



## Interactive effects of maternal and environmental exposure to coal combustion wastes decrease survival of larval southern toads (*Bufo terrestris*)

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### ABSTRACT

We conducted a mesocosm study to assess the individual and interactive effects of previous maternal exposure and larval exposure to trace element-laden sediments on southern toads (*Bufo terrestris*). Previous maternal exposure to coal combustion wastes (CCW) reduced larval survival to metamorphosis up to 57% compared to larvae of unexposed females. Larvae reared on CCW accumulated significant concentrations of trace elements resulting in extended larval periods, reduced growth rates, and reduced mass at metamorphosis. However, the effects were dependent on age of sediments, suggesting the effects of contaminants from CCW may be partially ameliorated over time through the reduced bioavailability of trace elements in aged CCW. Most importantly, maternal exposure to contaminants coupled with larval exposure to fresh CCW interacted to reduce survival to metamorphosis by 85% compared to reference conditions. Our study yields further evidence that disposal of CCW in aquatic basins potentially creates ecological traps for some amphibian populations.

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### 1. Introduction

Environmental contamination is thought to be one of the greatest contributors to worldwide amphibian population declines (Collins and Storer, 2003; Hoffmann et al., 2010; Stuart et al., 2004). Environmental contaminants come from many sources, but coal fired power plants are one of the largest producers of contaminated solid wastes in the U.S. (USDOE, 2005). Coal combustion wastes (CCW) contain high concentrations of trace elements (e.g., arsenic (As), mercury (Hg), selenium (Se)) and are often disposed of in open aquatic settling basins (Rowe et al., 2002). Amphibians and other wildlife using these basins can accumulate elevated concentrations of trace elements, resulting in adverse effects on survival, growth, development, behavior, performance, and recruitment (Hopkins et al., 2000, 2006; Raimondo and Rowe, 1998; Rowe et al., 1996). Taken together these studies suggest that CCW contaminated wetlands may serve as ecological traps to amphibian populations (Roe et al., 2006; Rowe and Hopkins, 2003; Snodgrass et al., 2003, 2004; Snodgrass and Hopkins, 2005).

The majority of research investigating the effects of contaminants on amphibians has focused on environmental exposure (i.e., sediment, soil, and water) or trophic uptake (Linder and Grillitsch, 2000),

but other routes of exposure have gained recent attention. Although environmental exposure to contaminants often elicits adverse effects, parental factors such as maternal transfer of contaminants may also negatively affect reproduction and development (Hopkins et al., 2006). Previous studies have demonstrated that adult amphibians can accumulate contaminants and transfer them to their eggs (Kadokami et al., 2004; Bergeron et al., 2010), ultimately leading to reduced hatching success and offspring viability (Kotyzova and Sundeman, 1998; Hopkins et al., 2006; Bergeron et al., 2011a). In addition, the consequences of previous maternal exposure may become apparent later in development of surviving offspring (i.e., long term and/or latent effects; Bergeron et al., 2011b). For example, larvae of female American toads (*Bufo (Anaxyrus) americanus*; hereafter *Bufo*) exposed to Hg grew more slowly as embryos and were smaller at metamorphosis than larvae from unexposed females (Bergeron et al., 2011b). Further, amphibians born into the same environment as their parents can be exposed to contaminants of maternal origin and from the environment, making it important to identify interactions between maternal and environmental or dietary exposure (Bergeron et al., 2011b). For instance, maternal Hg exposure in *B. americanus* had a greater influence on offspring health than larval dietary exposure. However, the two routes of exposure acted synergistically, reducing survival by 50% compared to reference larvae (Bergeron et al., 2011b).

