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Short Communication

MATERNAL TRANSFER OF SELENIUM IN *ALLIGATOR MISSISSIPPIENSIS* NESTING DOWNSTREAM FROM A COAL-BURNING POWER PLANT

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Abstract—Selenium (Se) is embryotoxic in many oviparous vertebrates, but little is known about maternal transfer of Se and its impact in reptiles. Over a four-year period, we collected three clutches of eggs of the American alligator (*Alligator mississippiensis*) from a single nest at a site contaminated with Se and compared egg and hatchling Se concentrations and clutch viability from this nest to nests downstream from the contaminated site (two clutches from two nests) and at a reference site (two clutches from two nests). Eggs and hatchlings from the nest at the Se-contaminated site and downstream nests had elevated Se concentrations (2.1–7.8 ppm) and lower viability (30–54%) compared to reference nests (1.4–2.3 ppm and 67–74% viability), but Se concentrations did not exceed reproductive toxicity thresholds established for other oviparous vertebrates. Selenium concentrations were higher in chorioallantoic membranes of eggs from Se-contaminated sites, suggesting that this tissue may be useful as a nondestructive index of Se exposure for embryos of *A. mississippiensis*. Examination of these data suggests that further studies on uptake, accumulation, and reproductive success of crocodilian embryos exposed to excessive Se are warranted.

Keywords—*Alligator mississippiensis* Chorioallantoic membrane Crocodylia Maternal transfer Selenium

INTRODUCTION

Maternal transfer can be a significant source of exposure to potentially toxic substances for crocodilian embryos and hatchlings. Most studies of maternal transfer of environmental pollutants in crocodilians have focused on organic contaminants and mercury [1–8], but nothing is known about maternal transfer of selenium (Se) in crocodilians inhabiting Se-contaminated sites despite evidence that Se is embryotoxic to other oviparous organisms [9,10]. Selenium contamination in aquatic habitats is a growing concern worldwide as a consequence of mining, industrial, and agricultural discharges and runoff; oil procurement, transport, refining, and utilization; and the disposal of fly ash from electric power–generation facilities [11–13]. Because crocodilian embryos are particularly sensitive to some environmental contaminants (e.g., organic compounds [14,15]), it is important that maternal transfer of other contaminants, such as Se, be examined with respect to its potential effects on juvenile recruitment.

A single American alligator (*Alligator mississippiensis*) was observed to repeatedly nest in a swamp downstream of a coal-burning electric power plant, a Se-contaminated site that has been the subject of many previous toxicological investigations [16–20]. No other alligator nested in this swamp during the study. Although obvious limitations exist to what can be achieved when working with a single individual, this female provided us with a novel opportunity to determine if excessive Se is maternally transferred in *A. mississippiensis*, and whether the chorioallantoic membrane (CAM; the extraembryonic membrane often remaining in the egg shell after hatching) has potential as a nondestructive index of Se exposure for embryos of *A. mississippiensis*.

MATERIALS AND METHODS

We collected eggs of *A. mississippiensis* in 1997 through 2000 from three sites in South Carolina, USA: a 2-ha drainage swamp (ABS) that receives effluent from a coal-burning electric power plant; 2.0 to 2.5 km downstream from the power plant along Beaver Dam Creek (BDC), a tributary of the Savannah River into which surface water from the ABS drains; and a nearby reference pond (REF). Seven clutches were collected, three from the only nest that exists in the ABS site (1997, 1999, and 2000), and one each from two different nesting locations in the BDC site (1997 and 2000) and two nesting locations in the REF site (1997 and 1999). Although we cannot be certain, the three clutches from ABS were likely from the same female, whereas the four clutches from BDC and REF were likely from different females. The coal-burning plant is located on the U.S. Department of Energy's Savannah River Site. Previous investigations, many of which were conducted simultaneously with this study, documented high concentrations of Se in water, sediments, and biota from ABS and from BDC relative to reference sites historically unpolluted with coal combustion wastes. Site descriptions and a detailed discussion of pollutant levels at the contaminated site can be found in previous publications [16–20].

