Arch. Environ. Contam. Toxicol. 40, 531–536 (2001)
DOI: 10.1007/s002440010206

ARCHIVES OF
Environmental
Contamination
and Toxicology

Accumulation and Selective Maternal Transfer of Contaminants in the Turtle *Trachemys scripta* Associated with Coal Ash Deposition

R. D. Nagle,[1,2] C. L. Rowe,[1,3] J. D. Congdon[1]

[1] University of Georgia, Savannah River Ecology Laboratory, Drawer E, Aiken, South Carolina 29802, USA
[2] Environmental Science and Studies, Juniata College, Huntingdon, Pennsylvania 16652, USA
[3] University of Maryland, Center for Environmental Science, Chesapeake Biological Laboratory, PO Box 38, Solomons, Maryland 20688, USA

Received: 1 August 2000/Accepted: 15 December 2000

Abstract. Coal combustion wastes are enriched in a number of potentially toxic compounds and may pose risks to biota exposed to the wastes. Slider turtles (*Trachemys scripta*) are common inhabitants of coal ash settling basins in South Carolina, USA, where they feed on contaminated prey items and accumulate high levels of potentially toxic compounds in their tissues. Furthermore, female sliders sometimes nest in contaminated spill piles and thus may expose embryos to contaminated soils. We examined two potential pathways by which female *T. scripta* may influence the survivorship and quality of their offspring in a contaminated habitat: (1) nesting in contaminated soil and (2) maternal transfer of pollutants. Eggs were collected from turtles captured in coal ash–polluted or unpolluted sites; individual clutches were incubated in both ash-contaminated and uncontaminated soil in outdoor, artificial nests. Incubation in contaminated soil was associated with reduced embryo survivorship. Adult females from the polluted site accumulated high levels of As, Cd, Cr, and Se in their tissues, yet Se was the only element transferred maternally to hatchlings at relatively high levels. Hatchlings from polluted-site females exhibited reduced O_2 consumption rates compared to hatchlings from reference sites. Relatively high levels of Se transferred to hatchlings by females at the ash-polluted site might contribute to the observed differences in hatchling physiology.

Coal combustion remains the primary source of electrical energy in the United States (US EPA 1997), and global reliance on coal continues to increase (US DOE 1995). More than 80 million tons of coal ash, the primary solid waste product from coal combustion, is produced annually in the United States (US EPA 1997), and more than 70% of combustion facilities manage their wastes on site (US EPA 1988). Large volumes of water are often required for the disposal of coal combustion wastes, thus landfills and settling basins containing coal ash are invariably located adjacent to aquatic habitats, including lakes, rivers, and streams. Furthermore, because coal ash contains high levels of potentially toxic trace elements, containment and disposal procedures can be environmentally problematic. Impacts on ecosystems include contamination of soils, surface water, and groundwater; reductions in plant establishment and growth; and accumulation of potentially toxic trace elements throughout the food chain (Carlson and Adriano 1993).

Elevated levels of heavy metals and other trace elements found in coal ash are a source of risk to the biota associated with disposal facilities. Benthic invertebrates inhabiting coal ash settling basins accumulate high levels of As, Cd, Cr, and Se (Cherry *et al.* 1979; Guthrie and Cherry 1979; McCloskey and Newman 1995) and represent primary vectors of contaminant transfer to vertebrate organisms (Lemly and Smith 1987). Recent studies have linked coal ash exposure with morphological and physiological anomalies in amphibians (Rowe *et al.* 1996, 1998; Hopkins *et al.* 1997, 1998, 1999a; Raimondo *et al.* 1998). Furthermore, fish exposed to coal ash waste accumulate (Besser *et al.* 1996) and subsequently transfer high levels of Se to offspring, with detrimental effects on embryo survivorship and development (Gillespie and Baumann 1986).

Turtles are a major faunal component of many freshwater ecosystems, particularly those of the southeastern United States (Congdon *et al.* 1985). Because turtles occupy middle to upper-level positions in the food web and have long lifespans, they are excellent organisms for contaminant monitoring (Bishop *et al.* 1991, 1995; Struger *et al.* 1993). Female snapping turtles exposed to organic pollutants in their diet have been shown to transfer substantial levels to eggs, with deleterious effects on embryo development (Bishop *et al.* 1991, 1995; Struger *et al.* 1993). Because polychlorinated biphenyls (PCBs) and other organic contaminants are maternally transferred to eggs in relative proportion to the levels at which adult females are exposed, turtle eggs provide a useful biological monitoring tool (Bishop *et al.* 1991, 1995; Struger *et al.* 1993). Levels of heavy metals and other trace elements have been examined in eggs of the sea turtle *Caretta caretta* (Stoneburner *et al.* 1980) and the freshwater turtle *Trachemys scripta* (Burger and Gibbons

Correspondence to: R. D. Nagle

Reprint requests: Reprints Department, SREL, Drawer E, Aiken, SC 29802

1998). However, relationships between levels of inorganic contaminants in female turtles and those maternally transferred to offspring have not previously been established.

