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Maternal Transfer of Contaminants and Reduced Reproductive Success of Southern Toads (*Bufo [Anaxyrus] terrestris*) Exposed to Coal Combustion Waste

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Supporting Information

ABSTRACT: Bioaccumulation of contaminants and subsequent maternal transfer to offspring are important factors that affect the reproductive success of wildlife. However, maternal transfer of contaminants has rarely been investigated in amphibians. We examined maternal transfer of trace elements in southern toads (*Bufo[Anaxyrus] terrestris)* residing in two locations: (1) an active coal combustion waste (CCW) disposal basin and adjacent 40-ha floodplain contaminated with CCW over 35 years ago and (2) an uncontaminated reference site. Our study is among the few to document tissue concentration-dependent maternal transfer of contaminants and associated adverse effects in amphibians. We found that females collected from the CCW-contaminated area had elevated concentrations of Ni, Se, and Sr; these females also transferred elevated levels of Cu, Pb, Se, and Sr to their eggs compared to females from the reference site. Overall reproductive success, estimated as a function of clutch size and offspring viability, was reduced by 27% in clutches collected from parents from the contaminated site compared to the reference site. Offspring viability negatively correlated with female and/or egg concentrations of Se and Ni. Reproductive success negatively correlated with Se and Cu concentrations in females, and Se concentrations in eggs. Our study highlights how exposure to CCW can negatively affect amphibian reproduction.

■ INTRODUCTION

Bioaccumulation of contaminants in adult amphibians and subsequent maternal transfer to offspring may be an important factor that affects reproductive success. However, the majority of research investigating the effects of contaminants on amphibians has focused on environmental (e.g., aqueous) exposure or trophic uptake and has been heavily biased toward studies during the embryonic and larval stages.[1,2] Although environmental exposure to contaminants can have adverse effects on all life stages, maternal transfer of contaminants is an alternative route of exposure that may also negatively affect clutch characteristics and offspring development.[3,4] Previous studies have demonstrated that adult female amphibians accumulate contaminants and transfer them to their eggs,[5−7] which can ultimately lead to reduced hatching success and offspring viability.[4,8−10] For instance, female African clawed frogs (*Xenopus laevis)* exposed to cadmium (Cd) in laboratory experiments maternally transferred Cd to their eggs, which caused embryological malformations.[8] Similarly, female eastern narrowmouth toads (*Gastrophryne carolinensis)* collected from a coal ash settling basin accumulated and transferred selenium (Se) and strontium (Sr) to their eggs, which experienced 19% lower viability than those from a reference site.[4]

Environmental contaminants are thought to be among the major contributors to amphibian declines around the world.[11−15] Although environmental contaminants come from many sources, coal-fired power plants are one of the largest producers of solid waste in the U.S.[16] and this waste has been shown to produce a wide array of adverse effects on amphibians. Coal combustion waste (CCW) contains high concentrations of trace elements (e.g., arsenic [As], Se, Sr) and is often discharged into aquatic settling basins for disposal.[17] Amphibians and other wildlife that are attracted to these CCW disposal basins can accumulate elevated concentrations of trace elements in their tissues, resulting in adverse effects on survival, growth and development, behavior, performance, and recruitment.[4,18−28] Taken together, these studies suggest that CCW-contaminated impoundments and wetlands not only influence the health of individual amphibians but may also serve as ecological traps for amphibian populations.[22,26,27]

The objectives of this research were to determine if female southern toads (*Bufo [Anaxyrus] terrestris*; hereafter *Bufo*) exposed to CCW transfer trace elements to their offspring and to assess whether these contaminants negatively correlate with reproductive success. To achieve our objectives, we examined

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maternal transfer of contaminants in southern toads residing in two locations: (1) an active CCW disposal system, which includes an adjacent 40-ha floodplain contaminated with CCW over 35 years ago, and (2) an uncontaminated wetland/upland reference site. We tested two hypotheses. First, we hypothesized that female southern toads exposed to CCW would maternally transfer trace elements to their eggs in a tissue concentration-dependent manner (i.e., concentrations in eggs would be correlated with concentrations in females). Second, we hypothesized that, compared to reference animals, females from the CCW-contaminated site would have reduced clutch sizes and hatching success, and their offspring would have increased incidence of abnormalities, ultimately resulting in reduced offspring viability and reproductive success.

■ MATERIALS AND METHODS

Study Species. The southern toad is a common anuran in the southeastern U.S. that is well suited for this research because they are locally abundant, reproduce readily in captivity, have a short embryonic period, and have been the subjects of previous research investigating the effects of CCW.[21,26] Similar to many other amphibians, southern toads have a complex life cycle consisting of aquatic larvae and terrestrial adults. Adult southern toads prefer terrestrial habitats with sandy soils in the nonbreeding season, and migrate up to 1.6 km to aquatic breeding sites in the spring and summer.[29,30]

Study Sites. The two collection sites in our study are located on the Savannah River Site near Aiken, South Carolina. The CCW-contaminated site is associated with the D-area steam generation facility, which includes a disposal area where sluiced CCW is discharged into a series of open settling basins, and effluents from these basins flow into Beaver Dam Creek and eventually the Savannah River. Presumed CCW contaminated toads were collected from (1) an ash settling basin that is partially filled (∼7 ha) with CCW and has become revegetated and (2) a floodplain wetland approximately 600 m from the ash basin that received CCW effluents from the 1950s through the 1970s. Sediments in this area are comprised almost entirely of CCW that is enriched with trace elements, the primary contaminants of concern in this waste material.[4,17] Most organic contaminants are volatilized during the combustion process, and the remaining organic compounds (e.g., PAHs) in CCW tend to not be readily bioavailable.[31−34] The reference site was Rainbow Bay, a ∼1-ha Carolina bay wetland,[35,36] with no previously known contamination,[37,38] located approximately 12 km from the contaminated site. Both locations contain amphibian breeding sites that are surrounded by mixed pine−hardwood forest with some open field habitats.

Experimental Design and Data Collection. We collected adult male and female southern toads from April 8 to May 31, 2010, from the CCW-contaminated site and the reference site as they entered the sites during breeding migrations. We transported toads to the lab where we measured the snout−vent length (SVL, ± 0.5 mm) and the whole body wet mass (±0.01 g) of each female. We then injected males and females with human chorionic gonadotropin (males 100 IU, females 250 IU). Females were randomly paired with males from the same site and placed in plastic containers with 5−10 cm of well water for breeding. By allowing females from all sites to oviposit in uncontaminated well water, we were able to isolate maternal transfer as the primary source of trace element contamination to the eggs. After pairs bred, we released males at their location of capture, and kept females in plastic containers in the lab for 48 h to void their remaining gut contents. We euthanized females by immersion in buffered MS-222 and freeze-dried their carcasses for subsequent elemental analysis.