Eggs were collected from nests each year between June 22 and August 8. Complete clutches were placed in plastic bins, cushioned with natural nest material, and transported (avoiding vibrations and rotation) to the Savannah River Ecology Laboratory, near Aiken (SC, USA), where they were counted and weighed. Three to five eggs from each clutch were frozen for trace element analysis. The remaining eggs were placed in incubators (32–34°C; temperatures that produce mostly males [21]) containing moist vermiculite. Eggs from each clutch were spread across multiple incubators and mixed with eggs from

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Table 1. Egg and hatchling parameters for *Alligator mississippiensis* from a coal-ash–polluted swamp (ash basin swamp [ABS]), a site downstream of the polluted swamp (Beaver Dam Creek [BDC]), and an unpolluted reference pond (REF) in South Carolina, USA[a]

		Clutch and egg parameters					Hatchling parameters		
Site	Year	Clutch size	Clutch mass (g)	Egg mass (g)	% Hatching	Incubation period (d)	Mass (g)	SVL[b] (mm)	Total length (mm)
ABS[c]	1997	30	2,099	70.0 (0.5)	53.8	64.2 (0.5)	45.1 (0.5)	112.0 (0.5)	233.0 (0.9)
ABS[c]	1999	35	2,430	69.4 (0.4)	50.0	66.7 (0.7)	47.1 (0.6)	114.6 (0.7)	240.6 (1.7)
ABS[c]	2000	29	2,034	70.1 (0.6)	46.2	61.4 (0.9)	45.7 (0.07)	115.4 (1.2)	237.8 (2.1)
BDC	1997	44	4,367	99.3 (0.8)	53.8	43.3 (0.5)	62.7 (0.9)	124.5 (0.9)	258.0 (2.2)
BDC	2000	46	4,365	95.7 (0.5)	30.0	60.0 (1.1)	60.0 (0.8)	124.9 (0.8)	258.8 (1.5)
REF	1997	31	2,011	64.9 (0.6)	66.7	62.3 (0.4)	40.8 (0.5)	113.8 (0.6)	236.0 (1.2)
REF	1999	41	3,438	83.9 (0.9)	73.7	66.4 (0.6)	56.3 (0.4)	122.2 (0.5)	255.3 (1.1)

[a] Values are means (± 1 standard error).
[b] SVL = snout–vent length.
[c] Eggs were collected from the same nest at ABS in all three years.

other clutches. Egg viability was monitored throughout the incubation period, and eggs were periodically sprayed to maintain similar moisture conditions in all incubators. Eggs that were obviously inviable (severe discoloration or putrid odor) were removed and frozen. Upon hatching, alligators were measured (mass, snout–vent length, and total length), and three hatchlings were frozen for trace element analysis. Only CAMs left in the eggshell were removed and frozen for trace element analysis, resulting in five CAMs from ABS, two from BDC, and three from REF sites. Clutch viability was calculated as the proportion of eggs in a clutch (excluding eggs removed upon collection for trace element analysis) that produced viable hatchlings. All hatchlings not used for trace element analysis were released at their respective nests.

Tissues (egg contents minus shells, hatchlings, and CAMs) were lyophilized and homogenized before being digested and analyzed for trace element concentrations by using inductively coupled plasma–mass spectrometry (Perkin-Elmer, Norwalk, CT, USA). For a detailed description of digestion and analysis procedures, refer to Hopkins et al. [22]. Mean percent recovery for Se in certified reference materials was 106%. Mean instrument detection limits of Se in eggs, CAMs, and hatchlings were 0.155, 0.139, and 0.596 ppm, respectively. All Se concentrations (ppm) are presented as mean ± 1 standard error on a dry mass basis.