Slider turtles (*T. scripta*) are common inhabitants of coal ash settling basins on the Savannah River Site near Aiken, SC. Preliminary field investigations indicated that adult females at the site accumulated high levels of many trace elements in their tissues and also nested in contaminated coal ash spill piles. Spill piles are characterized by unvegetated, loose soil in open-canopy areas, with substrate characteristics similar to preferred turtle nesting sites. We subsequently questioned: (1) whether adult females inhabiting the coal ash–polluted site transfer trace elements to offspring, and whether or not such contamination affects hatchling survivorship or quality (*e.g.*, indicators of reduced quality may include small body size, morphological abnormalities, or altered metabolic rates); and (2) whether embryos that develop in coal ash–polluted soil exhibit reduced survivorship or incorporate trace elements from contaminated soil. We therefore conducted an experiment to simultaneously examine maternal transfer of inorganic pollutants to offspring, the viability of *T. scripta* eggs developing in coal ash, and whether eggs uptake trace elements from contaminated soil and incorporate them into embryonic tissues during development.

Materials and Methods

Site Description

On the Savannah River Site, basins in D-area receive sluiced fly ash from a coal-burning steam plant that has been in operation since 1952. The slurry is channeled through two settling basins (approximately 20 ha total area), then drainage water moves through a swamp (approximately 2 ha) that drains into Beaver Dam Creek, a tributary of the nearby (<1 km) Savannah River. Because water and sediments in the settling basins and drainage swamp are enriched in trace elements, aquatic and terrestrial organisms inhabiting these areas are chronically exposed to high levels of potentially toxic compounds (Rowe *et al.* 1996, 1998; Hopkins *et al.* 1998, 1999b; Raimondo *et al.* 1998).

Field-Collected Females and Selected Prey Items

Adult female *T. scripta* were collected from Aiken County, SC, using baited hoop traps. Females were collected during 1993 ($n = 3$) and 1994 ($n = 1$) from the D-area ash basins on the Savannah River Site (hereafter referred to as site "ASH"), and during 1993 only ($n = 3$) from the Old Jackson Canal swamp, an unpolluted reference site near Jackson, SC ("REF"). Turtles were killed by freezing, then thawed, and gut contents were examined to identify major food items. Liver samples were removed, lyophilized, and homogenized for trace elements analysis. Levels of As, Cd, Cr, Cu, and Se (μg/g dry mass) were determined by ICP-MS at the Soil and Crop Sciences Division of the University of Georgia. Samples of major prey items were also collected from each site and analyzed for trace elements (as above).

Hatchling Experiment

In the spring of 1995, we collected female *T. scripta* ($n = 10$) with baited hoop traps from ASH and two reference sites (REF sites, collectively): the Old Jackson Canal Swamp and Snipes Pond, an unpolluted site near Couchton, SC. Turtles were x-radiographed to determine the presence of shelled eggs (Gibbons and Greene 1979; Hinton *et al.* 1997). Because turtle embryos (within eggs) experience preovipositional arrest during late gastrulae in the oviducts, and further differentiation ceases until the eggs are laid (Ewert 1985), we were able to standardize the beginning of the incubation period by holding females in outdoor enclosures for a maximum of 5 days prior to the induction of oviposition. On May 28, 1995, gravid females were induced to oviposit via injections of synthetic oxytocin (Ewert and Legler 1978).

We obtained 18 eggs (four clutches) from females inhabiting site ASH and 18 eggs (six clutches) from females inhabiting REF sites. Eggs from each clutch were assigned to one of two treatments: incubation in ash-contaminated soil (ASH soil) or incubation in soil obtained from an unpolluted reference site (REF soil). On May 29, 1995, eggs were distributed among three replicate artificial nests and allowed to incubate under field conditions. Nests were divided in half by a 30-cm-tall piece of tin flashing buried to a depth of about 27 cm. On one half of the nest, ASH soil was filled to a depth of approximately 12 cm; on the other half of the nest, REF soil filled to the same depth. Two samples of each treatment soil were taken for trace element analysis. Artificial nests were completely surrounded by a minimum of 20 cm of treatment soil on each side. ASH soil was dark-gray compared to the light-beige color of REF soil, composed predominantly of sand. Therefore, to expose eggs from both substrate treatments to similar thermal conditions, all nests were covered with a thin layer (approximately 1 cm) of sand from an unpolluted site. Nests were enclosed in cages (approximately 1 m^3) of 2.5×5 cm welded wire to exclude predators.

