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Maternal Transfer of Contaminants to Eggs in Common Grackles (*Quiscalus quiscala*) Nesting on Coal Fly Ash Basins

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Abstract. Coal combustion is a major source of trace elements to the environment. Aquatic disposal of wastes from this process can result in reproductive failure in many wildlife species, but little is known regarding impacts on avian fauna. Individual eggs were collected from common grackles (*Quiscalus quiscala*) nesting in association with coal fly ash settling basins and a reference site to determine if females from the contaminated site transfered trace elements to their eggs. Whole clutches were also collected from both sites to examine inter- and intra-clutch variability of maternally transferred contaminants. Selenium was the only trace element found in significantly higher concentrations in ash basin eggs ($\bar{x} = 5.88 \pm 0.44$ μg/g DW) than in reference eggs ($\bar{x} = 2.69 \pm 0.13$ μg/g DW). Selenium concentrations in eggs from the ash basins were above background levels, but did not exceed higher proposed Se toxicity thresholds. Inter- and intra-clutch variation was higher for ash basin clutches than reference clutches. The relationship between selenium concentrations and laying order (estimated by egg mass) was not statistically significant, but increased Se concentration in the second egg of most ash basin clutches followed by declining concentrations in subsequent eggs suggested that further examination of this pattern might be warranted.

The disposal of slurried coal combustion waste (hereafter, ash) into open settling impoundments is a well-documented contaminant exposure pathway for fish and wildlife (Hopkins *et al.* 1998, 1999; Rowe 1998; Rowe *et al.* 1996; Lemly 1996). These wastes contain many potentially toxic trace elements, including As, Cd, Cr, Cu, Se, and Sr that are readily accumulated by organisms (Rowe *et al.* 2002). Of the elements accumulated by organisms in aquatic ash disposal systems, Se is of particular concern because it readily bioaccumulates and can severely impair reproduction (Rowe *et al.* 2002). Indeed, fish and reptiles exposed to ash transfer Se to their offspring (Lemly 1993; Nagel *et al.* 2001). In some ash-affected systems such as Belews Lake, NC, Se accumulation and maternal transfer by fish was sufficient to cause teratogenic effects, failed reproduction, and local extinctions (Gillespie and Bauman 1986; Lemly 1993).

Birds appear to be particularly vulnerable to excessive Se, and could thus be affected by aquatic ash disposal. Studies in agricultural systems, such as the Kesterson Reservoir (California, USA), indicated that Se is readily accumulated by birds, maternally transferred to their eggs and young, and can result in teratogenic effects and reduced reproductive success (Ohlendorf 2002; Skorupa and Ohlendorf 1991). Aquatic ash disposal sites are frequently used by birds as foraging and breeding areas. The adverse effects on bird health and reproduction observed in Se-contaminated agricultural systems suggest that aquatic disposal of ash in settling basins may similarly result in Se exposure in avian species with potential reproductive consequences. Avian studies pertaining to uptake and effects of Se from ash are generally lacking, but King (1988) reported a >50% depression in red-winged blackbird (*Agelaius phoeniceus*) egg hatchability associated with Se transfer to eggs following discharge of ash into Martins Reservoir, TX.

As an initial assessment of ash-related contaminant exposure in an avian species, we analyzed eggs of common grackles (*Quiscalus quiscala*) nesting at coal ash settling basins for trace elements to determine the occurrence and extent of maternal transfer of pollutants. Common grackles are medium-sized blackbirds known to nest in and around open wetlands (Peer and Bollinger 1997). Grackles are granivorous during the winter and migratory seasons, but like many avian species they shift to a diet of insects and other invertebrates during the breeding season. We hypothesized that birds foraging and breeding in ash-contaminated wetlands would accumulate contaminants and transfer trace elements to their offspring.