Forty-four females deposited clutches of eggs: 15 from the reference site and 29 from the CCW-contaminated site. To assess maternal transfer and the effect of contaminants on the reproductive success of southern toads, we enumerated the total number of eggs in each clutch. We then removed a subsample of 500 fertile eggs from each clutch. The remaining eggs from each clutch were freeze-dried for elemental analysis. Each subsample of 500 fertile eggs was held in a plastic container with 3 L of aged well water at 25 °C until hatching. We counted the number of hatchlings in each subsample and calculated the fraction of the 500 that hatched successfully. To quantify morphological abnormalities, we fixed hatchlings in MEMFA (0.1 M MOPS, pH 7.4, 2.0 mM EGTA, 1.0 mM $MgSO_4$, 3.7% formaldehyde) for 24 h, and preserved them in 100% ethanol.[4] Using a dissecting microscope, we examined each hatchling for morphological abnormalities (axial, adema, oral, and blisters) following the methods of Bantle et al.[39] and ASTM.[40] To estimate offspring viability, we calculated the fraction of the 500 eggs from each clutch that successfully hatched and had no morphological abnormalities.[4] Finally, to estimate overall reproductive success of each female (i.e., predicted number of morphologically normal hatchlings), we multiplied clutch size by offspring viability.

Sample Preparation and Elemental Analysis. For elemental analyses, we freeze-dried all tissue samples and then individually digested subsamples of each homogenized female carcass (∼250 mg) and eggs in 10 mL of trace metal grade nitric acid (70% HNO_3) using microwave digestion (MarsExpress, CEM Corp., Matthews, NC). After digestion, we brought each sample to a final volume of 15 mL with 18 MΩ deionized water. We analyzed samples using three separate runs on an inductively coupled plasma mass spectrometer (ICP-MS) (Perkin-Elmer, Norwalk, CT) to determine elemental concentrations of As, Cd, chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), nickel (Ni), Se, Sr, vanadium(V), and Zinc (Zn) in each sample. For quality control, we included certified reference material (TORT-2 and LUTS; National Research Council of Canada, Ottawa, Canada) in each analysis. Mean percent recoveries for elements in standard reference material ranged from 81 to 111%. Minimum detection limits in tissue samples for the three runs averaged: As, 0.30; Cd, 0.03; Cr, 0.23; Cu, 0.69; Hg, 0.18; Ni, 0.28; Pb, 0.33; Se, 0.65; Sr, 0.85; V, 0.53; and Zn, 3.23 μg/g dry mass. The analytical error, expressed as relative percent difference for independent analyses of replicate dilutions, ranged from 1.8% for V to 17.0% for Pb. We report elemental concentrations in this manuscript on a dry mass basis (μg element/g dry mass); see the Supporting Information.

Statistical Analyses. We performed statistical analyses using SAS 9.3 (SAS Institute, Cary, NC). To test for a location effect on clutch size, we used PROC GLM, with SVL as a covariate in the model; the SVL by location interaction was tested (p = 0.4197), the slopes were determined to be equal, and the interaction term was removed from the model. Clutch size residuals were normally distributed, and no transformation was applied to clutch size. To test the proportion of eggs hatching and the proportion of hatchlings with abnormalities, we used PROC LOGISTIC with events/trials syntax to test the binary responses (live or dead, abnormal or normal); we tested for model goodness-of-fit and used the Williams' method to

Table 1. Elemental Composition of Post-Ovipositional Female Southern Toads and Their Eggs Collected from a Reference Site (Reference) and a Coal-Fly-Ash Contaminated Area (CCW)[a]

	females (μg/g)		eggs (μg/g)	
	reference	CCW	reference	CCW
element	n = 15	n = 29	n = 15	n = 27
As	^{A}0.39 ± 0.03 (1)	^{A}0.64 ± 0.07 (10)	^{A}0.34 ± 0.02 (7)	^{A}0.58 ± 0.10 (20)
Cd	^{A}0.25 ± 0.04 (0)	^{A}0.15 ± 0.01 (0)	^{A}0.02 ± 0.01 (11)	^{A}0.04 ± 0.01 (10)
Cr	^{A}2.32 ± 0.20 (0)	^{A}2.15 ± 0.14 (0)	^{A}2.00 ± 0.09 (0)	^{A}2.29 ± 0.06 (0)
Cu	^{A}11.61 ± 1.43 (0)	^{A}15.12 ± 1.74 (0)	^{A}12.87 ± 0.70 (0)	^{B}19.98 ± 0.90 (0)
Hg	^{A}0.63 ± 0.05 (0)	^{A}0.63 ± 0.04 (1)	^{A}0.32 ± 0.02 (0)	^{A}0.39 ± 0.02 (8)
Ni	^{A}0.44 ± 0.09 (4)	^{B}1.54 ± 0.21 (2)	^{A}0.62 ± 0.05 (13)	^{A}0.80 ± 0.10 (12)
Pb	^{A}1.15 ± 0.10 (1)	^{B}0.75 ± 0.11 (4)	^{A}0.54 ± 0.10 (4)	^{B}1.29 ± 0.10 (3)
Se	^{A}2.22 ± 0.12 (0)	^{B}4.23 ± 0.23 (0)	^{A}3.80 ± 0.23 (0)	^{B}5.28 ± 0.31 (0)
Sr	^{A}64.79 ± 5.38 (0)	^{B}148.59 ± 10.78 (0)	^{A}BDL (all)	^{B}2.27 ± 0.11 (11)
V	^{A}0.64 ± 0.04 (6)	^{A}0.89 ± 0.08 (11)	^{A}0.56 ± 0.05 (9)	^{A}1.00 ± 0.10 (13)
Zn	^{A}138.57 ± 9.14 (0)	^{A}118.10 ± 5.43 (0)	^{A}411.85 ± 14.82 (0)	^{A}417.69 ± 14.88 (0)

[a]Data presented are means ±1 SE (# BDL) of samples that were above the method detection limit; statistical analyses for differences between sites included below detection limit values, as described in the Methods. Different letters indicate significant differences at the α = 0.0045 level.

correct for overdispersion. Initial LOGISTIC models included SVL to test for a body size effect on hatching success or malformations; SVL was nonsignificant (p > 0.87 in both tests) and removed from the model, and the scale parameter weighting factor was applied to the reduced model.