After day 50 of incubation, nests were checked weekly for indications that hatchling development was nearly complete (*i.e.*, exfoliation of eggshell mineral layers). Such indications were apparent on August 4, 1995, and all eggs were excavated and taken to the laboratory. Eggs were placed on wet paper towels in plastic containers at approximately 20°C. When hatchlings emerged from eggs, we measured hatchling wet mass and plastron and carapace length. Hatchlings were examined for external developmental and morphological abnormalities, including anomalous scute patterns and malformations of the eyes, jaws, feet, limbs, and bridge.

Hatchlings were held for 5 days at 25°C in an environmental chamber with a 12:12 h light:dark cycle, and then metabolic rates (O_2 consumption) were determined for each individual at 25°C using a Micro-Oxymax closed-system respirometer (Columbus Instruments, Columbus, OH). Next, we warmed hatchlings at a constant rate over a period of 24 h to 30°C, held them for an additional 4 days at 30°C, and determined their metabolic rates at 30°C. Oxygen consumption rates (ml/h) of individual turtles were recorded at 1-h intervals for 24∼30 h. A zinc-air cell battery that consumed a known quantity of oxygen was run in conjunction with each group of hatchlings as a reference standard.

Following metabolic measurements, hatchlings were killed by freezing. Carcasses were lyophilized for 96 to 120 h, then ground with a tissue homogenizer. Dried homogenates were digested and analyzed for As, Cd, Cr, Cu, and Se using ICP-MS.

Data Analysis

To determine differences in trace element levels among adult females, prey items, and hatchlings, trace element data were log transformed and compared using one-way ANOVA. We compared hatchlings between two treatments: (1) Incubation Substrate treatment, which consisted of incubation in ASH or REF soil, and (2) Maternal Residence treatment, which consisted of ASH or REF maternal capture location. Because measures of hatchling body size were highly correlated, we compared plastron length, carapace length, and body mass of hatchlings among treatments using MANOVA. Analysis of survival data were conducted using likelihood ratio chi-square (G) tests on counts of surviving and nonsurviving embryos. Metabolic rates of hatchlings from the two treatments were compared using ANCOVA with body mass used as a covariate. All statistical tests were performed using SAS 6.10 for Windows (Sas Institute, 1994). Because of low sample sizes, statistical power to detect differences was quite low. Thus, only the most substantial effects would be judged statistically significant. Levels of significance were set at $\alpha = 0.05$.

Results

Adult Females

Concentrations of As, Cd, Cr, and Se were significantly higher in adult female *T. scripta* from site ASH compared to those from REF sites (Table 1). Gut contents of adult female *T. scripta* from ASH included Asiatic clams (*Corbicula fluminea*), crayfish (*Procambarus acutus*), and small amounts of unclassified vegetation. Adult females from REF contained food items including crayfish, aquatic insects, and varying amounts of vegetation. Clams and crayfish sampled from ASH contained high levels of many trace elements compared to those from REF (Table 1).

Incubation Substrate Treatment

Trace element concentrations of incubation substrate samples are summarized in Table 1. Mean levels of As, Cr, Cu, and Se were substantially higher in ASH soil than in REF soil.

Incubation period and physical characteristics of hatchlings incubated in ASH soil were similar to those of hatchlings incubated in REF soil (Table 2). However, survivorship of embryos incubated in ASH soil was significantly reduced compared to that of embryos incubated in REF soil ($G = 4.98$, $p < 0.05$; Table 2). No differences in hatchling whole-body trace element levels were found between those incubated in ASH soil and those incubated in REF soil (Table 3).

Maternal Residence Treatment

Egg and hatchling characteristics of females obtained from ASH were similar to those obtained from females from REF (Table 2). Incubation period was also similar between hatchlings from ASH and REF females (Table 2). Embryo survivorship of eggs from ASH females was not significantly different from that of REF females ($G = 0.53$, $p > 0.05$; Table 2). Eggs that did not hatch exhibited decomposition to such an extent that we were unable to determine the degree of embryo development and whether or not abnormalities had occurred. One hatchling from a REF female was found to have anomalous vertebral scutes; no other developmental abnormalities were found among hatchlings from either ASH or REF females.

Levels of Se in hatchlings derived from ASH females (regardless of incubation substrate type) were significantly higher than those found in hatchlings from REF females (Table 3). Although the majority of variance (90.8%) in hatchling whole-body Se levels was due to maternal residence effects, a significant portion of the remaining variance (6.3%) was due to individual females within sites (nested ANOVA: $F_{4,6} = 5.44$, $p < 0.05$). Despite differences in hatchling whole-body Se levels, however, no significant differences were found in levels of other trace elements (As, Cd, Cr, and Cu) between hatchlings from ASH and REF females (Table 3).