The objectives of our study were to assess the individual and interactive effects of previous maternal exposure and subsequent

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environmental (i.e., sediment) exposure to trace elements on survival, growth, and performance of larval southern toads (*Bufo terrestris*). We also sought to determine whether reduced availability of trace elements in aged surface sediments would mitigate the effects on amphibians. We conducted a factorial mesocosm experiment designed to simulate conditions in 1) a CCW settling basin, 2) a natural wetland contaminated with CCW that has undergone natural succession for over 35 years, and 3) an uncontaminated reference wetland. We hypothesized that maternal and environmental exposure to trace elements would each independently prolong the larval period and decrease survival, growth rate, size, and performance of metamorphs. Furthermore, we predicted that the interaction of maternal and environmental exposure would exacerbate the individual effects of contaminants on larvae. Because the availability of trace elements in contaminated wetlands may decrease over time, due to downward leaching from surface sediments and other biogeochemical processes (Sandhu et al., 1993), we hypothesized that the effects of contaminants would be less pronounced in larvae reared on wetland sediments contaminated with ash deposited more than 35 years ago compared to sediments from an active ash settling basin.

## 2. Materials and methods

### 2.1. Study species

*Bufo terrestris* is a common anuran in the southeastern U.S. that like many other amphibians, has a complex life cycle consisting of aquatic larvae and terrestrial adults. Their home range extends up to 1.6 km from wetlands during the non-breeding season (Bogert, 1947) and adults migrate in the spring and summer to aquatic breeding sites (Jensen, 2008). Females lay 2500 to 4000 eggs which hatch in 2–4 days. Larvae graze mostly on aquatic vegetation and algae, exhibit rapid growth, and have a relatively short larval period (30–55 days; Jensen, 2008). After metamorphosis juveniles move on to land and migrate across the landscape. Once they mature, most individuals return to breed in their natal wetland, while others disperse to neighboring wetlands (Breden, 1987; Berven, 1990). Thus, adults may inhabit and breed in both contaminated and uncontaminated wetlands occurring within a few square kilometers of one another.

### 2.2. Study sites

We collected adult *B. terrestris* from three locations on the Savannah River Site near Aiken, SC, USA: the D-area ash basin, the ash plume wetland, and a reference site (Ellenton Bay, a nearby uncontaminated Carolina bay wetland). The two contaminated sites (ash basin and ash plume wetland) are associated with a coal powered steam generation facility, which includes a disposal area where sluiced CCWs are discharged into a series of open settling basins. Sediments in this basin are comprised entirely of CCWs that are enriched with trace elements (Rowe et al., 2002; Hopkins et al., 2006) and extremely low in organic matter (Hopkins et al., 2004). The second contaminated site is the ash plume wetland, a natural wetland in the Savannah River floodplain that was contaminated when CCWs were discharged into it during the 1950's to early 1970's (Roe et al., 2005). Since the cessation of CCW discharge into the floodplain, vegetational succession has occurred in the ash plume wetland, and possible attenuation of trace elements in the sediments (Sandhu et al., 1993) could create an environment that is less contaminated than the active ash basins. Coal combustion wastes in the ash plume sediments extend to a depth of 2.7 m and cover approximately 40 ha, 30% of which is occasionally inundated when the Savannah River floods into its surrounding floodplain. A thin layer (~2.5 cm) of organic material covers the CCW and the ash plume wetland is vegetated with a mixed floodplain flora community (Roe et al., 2005). The reference site, Ellenton Bay, is a 10-ha natural Carolina bay wetland with no known historical contamination, located approximately 3 km from the D-area facility (Sharitz, 2003; Sharitz and Gibbons, 1982). All three sites are surrounded by mixed pine-hardwoods and open field habitats.

### 2.3. Experimental design and data collection

In February 2009, we created outdoor mesocosms using polyethylene cattle tanks (1.85 m diameter, 1480 L volume). We randomly assigned each mesocosm two treatment components, sediment type and female origin, yielding a 3×3 completely randomized design replicated four times ( $n = 36$  mesocosms). We filled mesocosms to a depth of 5 cm with 93 L of sediment from either the ash basin, ash plume wetland, or commercially available river sand. To simulate the low organic content of CCW, we used river sand as reference sediments instead of sediments

from a natural wetland, which are typically high in organic content (Sharitz and Gibbons, 1982). The initial conductivity of water containing CCWs can be quite high, thus to help desalinate the water, we filled each mesocosm with 500 L of well water, drained each mesocosm, and refilled them with 1000 L of well water. To provide nutrients to the system, we added 1.5 kg of dry leaf litter from an uncontaminated source to each mesocosm. We inoculated each mesocosm with 10 L of pond water collected from a nearby unpolluted wetland to establish phytoplankton populations.