Analyses that involved trace element concentrations were constrained by the occurrence of samples that were below the instrument detection limit (BDL) for certain elements and/or locations. Of the 11 trace elements we analyzed, four (As, Ni, Pb, V) had values BDL for >10% of female tissue samples in one or both groups (reference versus CCW contaminated locations), and seven (As, Cd, Hg. Ni, Pb, Sr, V) were BDL for >10% of egg samples. Consequently, in our initial exploratory analyses of trace element levels in females and their eggs we used nonparametric Spearman rank correlations, with α = 0.0045 to adjust the family wise error for 11 tests. For trace elements that showed significant correlations between female and egg concentrations (Cu, Zn, Se, Sr, Pb), we used PROC REG on the natural-log transformed subset of values above BDL to determine female allocation to eggs, as indicated by the regression coefficient in a forced no-intercept model.

We compared trace element levels in females between the two locations for all elements, also with α = 0.0045. Due to the number of left-censored (i.e., BDL) data, we used non-parametric Wilcoxon tests in PROC LIFETEST, in which a modified survival analysis can be used to examine group differences in element concentrations that include several values for BDL from separate analytical runs, where each group has ≥10 samples.[41] Trace elements that did not differ between females from the two locations were deleted from further analysis. For the elements (Se and Cu) that were both maternally transferred and also differed between locations, we examined rank correlations between egg and female elemental concentrations and viability and reproductive success (corrected for SVL in PROC CORR).

■ RESULTS

Trace Element Concentrations in Females. Four of 11 elemental concentrations in adult females varied between the contaminated and the reference site. Specifically, concentrations of Ni, Se, and Sr were higher in females from the contaminated site compared to reference females (PROC LIFETEST Wilcoxon tests: χ^2 >10.9, df = 1, P < 0.0009 for all tests; Table 1). Nickel concentrations in females from the contaminated site were 3.5× (Figure 1B), Se 1.9×, and Sr 2.3× greater than in females from the reference site. In contrast, Pb concentrations were 60% higher in reference females than in females from the contaminated site (Table 1).

Maternal Transfer of Trace Elements. Elemental concentrations in eggs varied between sites. Eggs from females collected from the contaminated site contained higher concentrations of Cu, Pb, Se, and Sr compared to eggs from reference females (PROC LIFETEST Wilcoxon tests: χ^2 > 8.9, df = 1, P < 0.003) for all tests; Table 1). Copper, Se, and Sr concentrations in eggs from the contaminated site were 60%, 39%, and 93% higher than in reference eggs, respectively (Figure 1; Table 1). Despite the higher Pb concentrations in reference females, the concentration of Pb in eggs was 160% higher in eggs from contaminated females than in eggs from reference females.

Maternal transfer was also supported by correlations between trace element concentrations in females and their eggs. Concentrations of Cu, Se, Sr, and Zn in eggs were significantly positively related to the concentrations in females (Spearman rank correlations, r > 0.44, N = 43, P < 0.003 for all tests; Figure 2). In contrast, Pb concentration in eggs was negatively correlated with female concentrations (r = −0.44, P = 0.0039, N = 43). No-intercept regression analyses of log-transformed values above BDL showed that females varied their apportionment of these trace elements to their eggs. For Cu, Se, and Zn, slope parameter estimates (β) were >1 (Cu: β = 1.09 ± 0.03, $t_{1,42}$ = 33.87, P < 0.0001. Se: β = 1.19 ± 0.05, t = 24.27, P < 0.0001. Zn: β = 1.25 ± 0.01, t = 133.38, P < 0.0001). For a unit increase in female element concentrations, egg concentrations rose by approximately 1.1, 1.2, and 1.3 times for Cu, Se, and Zn, respectively. Females allocated very little Sr to their eggs compared to their Sr body concentrations (β = 0.13 ± 0.03, t = 4.08, P = 0.0009).

Effects on Reproduction and Offspring Viability. Female southern toads were similar in size between sites (SVL: $F_{1,42}$ = 0.01, P = 0.93. Mass adjusted for SVL: $F_{1,41}$ = 0.83, P = 0.3678. Table 2). There was a significant positive relationship between female SVL and clutch size (r^2 = 0.43, $F_{1,41}$ = 30.94, P < 0.0001). After accounting for female SVL,

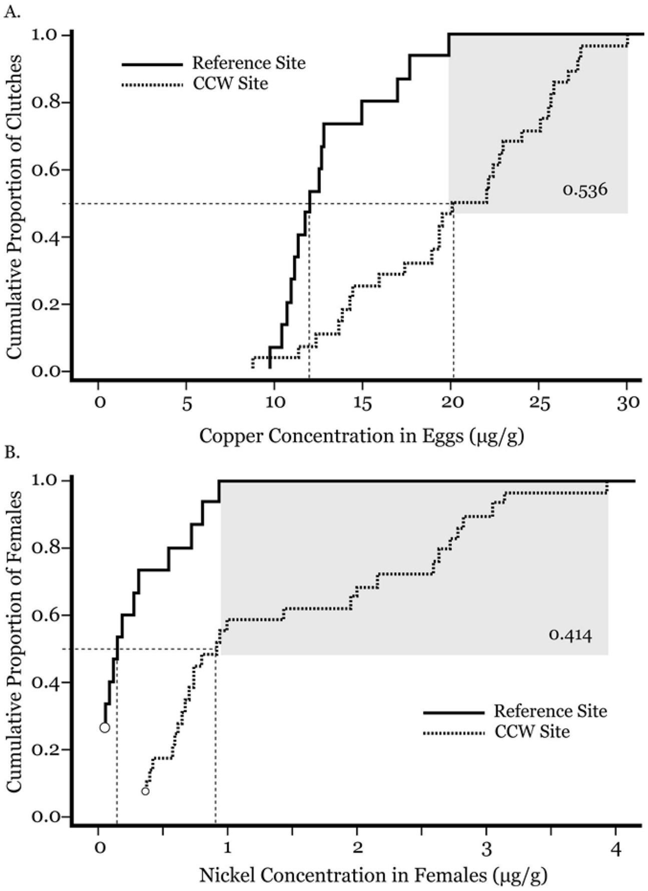

Figure 1. Differences between the coal ash contaminated area (CCW) and an uncontaminated reference site (reference) in (A) Cu concentration in eggs and (B) Ni concentrations in females. Sites were tested for differences in trace metal levels using the "flip" method (see ref 41) for left-censored (below detection limit) data. Original (=unflipped) data presented here for ease of interpretation. Fine-dashed lines represent the median metal concentrations for the two sites. Shaded areas depict the proportion of CCW samples (0.536 for Cu in eggs; 0.414 for Ni in females) that had concentrations higher than the maximum observed reference value. For Cu (A), no observations were below detection limit; open symbols for Ni (B) represent the BDL observations (4 from reference site, 2 from CCW).