RESULTS

Mean clutch incubation periods ranged from 43 to 67 d (Table 1). Incubation typically lasts 62 to 66 d [23], indicating that most clutches in this study were collected within a few days of laying. Mean clutch, egg, and hatchling sizes varied widely among sites (Table 1). In general, clutch, egg, and hatchling sizes were larger for BDC than for ABS and REF sites. Clutch viability ranged from 30 to 74%, but viability of REF clutches was higher than for ABS or BDC clutches (Table 1).

Selenium concentrations were generally higher in ABS eggs, hatchlings, and CAMs compared to BDC and REF sites. Mean Se concentrations in eggs and hatchlings from the ABS site were 2.4 to 3.7 times higher than those from BDC, and 3.0 to 5.5 times higher than those from REF sites (Table 2). Selenium concentrations in eggs and hatchlings from the ABS nest differed little over the four-year period. Mean Se concentrations in CAMs exceeded concentrations in eggs and hatchlings. Selenium concentrations of CAMs from the ABS, BDC, and REF sites were 27.2 ± 5.6 ppm, 6.7 ± 0.6 ppm, and 5.0 ± 0.9 ppm, respectively.

DISCUSSION

This is the first study to document maternal transfer of excessive Se in a crocodilian from a site known to be polluted with Se. Not surprisingly, Se concentrations in the eggs of *A. mississippiensis* from the ABS were the highest yet reported in a crocodilian. In contrast, Se concentrations in eggs from BDC and REF were generally comparable to concentrations in eggs collected from other sites uncontaminated with Se [2,3,12]. The female that transferred Se to her eggs probably accumulated Se from her diet, because reptiles in Se-contaminated habitats often accumulate high tissue burdens of Se through their food [17,19,24]. Whole-body Se concentrations of some common prey items for *A. mississippiensis*, such as crayfish, fish, frogs, and turtles, range between 10 and 37 ppm in the ABS [17,19]. Once high concentrations of Se accumulate in tissues, Se is readily transferred to developing embryos, most likely through incorporation of selenoamino acids into proteins such as vitellogenin [25]. Selenium in eggs of *A. mississippiensis* was likely maternally derived because eggs were in contact with contaminated nest substrate for a short

Table 2. Mean (± 1 standard error) selenium concentrations (ppm dry mass) of eggs and hatchlings of *Alligator mississippiensis* from a coal-ash–polluted swamp (ash basin swamp [ABS]), a site downstream of the polluted swamp (Beaver Dam Creek [BDC]), and an unpolluted reference pond (REF) in South Carolina, USA. Selenium concentrations were based on three to five eggs and three hatchlings per clutch

	Site (year)						
	ABS (1997)[a]	ABS (1999)[a]	ABS (2000)[a]	BDC (1997)	BDC (2000)	REF (1997)	REF (1999)
Egg	7.30 (0.18)	7.64 (0.14)	7.44 (0.40)	3.00 (0.03)	2.21 (0.19)	2.34 (0.04)	1.76 (0.10)
Hatchling	7.20 (0.27)	7.49 (0.12)	7.75 (0.23)	2.36 (0.25)	2.11 (0.12)	1.95 (0.08)	1.41 (0.03)

[a] Eggs were collected from the same nest at ABS in all three years.

period, and contact with Se-contaminated nest material did not account for additional Se uptake in turtles at this site [19].

Maternal transfer of Se has now been documented in diverse taxonomic groups including birds, crocodilians, turtles, and fish that use aquatic habitats near the same coal-burning power plant in South Carolina. Selenium concentrations in eggs and offspring of the ABS *A. mississippiensis* were higher than concentrations in common grackle eggs (*Quiscalus quiscala*; 5.8 ppm [26]), similar to those in eggs of slider turtles (*Trachemys scripta*; 7.4 ppm [19]), and lower than concentrations in offspring of eastern mosquitofish (*Gambusia holbrooki*; 15.9 ppm [27]) at the ABS site. Variation among taxa may reflect differences in physiology, ecology, or life history traits. Interestingly, maternal transfer of Se in the *A. mississippiensis* nesting in the ABS varied little over the four years of this study, suggesting that Se elimination during reproduction was perhaps in equilibrium with Se uptake or tissue burdens. Similarly, no temporal trends in Se transfer to eggs were observed in a snake fed constant levels of Se in the laboratory [22].