At the beginning of the study we collected sediment and water samples from each mesocosm for elemental analysis. We collected sediments using a modified 60 mL syringe inserted with backpressure into the sediment at six locations, one at each of the cardinal directions near the mesocosm periphery and two near the center. We homogenized the six subsamples (~15 mL/sample) from each mesocosm into a single sample (90 mL) and froze them for subsequent elemental analyses. We collected water samples from 15 to 20 cm beneath the surface near the center of the mesocosm using a clean 250 mL plastic bottle. We then acidified water samples to 2% acidity with ultra high pure nitric acid until elemental analyses were performed. We also monitored environmental conditions in each mesocosm weekly by measuring water temperature, specific conductance, dissolved oxygen, pH, and oxidation-reduction potential (ORP) using a YSI 556 MPS handheld probe (YSI Environmental Inc., Yellow Springs, OH).

To quantify periphyton abundance, we suspended a 12.7 × 17.8 cm plastic plate in each mesocosm on 15 April 2009. On 19 June 2009, we removed the plates, scraped both sides with a razor blade, and rinsed the material with deionized water. We dried each periphyton sample at 60 °C and then combusted them at 500 °C for 24 h. We measured periphyton dry mass to the nearest 0.1 mg before and after combustion and used the difference between the two as our estimate of organic content in periphyton.

We collected adult *B. terrestris* on 28–29 March 2009 from breeding congregations at the ash basin, ash plume, and reference site wetlands. We transported toads to the lab where we measured snout-vent length (SVL, mm) and weighed (mg) each female. We injected males and females with human chorionic gonadotropin (males 100, females 250 IU), and placed the breeding pairs in plastic containers with well water. Thirty-six females (12 from each site) deposited eggs. After pairs bred, we released males at their location of capture. We kept females in plastic containers in the lab for an additional 48 h to allow them to void their gut contents. We then euthanized females by immersion in buffered tricaine methanesulfonate (MS-222) and freeze-dried the carcasses for subsequent elemental analysis.

We transferred a subsample of 120–150 eggs from each female to a 19 L floating plastic container with screen sides and placed it in the mesocosm to which that female had been assigned. Clutches were not mixed; this approach allowed us to track offspring of individual females and subsequently evaluate female trace element profiles relative to the success of their young. After hatching in the flow-through container, 100 free-swimming larvae were transferred from the embryonic container into the mesocosm for the remainder of the larval period. We monitored larvae in mesocosms for evidence of initiation of metamorphosis (Gosner stage 42), at which time we set two minnow traps in each mesocosm to capture metamorphosing individuals. We collected metamorphs and transferred them to the laboratory where they were kept at 23 °C on moist paper towels in plastic containers. After complete tail resorption (typically 2–4 days) we weighed and measured each metamorph. In addition, we assessed performance on a subset of 30 metamorphs from each mesocosm by measuring hopping speed (distance covered in the first 30 s of a trial; Walton, 1988) and endurance (total distance covered before no longer responding to ten consecutive proddings) on a 3 m linear track (Beck and Congdon, 2000). In mesocosms that produced fewer than 30 metamorphs we measured performance of all metamorphs from that mesocosm. After performance tests, we euthanized metamorphs, pooled them by mesocosm into samples large enough for elemental analysis (~800 mg wet weight), and freeze-dried them for elemental analyses.

### 2.4. Sample preparation and element analysis

We individually digested a subsample (~250 mg) of each homogenized female carcass, each sample of pooled metamorphs, and mesocosm sediment samples in 10 mL of trace metal grade nitric acid (70% HNO<sub>3</sub>) using microwave digestion (MarsExpress, CEM Corp., Matthews, NC). After HNO<sub>3</sub> digestion, we brought tissue samples to a final volume of 15 mL and sediment samples to a final volume of 50 mL with 18 MΩ deionized water. Acidified water samples from mesocosms were not digested or diluted prior to analysis. We determined elemental concentrations of As, Hg, Se, strontium (Sr), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), vanadium (V), and zinc (Zn) in tissue, sediment, and water samples using inductively coupled plasma mass spectrometry (Perkin Elmer, Norwalk, CT). For quality control, we included certified reference material in each analysis (TORT-2 and LUTS; National Research Council of Canada, Ottawa, Canada). Mean percent recoveries for elements in certified reference material ranged from 84 to 95%. Minimum detection limits in water samples were: As, 0.08; Cd, 0.12; Cr, 0.02; Cu, 0.08; Ni, 0.10; Pb, 0.07; Se, 0.88; Sr, 3.92; V, 0.15; Zn, 0.56 µg/L. Detection limits in sediment and tissue samples were: As, 0.40; Cd, 0.27; Cr, 0.36; Cu, 0.39; Hg, 0.23; Ni, 0.39; Pb, 1.31; Se, 1.70; Sr, 2.86; V, 0.17; Zn, 2.35 µg/kg.