Methods

Study Sites

The D-area coal-fired power plant on the U.S. Department of Energy's Savannah River Site (SRS) in west-central South Carolina discharges slurried ash into a series of settling impoundments (two open basins and a drainage swamp) before surface waters enter Beaver Dam Creek and eventually the Savannah River. Grackles nest in shrubs and tall

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grasses on islands within, and edges around, these impoundments. Studies of contaminant uptake by other wildlife species at these basins confirmed the bioavailability of pollutants to wildlife (Hopkins *et al.* 1998, 1999; Rowe *et al.* 1996). The reference site, a series of small impoundments where grackles nest in dense shrubs, is approximately 16 km from the D-area power plant and has no historical input of ash.

Field Collections

Eggs were collected from 14 ash basin nests and 12 reference nests from 19 April 2000 through 19 June 2000. We randomly collected an egg from nests at both sites for overall site comparison of trace elements. Stage of embryo development/age since laying was not determined. We removed whole clutches from seven (four ash basin, three reference site) nests to examine inter- and intra-clutch variation in trace element concentrations. Single eggs were randomly selected from these whole clutches for inclusion in the overall site comparisons. All eggs were weighed (whole egg, including shell), then frozen prior to analyses.

Trace Element Analyses

Grackle egg contents from the polluted and reference sites were separated from shells and only contents were analyzed for trace elements. Egg content samples were lyophilized and homogenized prior to analysis. Egg homogenates were then digested and analyzed for trace element concentrations (As, Cd, Cu, Fe, Se, Sr, V, and Zn) according to the following procedures. Approximately 150 mg of sample were used for digestion. Nitric acid (2.5 mL) was added to samples before digestion in a microwave (CEM Corp., Matthews, NC, USA) with heating steps of 60, 60, 70, and 80% microwave power for 10, 10, 15, and 20 min, respectively. Next, 1.0 mL of H_2O_2 was added to the samples and microwaved at the same power and duration as the HNO_3 digestion. After digestion, samples were brought to a final volume of 10 mL with double distilled water. Trace element analysis was performed by ICP-MS (Perkin Elmer, Norwalk, CT, USA) on samples diluted 1:1 with double distilled water. Calibration standards covering a range of 1–500 μg/L were prepared daily by serial dilution of NIST traceable primary standards. Certified reference material (Tort 2; NRC, Ottawa, Canada) and blanks were included in the digestion and analysis procedure for quality control purposes. Mean percent recoveries for trace elements in tissue certified reference material ranged from 97–121%. Mean variability in percent recoveries of certified reference materials between digestion sets ranged from 5–15%. Instrument detection limits for As, Cd, Cu, Fe, Se, Sr, V, and Zn were 0.25, 0.04, 0.16, 2.95, 0.38, 0.05, 0.08, and 0.34 μg/kg dry mass, respectively.

Statistical Comparisons

Egg mass (wet weight, with shell) and trace element concentration data were tested for normality with Shapiro-Wilk tests. To meet the assumptions of normality, egg masses and element concentrations were log-transformed prior to analyses.

The majority of egg content samples from the ash basin (96% of 24 total eggs) and reference (82% of 22 total eggs) sites had Cd concentrations below the instrument detection limit (BDL). Thus, Cd was not included in statistical analyses. Arsenic concentrations for two reference eggs were BDL. For these eggs, we used one-half of the detection limit as the As concentration in the statistical comparison.

Egg mass and trace element concentration data were compared between sites using multivariate analysis of variance (MANOVA). We used linear regression analyses to test for relationships between ash basin egg mass and trace element concentrations (reference eggs were not tested). In addition, we used a series of linear regression analyses to examine relationships between individual trace elements in eggs from the ash basin.

For comparisons of whole clutches, all eggs within a clutch were analyzed for Se and the mean concentrations for clutches for each site were compared by ANOVA. To examine inter-clutch variation in Se concentrations (the only element maternally transferred to eggs, see below), we calculated coefficients of variation (CV = standard deviation × 100/mean) of the mean Se concentrations of clutches at each site. For comparisons of intra-clutch variation in Se concentrations, we calculated CVs of Se concentrations within individual clutches. The intra-clutch CVs for clutches were arcsine-square root transformed prior to comparison between sites by ANOVA. To examine the possible effect of laying order on intra-clutch variation, we first assigned an estimate of within-clutch laying order based on egg mass. Howe (1976, 1978) suggested that egg mass of *Q. quiscala* increases with laying order (last eggs heavier than first), but that this relationship was most significant in larger ($n > 4$ eggs) clutches. We then utilized a two-way ANOVA without replication (simple repeated measures ANOVA examining repeated eggs within unreplicated clutches) to test for potential effects of laying order (within clutch) and nest (among clutch) in ash basin clutches. Due to differences in clutch size, only the first three eggs in each clutch were included in this analysis.