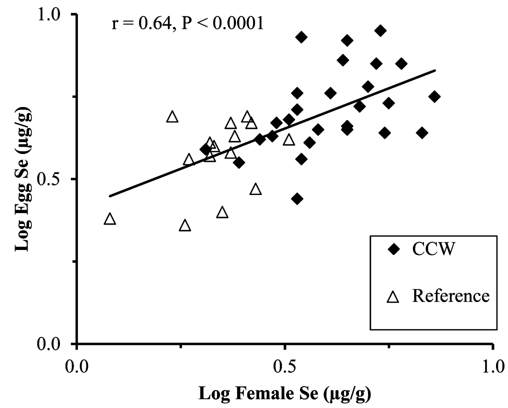

Figure 2. Relationship between log selenium concentrations (μg/g dry mass) in carcasses of female southern toads and their eggs collected from an coal ash contaminated area (CCW) and an uncontaminated reference site (reference). Correlation coefficient is Spearman rank correlation on untransformed data; no observations were below detection limit.

Table 2. Snout Vent Length (SVL), Mass, Clutch Size, Percent Hatching Success, Hatchling Abnormality Frequency, Offspring Viability, and Reproductive Success of Female Southern Toads Collected from a Reference Site (Reference) and a Coal-Fly-Ash Contaminated Area (CCW)[a]

	reference n = 15	CCW n = 29
SVL (mm)	^{A}65.3 ± 0.48	^{A}65.1 ± 1.0
mass (g)	^{A}30.38 ± 0.68	^{A}29.38 ± 1.31
clutch size (no. eggs)	^{A}4060 ± 98	^{A}3939 ± 233
hatching success (%)	^{A}91.1 ± 2.2	^{B}70.6 ± 5.8
abnormality frequency (%)	^{A}11.0 ± 1.2	^{A}11.1 ± 1.4
viability (%)	81.1 ± 2.4	62.6 ± 5.1
reproductive success (predicted no. viable offspring)	3307 ± 337	2410 ± 280

[a]SVL, hatching success, morphological abnormality frequency, and viability data are presented as means ±1 SE. Clutch size, mass, and reproductive success are presented as LS means adjusted for female SVL ± 1 SE. Different letters indicate significant differences between sites. Viability and reproductive success were derived from hatching success, and therefore also differed between sites.

average clutch size did not differ between contaminated and reference females ($F_{1,41}$ = 0.14, P = 0.7105; Table 2); female elemental concentrations were not correlated with clutch size (Spearman rank correlations, $r < |\pm 0.24|$, N = 44, $P \geq 0.12$ for all tests).

Hatching success of eggs from the contaminated females was reduced by 22% compared to reference females (Table 2; Wald χ^2 = 6.07, df = 1, P = 0.0137). Egg and female elemental concentrations were generally not correlated with hatching success at α = 0.0045 (Spearman rank correlations, $r < |\pm 0.37|$, N = 43 or 44, $P \geq 0.01$), with the exception of Ni concentration in eggs (Spearman rank correlation, r = −0.46, N = 43, P = 0.0023).

The frequency of developmental abnormalities in hatchlings (∼11%) was similar between sites (Wald χ^2 = 0.0002, df = 1, P = 0.9882; Table 2), and was not correlated with either female or egg elemental concentrations (Spearman rank correlations, $r < |\pm 0.33|$, N = 43 or 44, $P \geq 0.03$ for all tests). Axial malformations were the most abundant abnormality at all sites, comprising approximately 78% of observed abnormalities (Table 3).

Offspring viability (the percentage of successful hatchlings with no abnormalities) was reduced by 23% in females from the

Table 3. Percentage of Southern Toad Hatchlings from Females Collected from a Reference Site (Reference) and a Coal-Fly-Ash Contaminated Area (CCW) Displaying Morphologic Abnormalities[a]

	reference N = 15	CCW N = 27
axial	9.4 ± 0.33	9.1 ± 0.26
adema	1.0 ± 0.06	0.8 ± 0.04
oral	0.6 ± 0.05	0.7 ± 0.05
blister	0.1 ± 0.01	0.5 ± 0.02
total	11.1 ± 0.31	11.1 ± 0.27

[a]Data presented are means ±1 SE.

contaminated site compared to reference females (Table 2). Overall reproductive success (clutch size X % viability = number of morphologically normal hatchlings) was reduced by 27% in contaminated females compared to that in reference females (Table 2).

Selenium and Cu were the two elements that were both (1) different between locations in the tissue concentrations of females and (2) transferred at enhanced levels from females to their eggs. Egg Ni was the one element correlated with hatching success at the adjusted family wise error rate of $\alpha = 0.0045$, and it also differed between locations. Therefore, we correlated Se and Cu (in both females and in eggs), and Ni (in eggs) to offspring viability and reproductive success. Concentrations of Se in females and eggs correlated negatively with offspring viability (Spearman rank correlations: $r = -0.39$, $N = 44$, $P = 0.008$ for female Se concentration; $r = -0.36$, $N = 43$, $P = 0.019$ for egg Se) and overall reproductive success (Spearman rank correlation: $r = -0.31$, $N = 44$, $P = 0.043$ for female Se concentration, adjusting for SVL; $r = -0.43$, $N = 43$, $P = 0.0049$ for egg Se, Figure 3). Copper concentrations in females negatively correlated with reproductive success (Spearman rank correlation: $r = -0.37$, $N = 44$, $P = 0.015$) but were not related to viability; Cu in eggs did not correlate with either viability or reproductive success ($r \leq -0.06$, $N = 43$, $P \geq 0.69$). Nickel in eggs was negatively correlated with offspring viability (Spearman rank correlation: $r = -0.41$, $N = 43$, $P = 0.0066$) and weakly related to reduced reproductive success (Spearman rank correlation: $r = -0.29$, $N = 43$, $P = 0.06$).