At both 25°C and 30°C, metabolic rates of hatchlings from ASH females were reduced in comparison to those of hatchlings from REF females. Mass-specific O_2 consumption rates for hatchlings from ASH and REF females were 0.037 ± 0.007 and 0.044 ± 0.011, respectively, at 25°C, and 0.061 ± 0.007 and 0.073 ± 0.012, respectively, at 30°C. Metabolic rates of hatchlings from ASH and REF females were significantly different at 30°C ($F_{1,22} = 7.78$, $p = 0.01$), but not at 25°C ($F_{1,22} = 2.76$, $p = 0.11$).

Discussion

Female *T. scripta* from the ash-polluted site had elevated levels of As, Cd, Cr, and Se, compared to females from a reference population (Table 1). Stomachs of turtles from ASH contained large quantities of Asiatic clams and crayfish, prey items found to contain substantial trace element levels (Table 1). Through trophic transfer, elevated trace element levels in prey items are likely responsible for the high levels of trace elements found in adult ash basin turtles. For example, the primary pathways by which Se is transferred from sediments into food chains is uptake by bottom-dwelling invertebrates, detrital-feeding organisms, and rooted plants (Lemly and Smith 1987). Biomagnification of Se, at rates of up to four orders of magnitude from water concentrations to top-level predator whole-body concentrations, occurs among producers (algae and plants), lower consumers (invertebrates and forage fish), and top-level consumers (vertebrates; Lemly and Smith 1987).

Female turtles that inhabited the contaminated site and accumulated trace elements transferred Se to their offspring. Variation in maternal transfer of Se among individual females may have resulted from differences in dietary composition or differences in period of residency at the ASH site. Despite elevated levels of As, Cd, and Cr in adult females inhabiting the coal ash basins, however, we found that the levels of these elements transferred to hatchlings were similar to both the levels found in hatchlings from reference sites, and to the levels reported in egg contents and eggshells of *T. scripta* from other unpolluted sites (Burger and Gibbons 1998). Similarly, studies of birds have described little direct maternal transfer of As (Blus *et al.* 1977), Cd (Sell 1975; Hulse *et al.* 1980; Maedgen *et al.* 1982; Reid and Hacker 1982; Ohlendorf and Harrison 1986; Scheuhammer 1987), and Cr (Anderlini *et al.* 1972) to eggs.

Maternal transfer of Se to offspring is widespread among vertebrate taxa, having been documented in fish (Gillespie and Baumann 1986; Hermanutz *et al.* 1992), birds (Ort and Latshaw 1978; Magat and Sell 1979; Ohlendorf *et al.* 1986), and mammals (Hansson and Jacobsson, 1966; Underwood, 1977). Although Se is considered an essential micronutrient, it produces toxic effects at levels only moderately higher than those required for animal nutrition (Ganther 1974). Levels of Se

Table 1. Concentrations of trace elements (μg/g dry mass) in wild-caught female *T. scripta*, their prey items, and incubation substrate samples from ash-contaminated (ASH) and reference (REF) sites (values are means ±1 SE)

	Location	n	Element: As	Cd	Cr	Cu	Se
Adult females	ASH	4	9.56 ± 2.42	3.57 ± 1.46	6.19 ± 1.39	102.23 ± 44.86	37.18 ± 10.92
(livers)	REF	3	0.26 ± 0.08	0.17 ± 0.08	1.16 ± 0.31	52.73 ± 2.12	3.41 ± 0.72
			$F_{1,5} = 52.98$	$F_{1,5} = 19.12$	$F_{1,5} = 22.41$	$F_{1,5} = 0.61$	$F_{1,5} = 37.94$
			$p = 0.0008$	$p = 0.007$	$p = 0.005$	$p = 0.47$	$p = 0.002$
Prey items							
Asiatic clams	ASH	12	13.22 ± 1.48	4.02 ± 0.60	5.63 ± 1.16	64.87 ± 6.49	8.69 ± 0.22
(tissue only)	REF	10*	3.85 ± 0.29	1.39 ± 0.12	2.71 ± 0.46	55.61 ± 0.85	2.01 ± 0.21
			$F_{1,20} = 86.14$	$F_{1,20} = 44.77$	$F_{1,11} = 3.53$	$F_{1,11} = 0.52$	$F_{1,20} = 93.50$
			$p < 0.0001$	$p < 0.0001$	$p = 0.09$	$p = 0.49$	$p < 0.0001$
Crayfish	ASH	3	8.71 ± 0.80	2.78 ± 0.44	2.46 ± 0.40	158.52 ± 8.19	14.92 ± 0.93
(whole-body)	REF	3	0.99 ± 0.31	0.02 ± 0.01	1.59 ± 0.32	46.42 ± 8.87	1.50 ± 0.14
			$F_{1,4} = 44.77$	$F_{1,4} = 372.99$	$F_{1,4} = 3.03$	$F_{1,4} = 43.91$	$F_{1,4} = 407.09$
			$p < 0.0001$	$p < 0.0001$	$p = 0.16$	$p = 0.0027$	$p < 0.0001$
Incubation	ASH	2	25.08 ± 0.35	0.04 ± 0.001	6.92 ± 0.50	10.57 ± 0.35	2.56 ± 0.05
Substrate	REF	2	0.93 ± 0.01	0.03 ± 0.001	0.05 ± 0.02	2.23 ± 0.11	0.20 ± 0.001