## 2.5. Statistical analyses

We performed statistical analyses using SAS 9.1 (SAS Institute, Cary, NC). We examined the assumptions of homogeneity of variance and normality, and where deviations from these assumptions were found, we performed log or angular transformations to better meet the assumptions. For each mesocosm, we calculated percent survival to metamorphosis, average days to metamorphosis, mass, SVL, growth rate, hopping speed, endurance, and trace element composition of metamorphs. We used mesocosm-specific mean values as the unit of replication for all dependent variables in the models. In all statistical comparisons, we accepted statistical significance at  $\alpha = 0.05$  and we present data as mean  $\pm$  1 standard error (SE). When multivariate models were significant, we used individual ANOVAs followed by Bonferroni corrected pair-wise comparisons to identify differences among treatment groups.

To determine differences in the weekly mesocosm water chemistry parameters (e.g., temperature, dissolved oxygen), we used repeated measures MANOVA with sediment type as the independent variable and time as the repeated measure. We compared dry mass and organic content of periphyton among mesocosms using ANOVA with sediment type as the independent variable.

We compared trace element concentrations in metamorphs, sediments, and water among treatments (sediment type and female origin), using individual MANOVAs. To ensure that the trace element profiles of females from each study site were comparable across the three sediment treatments to which they were assigned, we first used individual MANOVAs for comparisons within each site. After confirming that trace element profiles of females within each site were equivalent, we then compared trace element concentrations in females among sites using MANOVA. For statistical comparisons, we assigned trace element concentrations below the instrument's detection limit (BDL) a value of half the minimum detection limit. However, if more than 10% of the samples from a treatment were BDL for a given element, we excluded that treatment from analysis of that element.

We compared percent survival to metamorphosis among mesocosm treatments using ANOVA. To better quantify the latent effect of previous maternal exposure on survival, we compared survival of larvae from the three female exposure groups that were raised only on reference sediments using contrast statements. To quantify the effects of environmental exposure on larval survival, we compared survival of larvae from each female group reared on their source sediment to survival when reared on the other two sediment types. Because survival within a mesocosm can influence density, which affects amphibian growth rate, size, and larval period (Travis, 1983; Wilbur, 1997) we initially compared SVL, mass, growth rate, and days to metamorphosis among treatments using MANCOVA with survival as the covariate (e.g., Parris and Semlitsch, 1998). However, because survival was influenced by the treatments, making it an inappropriate covariate (Cochran, 1957), and because survival was not significant in the multivariate or individual models, we removed survival from the model in the final analysis. Because size at metamorphosis can influence performance, we compared hopping speed and endurance of metamorphs among treatments using MANCOVA with SVL as the covariate.

## 3. Results

### 3.1. Water quality

Water quality changed over time and was dependent on sediment type (time: Pillai's trace = 0.99,  $F_{4, 318} = 131,635$ ,  $p < 0.001$ ; sediment:  $F_{2, 321} = 11.15$ ,  $p < 0.001$ ; time  $\times$  sediment: Pillai's trace = 0.92,  $F_{8, 638} = 68.06$ ,  $p < 0.001$ ). Briefly, water temperature,