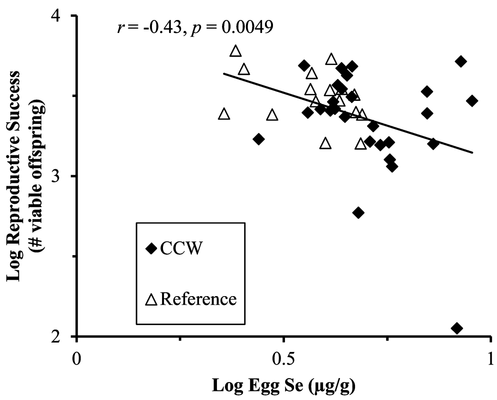

Figure 3. Relationship between log selenium concentrations (μg/g dry mass) in eggs and reproductive success of females collected from an coal ash contaminated area (CCW) and an uncontaminated reference site (reference). Correlation coefficient is Spearman rank correlation on untransformed data; no observations were below detection limit.

DISCUSSION

Our study is among the first to document correlations between contaminant concentrations in female amphibians, their eggs, and reduced reproductive success. Specifically, we found that female southern toads accumulated select trace elements from exposure to CCW, and subsequent maternal transfer of some of these elements was associated with reductions in hatching success (Ni), offspring viability (Ni, Se), and overall reproductive success (Se, Cu). Our findings demonstrate that exposure to CCW can have deleterious effects on amphibian reproductive success, underscoring the importance of determining how prevalent these effects are across species and CCW-disposal sites. Our results also highlight the need to determine whether these individual level effects influence populations.

Similar to other wildlife exposed to CCW, female southern toads inhabiting the CCW-contaminated site accumulated numerous trace elements and transferred them to their offspring. Female toads collected from the contaminated site transferred elevated concentrations of Cu, Pb, Se, and Sr to their eggs compared to eggs of females collected from the reference site. Copper and Se are essential micronutrients and may have been transferred to eggs as components of egg yolk proteins.[42,43] Also, Se was likely maternally transferred because it substitutes for sulfur in a variety of egg components.[44,45] In contrast, Pb is a nonessential heavy metal that can be toxic to vertebrates including humans even at low doses.[46,47] Although Pb concentrations were generally low and concentrations in females were similar between sites, females from the CCW-contaminated site transferred elevated concentrations of Pb to their eggs. Lead and Sr may have been transferred because they both substitute for calcium, which is an essential nutrient that plays an important role in cell division during embryonic development.[48,49]

Comparison of our results to studies of inhabitants from the same contaminated site suggests that southern toads may be more sensitive to CCW contamination than other vertebrates. Some vertebrate species appear unaffected by CCW exposure, and others can persist in the CCW-contaminated sites for long periods of time.[50−53] However, female eastern narrowmouth toads collected from the D-area ash basin experienced a 19% reduction in offspring viability compared to reference individuals.[4] In our study, southern toads collected from the contaminated site experienced a 23% reduction in offspring viability, resulting on average in a 27% reduction in reproductive success compared to reference toads. Previous studies demonstrated that southern toads also experience other adverse effects from exposure to CCW, such as abnormal hormone levels, decreased growth and development, alterations in behavior and performance,[54,55] and, ultimately, reduced survival to metamorphosis and recruitment into the terrestrial environment.[21,26] The underlying causes of differential sensitivity among species remain unknown but underscore the importance of considering diverse taxa when assessing risks from CCW exposure.

Of the elements maternally transferred, Se is of particular interest because it can disrupt embryonic development in oviparous vertebrates and has well-documented negative effects on reproductive success of fish and birds.[56−62] Reproductive toxicity thresholds based on egg Se concentrations for fish and birds are variable but typically range from 8 to 16 μg/g;[59,61,63−65] however, thresholds for amphibians are not established. In our study, southern toads exposed to CCW accumulated (carcass mean = 4.2 μg/g Se) and maternally transferred relatively low Se concentrations (egg mean = 5.3 μg/g Se) compared to other vertebrate species from the same site. In contrast, female eastern narrowmouth toads collected from the same ash basin accumulated very high levels of Se (42 μg/g) and transferred similar concentrations to their eggs.[4] Reptiles, fish, and birds from the ash basin transferred 5.9−15.9 μg/g of Se to their offspring.[52,53,66] Despite maternally transferring lower concentrations of Se than all of the above-mentioned taxa, southern toads experienced a significant reduction in reproductive success, which also correlated with Se concentrations in females and eggs. On average, reproductive success was reduced by 27% compared to that of reference females; moreover, the 75th percentile Se concentration resulted in a 35% reduction in reproductive success. It is possible that our findings are due to exposure to the suite of elements in CCW and/or the cotransference of several elements to eggs, not from Se alone. For instance, Ni concentrations in eggs negatively correlated with offspring viability. It is also possible that other effects of CCW could influence egg quality. For example, toads from this site are known to have high plasma corticosterone levels.[54] In birds, high maternal corticosterone levels can alter offspring phenotype and impair viability.[67] Disentangling the direct effects of Se from the cotransference of other elements and other physiological effects was not possible in a field study of this nature, but it is a fruitful line of investigation using controlled experiments.

Exposure to contaminants negatively affected southern toad reproductive success and could ultimately lead to population declines. Although population-level effects of CCW on southern toads remain unknown, pronounced reductions in reproductive success, such as those documented in our study, could impact local populations and potentially metapopulations.[68,69] This is particularly true if other adverse effects of maternal contaminant transfer emerge during larval and/or juvenile development. For example, in American toads (*Bufo americanus*), maternal Hg exposure and dietary exposure during larval development both had negative effects on offspring health. However, the two routes of exposure acted synergistically, decreasing survival by 50% compared to reference larvae.[10] Similarly, Metts et al.[26] found that previous maternal exposure to CCW interacted with larval exposure to reduce larval survival in southern toads by 85% compared to reference larvae reared on reference sediments.[26] Together, a 27% reduction in reproductive success and an 85% reduction in survival of remaining larvae to metamorphosis would result in very poor per capita production of recruits to the terrestrial environment. For example, a typical female producing 4000 eggs in the reference site would be expected to produce 2530 successful recruits, while a female from the contaminated site would be expected to produce only 275 recruits, nearly a 90% reduction in recruitment to the terrestrial environment. Such pronounced effects on recruitment could result in population declines, although reduced larval density due to mortality could decrease the strength of density-dependent interactions among larvae at the contaminated site, improving the survival of remaining larvae with no net effect on local population dynamics.[70] Future modeling studies that incorporate density-dependent larval dynamics and the individual-level responses we observed are needed to predict potential changes in population dynamics.[68−72]

In conclusion, bioaccumulation and maternal transfer of trace elements as a result of female exposure to CCW is an important factor that negatively affects amphibian reproductive success and may ultimately contribute to localized amphibian declines. Our results yield further evidence that CCW-contaminated basins and wetlands may be ecological traps for amphibians and other wildlife because animals residing in sites accumulate contaminants and experience deleterious effects, including reduced reproductive success. Our study also highlights the need for continued research on maternal transfer of contaminants in amphibians. The negative correlations between trace element concentrations and reproductive success suggest that maternal transfer of contaminants is at least partially responsible for reduced reproductive success; however, males were also collected from the same contaminated sites, and thus, paternal effects cannot be ruled out. Future studies examining paternal effects should be a high priority. Finally, investigating the effects of exposure to CCW on postmetamorphic terrestrial juveniles is necessary to determine if amphibian populations can persist in CCW-contaminated sites or whether they are sustained by immigration from nearby source populations.