* n = 3 reference individuals each for which Cr and Cu data were available.

Table 2. Egg and hatchling characteristics of hatchlings incubated in soil from ash-contaminated (ASH) and unpolluted (REF) sites, and of hatchlings from *T. scripta* females residing in ash-polluted (ASH) and unpolluted (REF) sites (values are clutch means ± 1 SE; N = number of clutches, n = number of eggs or hatchlings)

	Egg Characteristics				Hatchling Characteristics				
	N/n	Width (mm)	Length (mm)	Wet Mass (g)	N/n	Days to Hatching	Plastron Length (mm)	Carapace Length (mm)	Wet Mass (g)
Incubation substrate									
ASH	5/18	22.79 ± 0.24	37.10 ± 0.43	11.23 ± 0.20	4/10	80.20 ± 0.74	27.94 ± 0.45	30.34 ± 0.58	8.20 ± 0.41
REF	5/18	22.95 ± 0.27	37.27 ± 0.33	11.48 ± 0.18	5/16	79.50 ± 0.39	28.23 ± 0.45	30.36 ± 0.42	8.02 ± 0.23
		$p = 0.67$	$p = 0.76$	$p = 0.36$		$p = 0.37$	$p = 0.66$	$p = 0.97$	$p = 0.68$
Maternal residence									
ASH	4/18	23.19 ± 0.56	36.50 ± 1.12	11.16 ± 0.57	4/14	79.42 ± 0.28	28.09 ± 0.67	30.51 ± 0.61	8.02 ± 0.53
REF	6/18	22.06 ± 0.26	37.31 ± 0.54	11.31 ± 0.26	5/12	80.52 ± 0.79	27.82 ± 0.59	29.69 ± 0.61	7.77 ± 0.38
		$p = 0.14$	$p = 0.13$	$p = 0.79$		$p = 0.30$	$p = 0.77$	$p = 0.39$	$p = 0.71$

transferred to hatchling *T. scripta* during our study were comparable to values maternally transferred by some waterfowl species inhabiting irrigation ponds of central California (Ohlendorf *et al.* 1986). Incidences of embryo mortality and deformities were correlated with levels of maternal Se transfer (Ohlendorf *et al.* 1986). Similarly in mammals, exposure of adult females to high Se levels has been associated with developmental abnormalities in sheep embryos (Hunter 1957) and teratogenic effects in humans (Robertson 1970).

We failed to detect morphological abnormalities associated with high levels of Se in hatchlings from females inhabiting the ash-polluted site. However, hatchlings from ASH site females exhibited reduced O_2 consumption rates compared to hatchlings from reference sites. We know of no other data for any ectothermic organism associating metabolic depression with elevated levels of Se (nor of any studies examining such a relationship). However, in mammals, excess Se may result in electrocardiographic changes and decreased blood pressure (Heinrich and MacCannon 1961) and reduced growth and shortening of life-span (Glover *et al.* 1979).

The negative relationship between Se whole-body burden and O_2 consumption rate in hatchling turtles is interesting given responses of a number of other organisms to exposure to ash basin sediments. For example, exposure of tadpoles (*Rana catesbeiana*) to ash basin sediment increased metabolic maintenance costs by 30–175% depending on temperature conditions and exposure duration (Rowe *et al.* 1998). Similar responses have been determined for banded watersnakes (*Nerodia fasciata*; Hopkins *et al.* 1999b) and freshwater shrimp (*Palaemonetes paludosus*; Rowe 1998) exposed to coal ash. It seems probable that the increase in O_2 consumption rates observed in such organisms is a response to elevated levels of some element, or suite of elements, other than Se.

