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# Maternal transfer and embryonic assimilation of trace elements in freshwater turtles after remediation of a coal fly-ash spill

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ABSTRACT

Oviparous vertebrates maternally transfer elements to their offspring during egg production. Maternal transfer occurs because elements mimic, or are incorporated into, nutrients allocated to eggs, but likely differs among species depending on the quantities of specific nutrients allocated to eggs. Developing embryos are often assumed to assimilate all of the elements allocated to eggs, but this assumption has rarely been tested. We tested the hypothesis that maternal transfer and embryonic assimilation of trace elements differed between two species of freshwater turtles exposed to a recently-remediated coal fly-ash spill. *Sternotherus odoratus* transferred As, Se, and Zn, while *Trachemys scripta* transferred As, Hg, Se, Sr, and Zn. Logarithmic non-linear relationships between hatchling and egg concentrations indicated that turtles partially assimilated elements present in eggs. In systems contaminated with multiple trace elements, our data show that maternal transfer and embryonic assimilation are element- and species-specific, and may be inconsistent even among closely-related species.



## 1. Introduction

Maternal transfer is the primary route of exposure to bioaccumulative metals, metalloids, and trace elements (collectively referred to as trace elements for simplicity hereafter) in developing embryos of oviparous vertebrates (di Giulio and Tillitt, 1999). In oviparous amniote vertebrates (birds and most reptiles), trace elements are primarily transferred to offspring during egg formation, via vitellogenesis (yolk production), albumin deposition, or shell deposition (reviewed by Van Dyke et al., 2013a). Maternal transfer often occurs because trace elements have similar biochemical properties to essential elements with the same core charge. For example, Sr can replace Ca directly in biological tissues, while Se can replace S in the structure of some amino acids, like cysteine (reviewed by Van Dyke et al., 2013a). Thus, maternal transfer occurs because trace elements replace, or are incorporated into, essential nutrients that must be transferred to eggs to nourish developing offspring. However, maternal transfer also differs among trace elements (Chin et al., 2013; Guirlet et al., 2008; Hopkins et al., 2013a; Nagle et al., 2001; Páez-Osuna et al., 2010; Xu et al., 2006) because different trace elements replace or are incorporated into different nutrients. As a result, whereas the mechanisms of egg production, such as vitellogenesis, are conserved across oviparous amniotes, among-species differences in maternal transfer might be expected due to among-species differences in the proportions of specific nutrients allocated to eggs (Speake and Thompson, 1999, 2000; Thompson et al., 1999). Understanding these sources of variation in maternal transfer is therefore fundamental to determining which species inhabiting a contaminated area are likely to suffer reproductive consequences (Van Dyke et al., 2013a).

During development, embryos are usually assumed to assimilate maternally-transferred trace elements as they assimilate nutrients from yolk, albumin, and components of eggshell during embryogenesis. To our knowledge, only one study has examined whether embryos assimilate all of the trace elements present in eggs. Green sea turtle (*Chelonia mydas*) embryos from Malaysia bioaccumulated Cr, Cu, Mn, Se, and Zn from yolk, but the assimilation efficiency differed among elements (Ikonomopoulou et al., 2013). Hatchling and egg concentrations of Cr, Mn, and Zn were approximately equal, while hatchling concentrations of Cu were twice those of eggs, and hatchling concentrations of Se were 1.5 times greater than those of eggs (Ikonomopoulou et al., 2013).

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These data suggest that embryos may differentially assimilate maternally-transferred trace elements from yolk and albumin, which is a potentially overlooked source of variation in studies of ecotoxicological effects on reproduction. Additional studies are needed to determine whether this pattern is widespread in oviparous vertebrates, and if so, to identify whether selective assimilation (i.e., selective uptake or excretion by embryos) is the cause.

In the current study we quantified concentrations of 13 trace elements in the blood and claws of adults as well as the eggs and hatchlings of two species of freshwater turtles exposed to the 2008 Kingston, TN coal fly-ash spill. In December, 2008, the Kingston Fossil Plant accidentally discharged over 4 million $m^3$ of coal fly-ash into the Emory-Clinch-Tennessee River system in eastern Tennessee (TVA, 2009). Remediation efforts subsequently removed most of the ash by May, 2010 (~1 year prior to our study), but 400,000 $m^3$ of ash still remained in the system (Yankee et al., 2011) and trace elements in the ash may enter local food webs. We used this event as an opportunity to test three hypotheses regarding maternal transfer and embryonic assimilation of fly ash-derived trace elements. First, we examined maternal claw-hatchling and maternal blood-hatchling trace element concentration relationships to determine whether either maternal blood or claw concentrations were correlated with hatchling concentration, and thus indicated whether trace elements were maternally transferred to offspring in a concentration-dependent manner. Second, we compared maternal claw-hatchling and maternal blood-hatchling relationships between species to determine whether maternal transfer, based on these indices, differed. Third, we compared egg-hatchling trace element relationships between species to determine whether embryos of either species differentially assimilated trace elements from yolk. This analysis also allowed us to test whether hatchlings can be used for future maternal transfer analysis, rather than eggs, so that entire clutches can be incubated to examine hatching success. For maternal transfer analyses, we focused on blood and claws because they have been commonly used to develop nondestructive indices of trace element bioaccumulation in vertebrates (e.g., Bearhop et al., 2003; Hopkins et al., 2013b, 2007). Blood typically represents a snapshot of trace element exposure, while claws represent integrated exposure over long periods of time (~12 months; Aresco, 2005; Hopkins et al., 2013b). We focused on turtles because they are long lived, relatively sedentary, and often feed in association with the benthos (Ernst and Lovich, 2009), and are therefore particularly likely to bioaccumulate trace elements in areas affected by large-scale industrial spills. In addition, turtles produce all eggs within a clutch simultaneously and as a result have low within-clutch variance in maternally transferred trace elements, making them useful for comparing eggs and hatchlings from a single clutch (Van Dyke et al., 2013a).

