (2000) stated, "the presence of a substance in an organism is not an adverse biological effect in and of itself." Although mercury concentrations were found in alligator tissues from the Florida Everglades, populations there are stable with no indication of decline, suggesting no significant impact on repro-

Table 1. Mercury concentrations (ppm wet mass) in crocodilian eggs

Species	Location	n	Mean (± SE) Mercury Concentration	Range	Reference
American alligator					
(*Alligator mississippiensis*)	Florida, USA	4	0.54 ± 0.06	0.41–0.71	Ogden *et al.* 1974
	Florida, USA	34	ND	ND	Heinz *et al.* 1991
	South Carolina, USA	10	NR	0.01–0.02	Bowles 1996
American crocodile					
(*Crocodylus acutus*)	Florida, USA	5	0.09 ± 0.01	0.07–0.14	Ogden *et al.* 1974
	Florida, USA	9	0.13 ± 0.01	NR	Stoneburner and Kushlan 1984
Morelet's crocodile					
(*Crocodylus moreletii*)	Belize	31	0.07 ± 0.01	<0.02–0.23	This study
Nile crocodile					
(*Crocodylus niloticus*)	Zimbabwe	26	0.23 ± 0.03[a]	0.02–0.54	Phelps *et al.* 1986

[a] Based on dry mass.
ND = Not detected, NR = Not reported.

duction (Heaton-Jones *et al.* 1997). Likewise, although virtually every river in coastal South Carolina is contaminated with mercury (Facemire *et al.* 1995), alligator population densities there are equal to or exceed those of other states within the animal's range (Rhodes 1998). The limited data from the present study indicate mercury contamination in Morelet's crocodile eggs in northern Belize, suggesting chemical exposure in adult females, developing embryos, and neonates. However, Morelet's crocodiles in these areas have shown no overt signs of mercury toxicity (*e.g.*, emaciation, abnormal motor coordination; Eisler 1987) and no indication of population decline is evident (Rainwater *et al.* 1998; Platt and Thorbjarnarson 2000). Because mercury may have latent, chronic effects on wildlife (Eisler 1987; Wolfe *et al.* 1998), long-term monitoring and sampling of crocodilian populations are essential for detecting contaminant-induced impacts over time.

In this study, 31 (100%) Morelet's crocodile eggs from three localities in northern Belize were found to contain mercury. This constitutes the first report of mercury in crocodilian eggs from Central America and, to our knowledge, only the fifth report of mercury in crocodilian eggs worldwide (4 of 23 extant species; Ross 1998). Mean concentrations were among the lowest reported for crocodilians, but certain eggs contained levels comparable to those observed in other species. However, a paucity of information on the effects of mercury on crocodilians makes it difficult to speculate on the biological significance of these concentrations (Stoneburner and Kushlan 1984). Controlled laboratory studies and long-term population monitoring are warranted to fully understand the relevance of mercury in crocodilian eggs and the effects of mercury on crocodilians at both the individual and population levels.

Acknowledgments. We thank Barry Bowen (Gallon Jug) and Robert Noonan (Gold Button Ranch) for providing access to their property. We also thank Colin, Mark, and Monique Howells (Lamanai Outpost Lodge/Lamanai Field Research Center); Bruce and Carolyn Miller (Wildlife Conservation Society, Gallon Jug); and Robert Noonan (Gold Button Ranch) for generously providing accommodations in Belize during this project. Thomas Rhott and Travis Crabtree assisted in egg collections. This project was supported by Lamanai Field Research Center, Indian Church, Belize, the Royal Geographical Society, and U.S. EPA (grant no. R826310 to STM). SGP was supported by Wildlife Conservation Society. Additional support for TRR was provided by the ARCS Foundation, Lubbock, Texas.

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