Incubation of *T. scripta* embryos in ash-polluted soil was associated with an increased risk of embryo mortality. Although the nature of our experiment precludes assigning causative factors, one possible explanation for the observed mortality is the pozzolanicity of coal fly ash (*i.e.*, it exhibits cement-like characteristics under certain physical conditions; Carlson and Adriano 1993). Also, the fine particulate nature of coal fly ash (Capp 1978) may have prevented adequate gas exchange across the eggshell and eggshell membranes. Re-

Table 3. Effects of incubation substrate type and maternal residence site (ASH = ash basin; REF = unpolluted) on whole-body concentration (μg/g dry mass) of trace elements in hatchling *T. scripta* (values are clutch means ± 1 SE; n = 3 clutches per site, 2 hatchlings per clutch)

	Element				
	As	Cd	Cr	Cu	Se
Incubation substrate					
ASH	0.46 ± 0.07	0.03 ± 0.003	1.05 ± 0.21	5.14 ± 0.60	4.45 ± 1.49
REF	0.45 ± 0.07	0.03 ± 0.004	0.75 ± 0.10	4.86 ± 0.35	4.56 ± 1.43
	$F_{1,4}$ = 0.00	$F_{1,4}$ = 0.36	$F_{1,4}$ = 1.18	$F_{1,4}$ = 0.11	$F_{1,4}$ = 0.08
	p = 0.95	p = 0.57	p = 0.31	p = 0.75	p = 0.78
Maternal residence					
ASH	0.49 ± 0.03	0.03 ± 0.005	0.98 ± 0.24	5.58 ± 0.71	7.36 ± 1.52
REF	0.43 ± 0.04	0.03 ± 0.006	0.85 ± 0.02	4.42 ± 0.15	1.63 ± 0.13
	$F_{1,4}$ = 1.53	$F_{1,4}$ = 0.46	$F_{1,4}$ = 0.06	$F_{1,4}$ = 3.09	$F_{1,4}$ = 34.50
	p = 0.28	p = 0.54	p = 0.81	p = 0.15	p = 0.004

duced survivorship of embryos incubated in coal ash could have broad implications given (1) the preference of many turtle species to nest in disturbed, unvegetated soil (characteristic of coal ash); and (2) the close proximity of coal ash spill piles to many aquatic habitats.

We found no relationship between incubation substrate type and trace element contamination of developing offspring. We had predicted that elements dissolved in water may enter the egg via pores in the calcareous shell and become absorbed through shell membranes. Flexible-shelled turtle eggs have been shown to absorb a considerable amount of water during development (Packard and Packard 1980; Packard 1991). That the eggshells and eggshell membranes of *T. scripta* eggs provided an effective barrier to trace element transport suggests these structures may function to protect developing embryos from factors other than those previously described (such as invertebrate predators and microorganisms; Packard and Packard 1980).

Only one other study exists with which to compare levels of trace elements in freshwater turtle offspring. Burger and Gibbons (1998) reported trace element levels in slider turtle eggs and eggshells from unpolluted habitats in the same general area as our study, the Savannah River site. The levels of Cd found in hatchlings in our study from both reference and polluted sites were intermediate to those found in egg contents (higher) and eggshells (lower; Burger and Gibbons 1998). Compared with hatchlings in our study, Burger and Gibbons (1998) found somewhat lower levels of Cr and Se in egg contents and eggshells. Overall, however, our data are consistent with that of Burger and Gibbons (1998) and other reports (Stoneburner *et al.* 1980; Heinz *et al.* 1991) describing levels of Cd and Cr in reptile eggs at levels approximating 1 ppm or less. We found similarly low levels of Se in hatchlings from REF site females, but levels of Se in hatchlings from females inhabiting coal ash disposal basins were substantially higher.

The turtle hatchlings examined in our study contained elevated levels of only one of several trace elements found elevated in adults. We therefore conclude that although offspring *T. scripta* do not represent useful bioindicators of many of the inorganic contaminants found in coal ash, they do appear to be useful bioindicators of Se contamination.

Acknowledgments. Field and laboratory assistance was provided by R. Estes, D. Kling, O. Kinney, K. Komoroski, T. Sajwaj, and C. Salice. Earlier drafts of the manuscript were improved by comments from W. Hopkins, O. Kinney, and two anonymous reviewers. Research was supported by NSF Grant 9732138 and Financial Assistance Award Number DE-FC09-96SR18546 from the U.S. Department of Energy to the University of Georgia Research Foundation.