Trace element concentrations are presented as μg/g dry weight (DW). All results are reported as the mean ± standard error.

Results

Site Comparison

Egg masses did not differ between sites (ANOVA, $F_{1,24} = 1.89$, $p > 0.18$). Selenium concentrations were significantly higher in ash basin egg contents than in reference site egg contents (Table 1, Fig. 1). No other trace element concentrations in eggs were significantly different between sites.

Within eggs from the ash basins, we found no significant relationships between egg mass and any trace element concentrations ($r^2 < 0.32$, $p > 0.05$) or between any trace element concentrations in ash basin eggs ($r^2 < 0.15$; $p > 0.20$).

Inter- and Intra-Clutch Variation in Se

Mean Se concentrations of whole ash basin clutches ($n = 4$, $\bar{x} = 5.3 \pm 0.42$ μg Se/g DW) were significantly higher (ANOVA, $F_{1,6} = 15.45$, $p = 0.01$) than reference clutches ($n = 3$, $\bar{x} = 2.5 \pm 0.53$ μg Se/g DW). Inter-clutch variation of Se concentrations, as measured by CVs of mean clutch concentrations, was higher in ash basin clutches (range = 3.6–6.4 μg Se/g DW; CV = 24.1%) than reference clutches (range = 2.0–3.0 μg Se/g DW; CV = 18.6%).

Variation of Se concentrations within ash basin clutches was also high (Fig. 2). Coefficients of variation for the individual clutches ($n = 4$) ranged 10.8–34.8%, with an average CV of 23.6%. Variation within reference clutches ($n = 3$) was relatively narrow, ranging 7.9–18.9% with an average CV of only 13.2%. However, CVs did not differ between sites (ANOVA, $F_{1,6} = 2.75$, $p > 0.17$).

Selenium concentrations in ash basin clutches were not linearly

Table 1. Trace element concentrations[a] in common grackle (*Quiscalus quiscala*) egg contents from nests associated with coal fly ash basins and a reference site

	Ash Basin Nests (n = 14 nests)		Reference Nests (n = 12 nests)		
	$\bar{x}$	SE	$\bar{x}$	SE	p[b]
Selenium	5.88	0.44	2.69	0.13	<0.01
Arsenic[c]	0.39	0.12	0.46	0.15	0.74
Cadmium	BDL[d]		BDL[d]		
Copper	2.55	0.25	2.68	0.25	0.65
Iron	182.13	7.87	164.38	9.18	0.13
Strontium	7.68	1.52	5.06	0.87	0.15
Vanadium	0.21	0.02	0.23	0.02	0.52
Zinc	65.11	3.34	66.12	2.35	0.70

[a] All trace element concentrations are express as μg/g dry mass.
[b] Log-transformed trace element concentrations were compared by MANOVA.
[c] Two reference eggs had As concentrations below instrument detection limits. We used half the detection limit (detection limit = 0.25 μg As/kg) as the As concentration for these eggs in statistical analyses.
[d] BDL = below instrument detection limit (see Methods). A single reference egg had a Cd concentration above the BDL (no ash basin eggs had Cd concentrations above the BDL).