## 2. Methods

### 2.1. Sample collection

From April–July 2011 and 2012, we trapped turtles in the vicinity of the Kingston, TN Fossil Plant using hoop traps baited with sardines and/or chicken. We set traps in shallow-water areas (<1 m deep) in microhabitats suitable for turtles. We concentrated trapping among sections of the Emory (river km 0–5.5) and Clinch (river km 3–7) Rivers impacted by the coal fly ash spill (impacted), and within a section of the Tennessee River (river km 914–922) that was not impacted by the spill (reference; Fig. 1). Throughout the study, we maintained at least 15 traps at various sites along both the Emory and Clinch Rivers, and 15–20 traps at sites along the Tennessee River (45–50 total traps per day). We documented trap locations using GPS. We rebaited traps every 3 days, and rotated them among trapping locations depending upon trapping success. We collected turtles from traps once per day. Throughout the study area, we targeted gravid *Sternotherus odoratus* and *Trachemys scripta*, because *S. odoratus* is a benthic forager and is likely to ingest sessile fly ash directly, and *T. scripta* is by far the most commonly trapped turtle in the system. Prior data also suggest that their diets may overlap (Van Dyke et al., 2013b). Both species exhibit high site fidelity, and rarely travel more than 400 m among points of capture (Ernst, 1986; Schubauer et al., 1990), so turtles inhabiting areas impacted by a spill are likely to forage exclusively in the impacted area.

We processed turtles at a laboratory facility in Kingston, TN. We determined whether female turtles were gravid via palpation and/or x-ray radiography (Ecoray Ultralight 9020 HF). We weighed gravid female turtles with Pesola® scales (Baar, Switzerland) and then induced oviposition via subcutaneous injection of 20 mg/kg of oxytocin dissolved in deionized water (Ewert and Legler, 1978; Tucker et al., 2007). We placed injected females in plastic tubs with ~2 cm of dechlorinated water (Ewert and Legler, 1978), and placed the tubs in a dark room at ~25 °C. We checked females for deposited eggs every 2 h. When eggs were present, we gently dried and weighed them to the nearest 0.01 g. In 2011, we froze one egg from each clutch at −20 °C. We labeled all other eggs laid in 2011 and 2012 with their maternal ID and egg number, and incubated all eggs in hovobators at 25 °C with a substrate of 1:1 vermiculite:water (by mass). Upon hatching, we weighed and measured all hatchling turtles, and euthanized the first turtle to hatch via an overdose of isoflurane or MS-222. Other hatchlings were eventually released at the point of maternal capture. Because we previously demonstrated that within-clutch variation in concentrations of trace elements is low (Van Dyke et al., 2013a), we paired the single egg and hatchling from each clutch to examine the bioaccumulation of egg trace elements by embryos.

Once turtles had laid all of the eggs we had counted via initial x-rays, we palpated and x-rayed females again to ensure they had deposited all eggs in their clutches. We then measured carapace length (cm), carapace width (cm), and plastron length (cm) of females using forestry calipers. We removed the tips (top 2–3 mm) of all claws on the right rear foot (if present) from turtles for trace element analysis. We sampled blood (0.5–1.0 ml) from the cervical sinus for trace element analysis, using heparinized 1-ml tuberculin syringes fitted with 26.5-gauge hypodermic needles. After sampling, we released female turtles at the site of capture.

We froze and stored all blood, claw, egg, and hatchling samples at −20 °C. Prior to further manipulation, we removed eggshells from eggs and homogenized egg contents (yolk and albumin) by vortexing samples with Teflon beads. We freeze-dried all egg, claw, and hatchling samples to asymptotic mass. We homogenized hatchling turtles by grinding them with mortars and pestles while the hatchlings were submerged in liquid nitrogen. We then freeze-dried hatchling samples again to remove any water that condensed on supercooled samples during homogenization. All samples were shipped to Dartmouth College for trace element analysis.

### 2.2. Trace elements analysis

We quantified concentrations of As, Ba, Cd, Cr, Cu, Fe, Mn, Hg, Se, Sr, Tl, V, and Zn in blood, claw, egg, and hatchlings using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Trace Element Analysis Core at Dartmouth College. Prior to analysis, claws were washed in Triton X-100, rinsed in distilled water, and dried to remove external contamination. Claw, egg, and hatchling samples were weighed into a pre-weighed VWR trace metal clean polypropylene centrifuge tube and 0.5 ml of 9:1 $HNO_3$:HCl (Optima Grade, Fisher Scientific) was added. Individual egg and hatchling subsample weights were variable but were generally <0.05 g.