■ ASSOCIATED CONTENT

Supporting Information

Elemental concentration, female size, and reproductive data. This information is available free of charge via the Internet at http://pubs.acs.org/ .

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Linder, G.; Grillitsch, B. Ecotoxicology of metals. In *Ecotoxicology of Amphibians and Reptiles*; Sparling, D. W., Linder, G., Bishop, C. A., Eds.; SETAC Press: Pensacola, FL, 2000; pp 325−459.

(2) Birge, W. J.; Westerman, A. G.; Spomberg, J. A. Comparative toxicology and risk assessment of amphibians. In *Ecotoxicology of Amphibians and Reptiles*; Sparling, D. W., Linder, G., Bishop, C. A., Eds.; SETAC Press: Pensacola, FL, 2000; pp 727−792.

(3) Bernardo, J. Maternal effects in animal ecology. *Am. Zool.* **1996**, *36*, 83−105.

(4) Hopkins, W. A.; DuRant, S. E.; Staub, B. P.; Rowe, C. L.; Jackson, B. P. Reproduction, embryonic development, and maternal transfer of contaminants in the amphibian *Gastrophryne carolinensis*. *Environ. Health Perspect.* **2006**, *114*, 661−666.

(5) Kadokami, K.; Takeishi, M.; Kuramoto, M.; Ono, Y. Maternal transfer of organochlorine pesticides, polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and coplanar polychlorinated biphenyls in frogs to their eggs. *Chemosphere* **2004**, *57*, 383−389.

(6) Wu, J.-P.; Luo, X.-J.; Zhang, Y.; Chen, S.-J.; Mai, B.-X.; Guan, Y.-T.; Yang., Z.-Y. Residues of polybrominated diphenyl ethers in frogs (*Rana limnocharis*) from a contaminated site, South China: Tissue distribution, biomagnification, and maternal transfer. *Environ. Sci. Technol.* **2009**, *43*, 5212−7.

(7) Bergeron, C. M.; Bodinof, C. M.; Unrine, J. M.; Hopkins, W. A. Bioaccumulation and maternal transfer of mercury and selenium in amphibians. *Environ. Toxicol. Chem.* **2010**, *29*, 989−997.