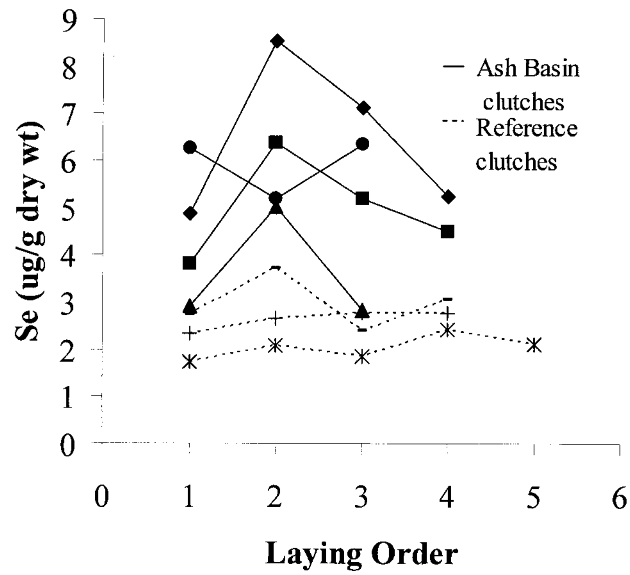

Fig. 2. Selenium concentrations in whole clutches of common grackle eggs relative to suggested egg laying order. Laying order is based on egg mass (Howe 1976, 1978)

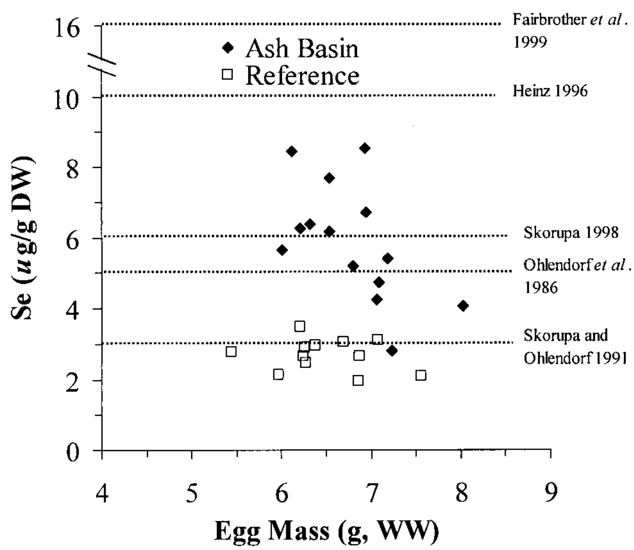

Fig. 1. Selenium concentrations in common grackle egg contents relative to total egg mass. Cited references indicate suggested background and toxic Se threshold levels discussed in text

related to laying order (ANOVA, $F_{2,6} = 2.68, p > 0.14$), but there was a marginal effect of nest (ANOVA, $F_{3,6} = 4.60, p < 0.06$). However, three of four ash basin nests exhibited a similar pattern of increasing Se concentration from the first to second egg, followed by declining Se concentrations in subsequent eggs (Fig. 2).

Discussion

Female grackles nesting around basins associated with the D-area coal burning power plant transferred significantly more Se to their eggs than reference birds, but no other trace elements differed in eggs between sites. It is possible that adult female grackles accumulated multiple elements from foraging at the ash basin site, but only transferred Se to their eggs. For example, Nagle *et al.* (2001) found that female slider turtles (*Trachemys scripta*) inhabiting these same (Savannah River Site) ash basins accumulated multiple contaminants (As, Cd, Cr, and Se), but Se was the only element maternally transferred to eggs. Avian studies of maternal transfer of the remaining trace elements examined in this study are generally lacking. Blus *et al.* 1977 suggested minimal maternal transfer of As by birds, but Stanley *et al.* (1994) documented As accumulation in egg contents of highly-dosed (≥25 μg/g in diet) mallards. Scheuhammer (1987) and Sell (1975) indicated little maternal transfer of Cd by avian species.

Selenium is known to readily accumulate in birds and be transferred to developing offspring (Heinz 1996). Maternal transfer of Se to avian eggs is well documented in field studies at the Kesterson National Wildlife Refuge (Ohlendorf *et al.* 1986) and Martins Reservoir (King 1988; King *et al.* 1994), and has been corroborated by laboratory studies involving captive mallards fed dosed food (Heinz *et al.* 1987, 1989). Transfer of sufficient Se can result in failed hatching, teratogenesis, oxidative stress, and other sublethal effects (Heinz 1996; Hoffman 2002). The species or chemical form of Se ingested also influences the occurrence and degree of effects, with organic forms (e.g., selenomethionine) generally considered more toxic to wildlife than inorganic forms such as selenite (Heinz *et al.* 1987). Both organic and inorganic forms of Se are available at the D-area ash site (Jackson *et al.,* in press).