Chorioallantoic membranes appear to have potential as indicators of Se exposure in embryos of *A. mississippiensis*, a finding in agreement with other investigations suggesting CAMs as useful, nondestructive indices of exposure to some organic contaminants for crocodilian embryos [4,5]. Alternatives to lethal sampling techniques are valuable when assessing contaminant exposure in rare wildlife, and conservation-oriented exposure assessments are particularly appropriate for crocodilian species, one third of which are endangered [12]. Although many studies have used CAMs to assess exposure to organic compounds, we know of only one other study [28] that used CAMs to determine whether embryos have been exposed to an inorganic pollutant (i.e., Hg in birds). In our study, Se was concentrated in CAMs to a greater extent than in whole egg contents and hatchlings. Although little is known about the exact composition of the CAM, it has a lower lipid content than the yolk [29]. Differential partitioning of Se into CAMs suggests the CAM may be rich in protein, where Se (a sulfur analog) tends to concentrate [30]. As a consequence of high Se partitioning in CAMs, these extraembryonic membranes may allow researchers to detect low levels of Se exposure in a nondestructive manner. Future studies that examine whether Se concentrations in CAMs and embryos are correlated would be useful for assessing the utility of CAMs as predictors of the extent of embryonic exposure to Se.

The suggestion has been made that reproductive parameters may be the most sensitive measures of Se toxicity to wildlife [9,10,22]. Egg and hatchling Se concentrations for *A. mississippiensis* from the ABS fell within the wide range of Se toxicity thresholds recommended for other oviparous vertebrates (fish and birds: 3–16 ppm [9,10,31–33]), but information on Se toxicity in reptiles is limited. In the only controlled study of maternal transfer of Se and reproduction in a reptile, no reproductive effects were observed in snakes transferring as much as 23 ppm of Se to eggs [22], a concentration three times higher than in eggs of *A. mississippiensis* from the ABS. However, extrapolating risks from surrogate taxa may be inappropriate because of fundamental differences in physiology among taxa [12,34]. The limited number of clutches precluded any statistical assessment of reproductive effects associated with Se in *A. mississippiensis*. General trends indicate that clutch viability from the contaminated ABS and downstream on BDC (30–54%) was lower compared to the REF site (67–74%; Table 1), and clutch, egg, and hatchling sizes were highly variable. Viability of artificially incubated clutches of *A. mississippiensis* from relatively pristine sites generally falls between 70 and 90% [15,35,36]. Whether reduced clutch viability at sites contaminated with coal-combustion wastes resulted from Se exposure is uncertain because numerous other factors, such as dietary nutrient deficiencies, genetic abnormalities, female size and age, population density, and the presence of other contaminants, may affect clutch viability in crocodilians [14]. Although numerous contaminants, including heavy metals and metalloids (e.g., As, Cd, Cr, Cu, and Se), can be found in high concentrations at fly-ash disposal sites, Se is the only of the above contaminants maternally transferred in high levels to fish, bird, and turtle embryos at our study site [19,26,27]. Variation in clutch, egg, and hatchling size and weight most likely reflects variation in size and age of females [37], for which we have no data.

Exposure of crocodilian embryos to Se at our study site likely is not an isolated case, but may be a widespread concern as a consequence of many human activities associated with Se pollution. Electricity generation by coal combustion; mining operations; and oil procurement, transport, and utilization are activities contributing to Se pollution that occur extensively in areas inhabited by crocodilians [13], and some of these activities have been identified as threats to crocodilian populations [12,34,38]. Although this study was limited in scope, we provide evidence that further research concerning maternal transfer of Se and reproductive toxicity in crocodilians, which are important components of many aquatic ecosystems, is warranted.

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