pH, and specific conductance increased in all mesocosms as summer progressed. We observed differences among mesocosm treatments for specific conductance ( $F_{2, 321} = 840.98$ ,  $p < 0.001$ ) and dissolved oxygen ( $F_{2, 321} = 17.0$ ,  $p < 0.001$ ), but not temperature, pH, or ORP (temp:  $F_{2, 321} = 0.08$ ,  $p = 0.92$ ; pH:  $F_{2, 321} = 0.99$ ,  $p = 0.37$ ; ORP:  $F_{2, 321} = 1.29$ ,  $p = 0.28$ ). Overall mean specific conductance in reference ( $48.3 \pm 0.6$  uS/cm) mesocosms was significantly lower than ash plume ( $57.1 \pm 0.4$  uS/cm), which was significantly lower than ash basin mesocosms ( $93.2 \pm 0.8$  uS/cm). Similarly, overall mean dissolved oxygen was significantly lower in reference compared to ash plume and ash basin mesocosms (reference:  $62.2 \pm 0.18\%$ , ash plume:  $68.8 \pm 0.13\%$ , ash basin:  $75.3 \pm 0.12\%$ ).

Periphyton dry mass was similar between ash plume ( $62.1 \pm 3.9$  mg) and reference ( $52.5 \pm 2.9$  mg) mesocosms, but was significantly reduced in the ash basin ( $25.9 \pm 3.3$  mg) mesocosms (dry mass:  $F_{2, 33} = 5.33$ ,  $p = 0.01$ ). In contrast, percent organic material in periphyton was similar between ash plume ( $30.1 \pm 0.7\%$ ) and ash basin ( $23.2 \pm 0.4\%$ ) mesocosms, but was significantly lower in reference mesocosms ( $11.1 \pm 0.3\%$ ; percent organic:  $F_{2, 33} = 35.20$ ,  $p < 0.001$ ).

### 3.2. Elemental concentrations in females

The females within each site that were allocated among the three sediment treatments had similar whole body trace element concentrations (in all three cases:  $p > 0.77$ ). However, as expected, we detected significant differences in elemental concentrations in adult females among sites (Pillai's trace = 1.33,  $F_{18, 52} = 5.74$ ,  $p < 0.001$ ). Specifically, female concentrations of Cu, Pb, Se, and Sr were significantly different among sites (in all cases:  $F_{2, 33} > 5.45$ ,  $p < 0.01$ ; Table 1). Female Se and Sr concentrations were 2–4 times higher in ash basin and ash plume wetland females compared with reference females (Table 1). Copper concentrations in ash plume females were two times that of reference and ash basin females (Table 1). Although Pb concentrations were generally low in all females, concentrations in reference females were 46% higher than ash basin females, and also elevated compared to ash plume females, which had Pb levels below detection limits (Table 1).

### 3.3. Elemental concentrations in mesocosm sediment

Elemental concentrations differed significantly among the sediments placed in mesocosms (Pillai's trace = 1.33,  $F_{22,48} = 35.39$ ,  $p < 0.001$ ). Concentrations of As, Cr, Cu, Ni, and Zn in sediments were similar between the ash basin and ash plume wetland mesocosms, but were significantly higher compared to reference sediments (in all cases:  $F_{2, 33} > 45.96$ ,  $p < 0.001$ ; Table 2). Sediment

**Table 1**

Elemental composition of post-ovipositional female *Bufo terrestris* collected 28–29 March 2009 from reference and CCW-contaminated sites, and their recently metamorphosed offspring reared in mesocosms containing sediments from reference and contaminated sites. Data presented are means  $\pm$  1 SE. BDL = below detection limit.