Our study clearly indicates that ash basin birds transferred Se to their eggs, but we did not determine if these concentrations affected their reproductive success. The concentration of Se in avian eggs associated with reduced productivity and/or teratogenesis has been debated (Fairbrother *et al.* 1999), due in part to apparent species-specific differences in responses to maternally transferred Se (Ohlendorf *et al.* 1986). Skorupa and Ohlendorf (1991) suggested that 3 μg/g Se (DW) in eggs was the background level for Se in aquatic bird eggs. Approximately 93% of ash basin eggs and 25% of reference site eggs were greater than this suggested background level. Ohlendorf *et al.* (1986) found that concentrations of 5 μg/g Se (DW) in eggs resulted in 20% mortality or deformity in black-necked stilt (*Himantopus mexicanus*) embryos. Approximately 71% of ash basin eggs had concentrations >5 μg/g, whereas all reference site eggs contained <4 μg/g Se. Skorupa (1998) later suggested 6 μg/g (DW) as a toxic threshold for Se in avian eggs. Fifty percent of the ash basin eggs had Se concentrations in excess of 6 μg/g. However, based on studies of captive mallards, Heinz (1996) suggested approximately 10 μg/g DW and Fairbrother *et al.* (1999) suggested 16 μg/g DW in eggs as the threshold for avian reproductive impairment. No eggs collected during this study surpassed these higher threshold concentrations.

Variation of Se concentrations within ash basin clutches was approximately twice the variation found within reference clutches, but was not statistically different. Selenium concentrations of eggs within individual ash basin clutches often straddled both sides of suggested toxicity thresholds. Smith *et al.* (1988) reported little variation in Se concentrations in egg samples (every fifth egg) from black-crowned night-heron (*Nycticorax nycticorax*) clutches produced by captive birds on constant doses of dietary Se, with a mean CV of only 16%, similar to the variation we found in our reference clutches (13.8%). Heinz *et al.* (1987) reported little variation in Se concentrations in clutches (every fourth egg sampled) from captive mallards fed sodium selenite. It is likely that variation among field sample clutches exceeded variation reported in laboratory studies due to a variety of factors, including differences in individual maternal exposure history. Unfortunately, other field studies of intra-clutch variation are lacking.

Our test of the relationship between Se concentration and laying order was limited by low sample sizes and our method of assigning laying order (estimated by egg mass; Howe 1976, 1978). Most of the clutches we utilized had ≤4 eggs and thus the strength of our assigned laying orders may have been limited. Heinz *et al.* (1987, 1989) and Stanley *et al.* (1994) did not find differences in Se transfer relative to laying order of dosed mallards. Also, Smith *et al.* (1988) did not find differences in Se concentrations within clutches produced by dosed black-crowned night-herons. Nonetheless, if our laying order assumptions are correct (that egg mass increased with laying order), three of four ash basin nests exhibited a similar pattern of increasing Se concentration from the first to second egg. Thus, while not statistically significant, our findings suggest a laying order effect may exist and additional studies on maternal transfer of Se relative to laying order may be warranted for this species.

Aquatic ash disposal sites attract birds and other wildlife for breeding, foraging, and overwintering activities. Because use of ash basins results in uptake and sometimes maternal transfer of contaminants, such disposal practices may impact wildlife at the individual and/or population level (Rowe *et al.* 2002). Our findings indicated that grackles nesting in proximity of coal fly ash basins transferred Se to their eggs. Although Se was the only contaminant transferred to eggs, it is possible that Se and other elements were also transferred by females to nestlings during feeding. Future studies are needed to ascertain whether maternal transfer of Se from ash basins affects avian embryonic development, and ultimately reduced reproductive success.

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