References

Anderlini VC, Connors PG, Risebrough RW, Martin JH (1972) Concentrations of heavy metals in some Antarctic and North American sea birds. In Parker BC Proceedings of the colloquium of conservation problems in Antarctica. Allen Press, Lawrence, KS

Besser JM, Giesy JP, Brown RW, Buell JM, Dawson GA (1996) Selenium bioaccumulation and hazards in a fish community affected by coal fly ash effluent. Ecotox Environ Safety 35:7–15

Bishop CA, Brooks RJ, Carey JH, Ng P, Nordstrom RJ, Lean DRS (1991) The case for a cause-effect linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra serpentina*) from Ontario, Canada. J Toxicol Environ Health 33:521–547

Bishop CA, Lean DRS, Brooks RJ, Carey JH, Ng P (1995) Chlorinated hydrocarbons in early life stages of the common snapping turtle (*Chelydra serpentina serpentina*) from a coastal wetland on Lake Ontario, Canada. Environ Toxicol Chem 14:421–426

Blus LJ, Neely BS, Jr, Lamont TG, Mulhern B (1977) Residues of organochlorines and heavy metals in tissues and eggs of brown pelicans, 1969–1973. Pestic Monit 11:40–53

Burger J, Gibbons JW (1998) Trace elements in egg contents and egg shells of slider turtles (*Trachemys scripta*) from the Savannah River Site. Arch Environ Contam Toxicol 34:382–386

Capp JP (1978) Power plant fly ash utilization for land reclamation in the eastern United States. In: Schaller FW, Sutton P (eds) Reclamation of drastically disturbed lands. Proceedings of a symposium held at the Ohio State Agricultural Research and Experiment Station, Wooster, Ohio, August 9–12, 1976. Sponsored by American Society of Agronomy, Crop Science of America, Soil Science Society of America, Madison, WI, pp 339–353

Carlson CL, Adriano DC (1993) Environmental impacts of coal combustion residues. J Environ Quality 22:227–247

Cherry DS, Larrick SR, Guthrie RK, Davis EM, Sherberger FF (1979) Recovery of invertebrate and vertebrate populations in a coal ash stressed drainage system. J Fish Res Board Canada 36:1089–1096

Congdon JD, Greene JL, Gibbons JW (1985) Biomass of freshwater turtles: a geographic comparison. *Am Midl Nat* 1986:165–173

Ewert MA (1985) Embryology of turtles. In: Gans C, Billett F, Maderson PFA (eds) Biology of the reptilia, vol. 14. John Wiley and Sons, New York

Ewert MA, Legler JM (1978) Hormonal induction of oviposition in turtles. Herpetologica 34:314–318

Ganther HE (1974) Biochemistry of selenium. In: Zingaro RA, Cooper WC (eds) Selenium. Van Nostrand Reinhold, New York

Gibbons JW, Greene JL (1979) X-ray photography: a technique to determine reproductive patterns of freshwater turtles. Herpetologica 35:86–89

Gillespie RB, Baumann PC (1986) Effects of high tissue concentrations of selenium on reproduction by bluegills. Trans Am Fish Soc 115:208–213

Glover J, Levander O, Parizek J, Vouk V (1979) Selenium. In: Friberg L, Nordberg GF, Vouk VB (eds) Handbook on the toxicology of metals. Elsevier, Amsterdam, pp 555–578

Guthrie RK, Cherry DS (1979) Trophic level accumulation of heavy metals in a coal ash basin drainage system. Water Res Bull 15:244–248

Hansson E, Jacobsson SO (1966) Uptake of [75Se] selenomethionine in the tissues of the mouse studied by whole-body autoradiography. Biochim Biophys Acta 115:285–293

Heinrich MA, MacCannon DM (1961) Respiratory responses to sodium selenite. Toxicol Appl Pharmacol 3:174–181

Heinz GH, Percival HF, Jennings M (1991) Contaminants in American alligator eggs from Lake Apapka, Lake Griffin, and Lake Okeechobee, Florida. Environ Monit Assess 16:277–285

Hermanutz RO, Allen KN, Roush TH, Hedtke SF (1992) Effects of elevated selenium concentrations on bluegills (*Lepomis macrochirus*) in outdoor experimental streams. Environ Toxicol Chem 11:217–224

Hinton TG, Fledderman PD, Lovich JE, Congdon JD (1997) Radiographic determination of fecundity: is the technique safe for developing turtle embryos? Chelon Conserv Biol 2:409–414

Hopkins WA, Mendonca MT, Congdon JD (1997) Increased circulating levels of testosterone and corticosterone in Southern toads, *Bufo terrestris*, exposed to coal combustion wastes. Gen Comp Endocrin 108:237–246

Hopkins WA, Mendonca MT, Rowe CL, Congdon JD (1998) Elevated trace element concentrations in southern toads, *Bufo terrestris*, exposed to coal combustion waste. Arch Environ Contam Toxicol 35:325–329

Hopkins WA, Mendonca MT, Congdon JD (1999a) Responsiveness of the hypothalamo-pituitary-interrenal axis in an amphibian (*Bufo terrestris*) exposed to coal combustion wastes. Comp Biochem Physiol C 122:191–196