(8) Kotyzova, D.; Sundeman, F. W. Maternal exposure to Cd (II) causes malformations of *Xenopus laevis* embryos. *Ann. Clin. Lab. Sci.* **1998**, *28*, 224–235.
(9) Bergeron, C. M.; Hopkins, W. A.; Bodinof, C. M.; Budischak, S. A.; Wada, H.; Unrine, J. M. Counterbalancing effects of maternal mercury exposure during different stages of early ontogeny. *Sci. Total Environ.* **2011**, *409*, 4746–4752.
(10) Bergeron, C. M.; Hopkins, W. A.; Todd, B. D.; Hepner, M. J.; Unrine, J. M. Interactive effects of maternal and dietary mercury exposure have latent and lethal consequences for amphibian larvae. *Environ. Sci. Technol.* **2011**, *43*, 3781–3787.
(11) Collins, J. P.; Storfer, A. Global amphibian declines: Sorting the hypotheses. *Diversity Distributions* **2003**, *9*, 89–98.
(12) Hoffmann, M.; Hilton-Taylor, C.; Angulo, A.; Böhm, M.; Brooks, T. M.; Butchart, S. H. M.; Carpenter, K. E.; et al. The impact of conservation on the status of the world's vertebrates. *Science* **2010**, *330*, 1503–1509.
(13) Stuart, S. N.; Chanson, J. S.; Cox, N. A.; Young, B. E.; Rodriguez, A. S. L.; Fischman, D. L.; Waller, R. W. Status and trends of amphibian declines and extinctions worldwide. *Science* **2004**, *306*, 1783–1786.
(14) Hof, C.; Araujo, M. B.; Jetz, W.; Rahbek, C. Additive threats from pathogens, climate and land-use change for global amphibian diversity. *Nature* **2011**, *480*, 516–519.
(15) Voyles, J.; Young, S.; Berger, L.; Campbell, C.; Voyles, W. F.; Dinudom, A.; Cook, D.; Webb, R.; Alford, R. A.; Skerratt, L. F.; Speare, R. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science* **2009**, *326*, 582–585.
(16) Energy Information Administration. *EIA-767 Data Files: Annual Steam-Electric Plant Operation and Design Report*; U.S. Department of Energy: Washington, DC, 2005.
(17) Rowe, C. L.; Hopkins, W. A.; Congdon, J. D. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: A review. *Environ. Monit. Assess.* **2002**, *80*, 207–276.
(18) Hopkins, W. A.; Congdon, J. D.; Ray, J. K. Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal-burning power plant. *Environ. Toxicol. Chem.* **2000**, *19*, 862–868.
(19) Raimondo, S. M.; Rowe, C. L. Exposure to coal ash impacts swimming performance and predator avoidance in larval bullfrogs (*Rana catesbeiana*). *J. Herpetol.* **1998**, *32*, 289–292.
(20) Rowe, C. L.; Kinney, O. M.; Fiori, A.; Congdon, J. D. Oral deformities in tadpoles (*Rana catesbeiana*) associated with coal ash deposition: Effects on grazing ability and growth. *Freshwater Biol.* **1996**, *36*, 723–730.
(21) Rowe, C. L.; Hopkins, W. A.; Coffman, V. R. Failed recruitment of southern toads (*Bufo terrestris*) in a trace element-contaminated breeding habitat: direct and indirect effects that may lead to a local population sink. *Arch. Environ. Contam. Toxicol.* **2001**, *40*, 399–405.
(22) Roe, J. H.; Hopkins, W. A.; DuRant, S. E.; Unrine, J. M. Effects of competition and coal-combustion wastes on recruitment and life history characteristics of salamanders in temporary wetlands. *Aquat. Toxicol.* **2006**, *79*, 176–184.
(23) Snodgrass, J. W.; Hopkins, W. A.; Roe, J. H. Relationships among developmental stage, metamorphic timing, and concentrations of elements in bullfrogs (*Rana catesbeiana*). *Environ. Toxicol. Chem.* **2003**, *22*, 1597–1604.
(24) Snodgrass, J. W.; Hopkins, W. A.; Broughton, J.; Gwinn, D.; Baionno, J. A.; Burger, J. Species-specific responses of developing anurans to coal combustion wastes. *Aquat. Toxicol.* **2004**, *66*, 171–182.
(25) Snodgrass, J. W.; Hopkins, W. A. Influence of larval period on responses of overwintering green frog (*Rana clamitans*) larvae exposed to contaminated sediments. *Environ. Toxicol. Chem.* **2005**, *24*, 1508–1514.
(26) Metts, B. S.; Buhlmann, K. B.; Scott, D. E.; Tuberville, T. D.; Hopkins, W. A. Interactive effects of maternal and environmental exposure to coal combustion wastes decrease survival of larval southern toads (*Bufo terrestris*). *Environ. Pollut.* **2012**, *164*, 211–218.
(27) Rowe, C. L.; Hopkins, W. A. Anthropogenic activities producing sink habitats for amphibians in the local landscape: A case study of lethal and sublethal effects of coal combustion residues in the aquatic environment. In *Amphibian Decline: An Integrated Analysis of Multiple Stressor Effects*; Linder, G., Krest, S. K., Sparling, D. W., Eds.; SETAC Press: Pensacola, FL, 2003; pp 271–281.
(28) Roe, J. H.; Hopkins, W. A.; Jackson, B. P. Species- and stage-specific differences in trace element tissue concentrations in amphibians: implications for the disposal of coal-combustion wastes. *Environ. Pollut.* **2005**, *136*, 353–363.
(29) Bogert, C. M. A field study of homing in the Carolina toad. *Am. Mus. Novit.* **1947**, *1355*, 24.
(30) Jensen, J. B. Southern toad *Bufo (Anaxyrus) terrestris*. In *Amphibians and Reptiles of Georgia*; Jensen, J. B., Camp, C. D., Gibbons, J. W., Elliott, M. J., Eds.; University of Georgia Press: Athens, GA, 2008; pp 44–46.
(31) U.S. EPA. *Wastes from the Combustion of Fossil Fuels*, EPA 530-R-99-010; U.S. Environmental Protection Agency: Washington, DC, 1999.
(32) Sandhu, S. S.; Mills, G. L. Chapter 17. Mechanisms of mobilization and attenuation of inorganic contaminants in coal ash basins. In *Emerging Technologies in Hazardous Waste Management*; Tedder, D. W.; Pohland, F. G., Eds.; American Chemical Society: Washington, DC, 1991; pp 342–364.
(33) Norton, D. M.; Mills, G. L. Polycyclic aromatic hydrocarbons in SRP aquatic systems derived from coal pile runoff. *193rd National Meeting*; American Chemical Society: Washington, DC, 1987; pp 120–122.
(34) Shorten, C. V.; Elzerman, A. W.; Mills, G. L. Methods for the determination of PAH desorption-kinetics in coal fines and coal contaminated sediments. *Chemosphere* **1990**, *20*, 137–159.
(35) Sharitz, R. R. Carolina bay wetlands: Unique habitats of the Southeastern United States. *Wetlands* **2003**, *23*, 550–562.
(36) Sharitz, R. R.; Gibbons, J. W. *The Ecology of Southeastern Shrub Bogs (Pocosins) and Carolina Bays: A Community Profile.* U.S. Fish and Wildlife Service, Division of Biological Services: Washington, DC, 1982.
(37) Semlitsch, R. D.; McMillan, M. A. Breeding migrations, population size structure, and reproduction of the dwarf salamander, *Eurycea quadridigitata*, in South Carolina. *Brimleyana* **1980**, *3*, 97–105.
(38) Pechmann, J. H. K.; Scott, D. E.; Semlitsch, R. D.; Caldwell, J. P.; Vitt, L. J.; Gibbons, J. W. Declining amphibian populations: The problem of separating human impacts from natural fluctuations. *Science* **1991**, *253*, 892–895.
(39) Bantle, J. A.; Dumont, J.; Finch, R.; Linder, G. *Atlas of Abnormalities: A Guide for the Performance of FETAX*; Oklahoma State Publications: Stillwater, OK, 1991.
(40) ASTM. *Standard Guide for Conducting the Frog Embryo Teratogenesis Assay-Xenopus (FETAX)*. American Society for Testing and Materials: Philadelphia, PA, 1998.
(41) Newman, M. C. Chapter 2: The measurement process. In *Quantitative Ecotoxicology*, 2nd ed.; CRC Press: Boca Raton, FL, 2012; pp 27–76.
(42) Kroll, K. J.; Doroshov, S. I. Vitellogenin: Potential vehicle for selenium in oocytes of the white sturgeon (*Acipenser transmontanus*). In *Acipenser*; Williot, P, Ed.; Cemagref Publishers: Montpellier, France, 1991; pp 99–106.
(43) Ghosh, P.; Thomas, P. Binding of metals to red drum vitellogenin and incorporation into oocytes. *Mar. Environ. Res.* **1995**, *39*, 165–168.
(44) Unrine, J. M.; Jackson, B. P.; Hopkins, W. A.; Romanek, C. Isolation and partial characterization of proteins involved in maternal transfer of selenium in the western fence lizard *(Sceloporus occidentalis)*. *Environ. Toxicol. Chem.* **2006**, *25*, 1864–1867.
(45) Unrine, J. M.; Jackson, B. P.; Hopkins, W. A. Selenomethionine biotransformation and incorporation into proteins along a simulated terrestrial food chain. *Environ. Sci. Technol.* **2007**, *41*, 3601–3606.
(46) Franson, J. C.; Pain, D. J. Lead in birds. In *Environmental Contaminants in Biota: Interpreting Tissue Concentrations*, 2nd ed.;