Hopkins WA, Rowe CL, Congdon JD (1999b) Elevated trace element concentrations and standard metabolic rates in banded watersnakes (*Nerodia fasciata*) exposed to coal combustion waste. Environ Toxicol Chem 18:1258–1263

Hulse M, Mahoney JS, Schroder GD, Hacker CS, Pier SM (1980) Environmentally acquired lead, cadmium, and manganese in the cattle egret, *Bubulcus ibis*, and the laughing gull, *Larus atricilla*. Arch Environ Contam Toxicol 9:65–78

Hunter D (1957) The diseases of occupation. Brown Publishers, Boston

Lemly AD, Smith GJ (1987) Aquatic cycling of selenium: implications for fish and wildlife. US Department of the Interior report 12, Washington, DC

Maedgen JL, Hacker CS, Schroder GD, Weir FW (1982) Bioaccumulation of lead and cadmium in the royal tern and sandwich tern. Arch Environ Contam Toxicol 11:99–102

Magat W, Sell JL (1979) Distribution of mercury and selenium in egg components and egg-white proteins. Proc Soc Exp Biol Med 161:458–463

McCloskey JT, Newman MC (1995) Sediment preference in the Asiatic clam (*Corbicula fluminea*) and viviparid snail (*Campeloma decisum*) as a response to low-level metal and metalloid contamination. Arch Environ Contam Toxicol 28:195–202

Ohlendorf HM, Harrison CS (1986) Mercury, selenium, cadmium and organochlorines in eggs of three Hawaiian seabird species. Environ Pollut Series B 11:169–191

Ohlendorf HM, Saiki MK, Aldrich TW (1986) Embryonic mortality and abnormalities of aquatic birds: apparent impacts of selenium from irrigation drainwater. Sci Total Environ 52:49–63

Ort JF, Latshaw JD (1978) The toxic level of sodium selenite in the diet of laying chickens. J Nutrition 108:1114–1120

Packard GC (1991) The physiological and ecological importance of water to embryos of oviparous reptiles. In: Deeming C, Ferguson MWJ (eds) Egg incubation, its effects on embryonic development in birds and reptiles. Cambridge University Press, Cambridge, UK

Packard GC, Packard MJ (1980) Evolution of the cleidoic egg among reptilian antecedents of birds. Amer Zool 20:351–362

Raimondo SM, Rowe CL, Congdon JD (1998) Exposure to coal ash impacts swimming performance and predator avoidance in larval bullfrogs (*Rana catesbeiana*). J Herp 32:289–292

Reid M, Hacker CS (1982) Spatial and temporal variation in lead and cadmium in the laughing gull, *Larus articilla*. Mar Pollut Bull 13:387–398

Robertson DSF (1970) Selenium—a possible teratogen. Lancet 1:518–519

Rowe CL (1998) Elevated standard metabolic rate in a freshwater shrimp (*Palaemonetes paludosus*) exposed to trace element–rich coal combustion waste. Comp Biochem Physiol A 121:299–304

Rowe CL, Kinney OM, Fiori AP, Congdon JD (1996) Oral deformities in tadpoles (*Rana catesbeiana*) associated with coal ash deposition: effects on grazing ability and growth. Freshwater Biol 36: 723–730

Rowe CL, Kinney OM, Nagle RD, Congdon JD (1998) Elevated maintenance costs in an anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during embryonic and early larval periods. Physiol Zool 71:27–35

Scheuhammer AM (1987) The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: a review. Environ Pollut 46: 263–295

Sell JL (1975) Cadmium and the laying hen: apparent adsorption, tissue distribution, and virtual absence of transfer into eggs. Poultry Sci 54:1674–1678

Stoneburner DE, Nicora MN, Bloud ER (1980) Heavy metals in loggerhead sea turtle eggs (*Caretta caretta*): evidence to support the hypothesis that demes exist in the western Atlantic population. J Herpetol 14:171–175

Struger J, Elliott JE, Bishop CA, Obbard ME, Norstrom RJ, Weseloh DV, Simon M, Ng P (1993) Environmental contaminants in eggs of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes–St. Lawrence river basin of Ontario, Canada (1981, 1984). J Great Lakes Res 19:681–694

Underwood EJ (1977) Trace elements in human and animal nutrition, 4th ed. Academic Press, New York

US DOE (1995) International energy outlook. Report DOE/EIA-0484(95), US DOE, Energy Information Adminstration, Washington, DC

US EPA (1988) Wastes from the combustion of coal by electric utility power plants. US Environmental Protection Agency Report 530-SW-88-002, US EPA, Washington, DC

US EPA (1997) Profile of the fossil fuel electric power generation industry. US Environmental Protection Agency Report 310-R-97-007, US EPA, Washington, DC