Beyer, W. N., Meador, J. P., Eds.; CRC Press: Boca Raton, FL, 2011; pp 563−593.
(47) ATSDR. *Toxicological Profile for Lead (Update)*; U.S. Department of Health and Human Services: Atlanta, GA, 2007.
(48) Goldstein, G. H. Evidence that lead acts as a calcium substitute in second messenger metabolism. *Neurotoxicology* **1993**, *14*, 97−101.
(49) Fluck, R. A.; Miller, A. L.; Jaffe, L. F. Slow calcium waves accompany cytokinesis in medaka fish eggs. *J. Cell Biol.* **1991**, *115*, 1259−1265.
(50) Rowe, C. L.; Kinney, O. M.; Congdon, J. D. Oral deformities in tadpoles of the bullfrog (*Rana categsbeiana*) caused by conditions in a polluted habitat. *Copeia* **1998**, 244−246.
(51) Rowe, C. L.; Kinney, O. M.; Nagle, R. D.; Congdon, J. D. Elevated maintenance costs in an anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during the embryonic and early larval periods. *Physiol. Zool.* **1998**, *71*, 27−35.
(52) Roe, J. H.; Hopkins, W. A.; Baionno, J. A.; Staub, B. P.; Rowe, C. L.; Jackson, B. P. Maternal transfer of selenium in *Alligator mississippiensis* nesting downstream from a coal-burning power plant. *Environ. Toxicol. Chem.* **2004**, *23*, 1969−1972.
(53) Staub, B. P.; Hopkins, W. A.; Novak, J.; Congdon, J. D. Respiratory and reproductive characteristics of eastern mosquitofish (*Gambusia holbrooki*) inhabiting a coal ash settling basin. *Arch. Environ. Contam. Toxicol.* **2004**, *46*, 96−101.
(54) Hopkins, W. A.; Mendonca, M. T.; Congdon, J. D. Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. *Gen. Comp. Endrocrinol.* **1997**, *108*, 237−246.
(55) Ward, C. K.; Appel, A. G.; Mendonca, M. T. Metabolic measures of male southern toads (*Bufo terrestris*) exposed to coal combustion waste. *Comparative Biochem. Physiol., Part A* **2006**, *143*, 353−360.
(56) Chapman, P. *Ecotoxicology of Selenium in the Aquatic Environment*. SETAC Press: Pensacola, FL, 2010.
(57) Gillespie, R. B.; Baumann, P. C. Effects of high tissue concentrations of selenium on reproduction in bluegills. *Trans. Am. Fish. Soc.* **1986**, *115*, 208−213.
(58) Janz, D. M.; Brooks, M. L.; Chapman, P. M.; DeForest, D. K.; Gilron, G.; Hoff, D.; Hopkins, W. A.; McIntyre, D. O.; Mebane, C. A.; Palace, V. P.; Skorupa, J. P.; Wayland, M. Selenium toxicity to aquatic organisms. In *Ecotoxicology of Selenium in the Aquatic Environment*; Chapman, P., Ed.; SETAC Press: Pensacola, FL, 2010; pp 141−231.
(59) Lemly, A. D. Selenium in aquatic organisms. In *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*; Beyer, W. N., Heinz, G. H., Redmon-Norwood, A. W., Eds.; Lewis Publishers: Boca Raton, FL, 1996; pp 427−445.
(60) Ohlendorf, H. M. Ecotoxicology of selenium. In *Handbook of Ecotoxicology*; Hoffman, D. J., Rattner, B. A., Burton, G. A., Cairns, J., Eds.; Lewis Publishers: Boca Raton, FL, 2003; 465−500.
(61) Skorupa, J. P. Selenium poinsoning of fish and wildlife in nature: Lessons from twelve real-world examples. In *Environmental Chemistry and Selenium*; Frankenberger, W. T. Jr., Engberg, R. A., Eds.; Marcel Dekker: New York, 1998; pp 315−354.
(62) Young, T. F.; Finley, K.; Adams, W. F.; Besser, J.; Hopkins, W. A.; Jolley, D.; McNaughton, E.; Presser, T. S.; Shaw, D. P.; Unrine, J. M. Chapter 3. What you need to know about selenium. In *Ecological Assessment of Selenium in the Aquatic Environment*; Chapman, P. M., Adams, W. J., Brooks, M., Delos, C. G., Luoma, S. N., Maher, W. A., Ohlendorf, H. M., Presser, T. S., Shaw, P., Eds.; SETAC Press: Pensacola, FL, 2010; pp 7−45.
(63) Heinz, G. H. Selenium in birds. In *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*; Beyer, W. N., Heinz, G. H., Redmon-Norwood, A. W., Eds.; Lewis Press: Boca Raton, FL, 1996; pp 447−458.
(64) Skorupa, J. P.; Ohlendorf, H. M. Contaminants in drainage water and avian risk thresholds. In *The Economics and Management of Water and Drainage in Agriculture*; Dinar, A., Zilberman, D., Eds.; Kluwer Academic: Dordrecht, Netherlands, 1991; pp 345−368.
(65) Fairbrother, A.; Brix, K. V.; Toll, J. E.; McKay, S.; Adams, W. J. Egg selenium concentrations as predictors of avian toxicity. *Hum. Ecol. Risk Assess.* **1999**, *5*, 1229−1253.
(66) Bryan, A. L., Jr.; Hopkins, W. A.; Baionno, J. A.; Jackson, B. P. Maternal transfer of contaminants to eggs in common grackles (*Quiscalus quiscala*) nesting on coal fly ash basins. *Arch. Environ. Contam. Toxicol.* **2003**, *45*, 273−277.
(67) Saino, N.; Romano, M.; Ferrari, R. P.; Martinelli, R.; Moller, A. P. Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. *J. Exp. Zool.* **2005**, *303A*, 998−1006.
(68) Willson, J. D.; Hopkins, W. A. Evaluating the effects of anthropogenic stressors on source-sink dynamics in pond-breeding amphibians. *Conserv. Biol.*, **2013**, in press.
(69) Willson, J. D.; Hopkins, W. A.; Bergeron, C. M.; Todd, B. D. Making leaps in amphibian ecotoxicology: Translating individual-level effects of environmental contaminants to population viability. *Ecol. Appl.* **2012**, *22*, 1791−1802.
(70) Vonesh, J. R.; De la Cruz, O. Complex life cycles and density-dependence: Population-level consequences of early mortality in amphibians. *Oecologia* **2002**, *133*, 325−333.
(71) Taylor, B. E.; Scott, D. E.; Gibbons, J. W. Catastrophic reproductive failure, terrestrial survival, and persistence of the marbled salamander. *Conserv. Biol.* **2006**, *20*, 792−801.
(72) Salice, C. J.; Rowe, C. L.; Pechmann, J. H. K.; Hopkins, W. A. Multiple stressors and complex life cycles: Insights from a population-level assessment of breeding site contamination and terrestrial habitat loss in an amphibian. *Environ. Toxicol. Chem.* **2011**, *30*, 2874−2882.