Element	Females ( $\mu\text{g/g}$ )			Metamorphs ( $\mu\text{g/g}$ )		
	Reference	Ash plume	Ash basin	Reference	Ash plume	Ash basin
As	BDL	BDL	BDL	<sup>A</sup> 0.47 $\pm$ 0.07	<sup>B</sup> 3.37 $\pm$ 0.02	<sup>C</sup> 7.44 $\pm$ 0.12
Cd	BDL	BDL	BDL	BDL	BDL	BDL
Cr	<sup>A</sup> 3.95 $\pm$ 0.32	<sup>A</sup> 2.65 $\pm$ 0.11	<sup>A</sup> 4.24 $\pm$ 0.23	<sup>A</sup> 1.65 $\pm$ 0.02	<sup>A</sup> 1.40 $\pm$ 0.02	<sup>A</sup> 2.16 $\pm$ 0.08
Cu	<sup>A</sup> 12.79 $\pm$ 0.58	<sup>B</sup> 26.23 $\pm$ 1.29	<sup>A</sup> 12.97 $\pm$ 0.53	<sup>A</sup> 5.54 $\pm$ 0.01	<sup>A</sup> 6.18 $\pm$ 0.01	<sup>A</sup> 13.13 $\pm$ 0.03
Hg	<sup>A</sup> 0.24 $\pm$ 0.01	<sup>A</sup> 0.22 $\pm$ 0.01	<sup>A</sup> 0.3 $\pm$ 0.01	BDL	BDL	BDL
Ni	<sup>A</sup> 2.42 $\pm$ 0.16	<sup>A</sup> 2.16 $\pm$ 0.10	<sup>A</sup> 3.48 $\pm$ 0.17	<sup>A</sup> 0.43 $\pm$ 0.04	<sup>A</sup> 0.34 $\pm$ 0.02	<sup>A</sup> 0.46 $\pm$ 0.02
Pb	<sup>A</sup> 0.94 $\pm$ 0.04	BDL	<sup>B</sup> 0.51 $\pm$ 0.04	BDL	BDL	0.17 $\pm$ 0.08
Se	<sup>A</sup> 1.62 $\pm$ 0.03	<sup>B</sup> 4.1 $\pm$ 0.19	<sup>C</sup> 9.41 $\pm$ 1.27	<sup>A</sup> 2.44 $\pm$ 0.13	<sup>B</sup> 8.07 $\pm$ 0.06	<sup>C</sup> 60.75 $\pm$ 0.07
Sr	<sup>A</sup> 72.04 $\pm$ 1.42	<sup>B</sup> 200.07 $\pm$ 7.26	<sup>B</sup> 162.33 $\pm$ 12.71	<sup>A</sup> 18.62 $\pm$ 0.04	<sup>B</sup> 94.63 $\pm$ 0.02	<sup>C</sup> 274.86 $\pm$ 0.05
V	<sup>A</sup> 0.17 $\pm$ 0.01	<sup>A</sup> 0.27 $\pm$ 0.03	<sup>C</sup> 0.96 $\pm$ 0.11	<sup>A</sup> 0.41 $\pm$ 0.14	<sup>A</sup> 0.25 $\pm$ 0.04	<sup>B</sup> 2.15 $\pm$ 0.13
Zn	<sup>A</sup> 90.73 $\pm$ 1.35	<sup>A</sup> 92.04 $\pm$ 1.64	<sup>A</sup> 98.56 $\pm$ 2.24	<sup>A</sup> 240.54 $\pm$ 0.23	<sup>A</sup> 113.72 $\pm$ 0.09	<sup>B</sup> 377.47 $\pm$ 0.42

<sup>a</sup> Significant differences indicated by varying letters.

**Table 2**Elemental concentrations of water and sediment from mesocosms. Data presented are means<sup>a</sup> ± 1 SE. BDL = below detection limit.

Element	Water (µg/L)			Sediment (µg/g)		
	Reference	Ash plume	Ash basin	Reference <sup>b</sup>	Ash plume	Ash basin
As	<sup>A</sup> 0.52 ± 0.04	<sup>B</sup> 1.76 ± 0.06	<sup>C</sup> 6.98 ± 0.22	<sup>A</sup> 1.58 ± 0.06	<sup>B</sup> 50.05 ± 1.34	<sup>B</sup> 44.15 ± 1.08
Cd	BDL	BDL	BDL	<sup>A</sup> 0.93 ± 0.01	<sup>B</sup> 1.21 ± 0.01	<sup>C</sup> 1.11 ± 0.01
Cr	<sup>A</sup> 0.27 ± 0.01	<sup>A</sup> 0.37 ± 0.01	<sup>A</sup> 0.49 ± 0.04	<sup>A</sup> 1.13 ± 0.32	<sup>B</sup> 23.09 ± 0.37	<sup>B</sup> 30.58 ± 0.36
Cu	<sup>A</sup> 1.65 ± 0.09	<sup>B</sup> 0.99 ± 0.03	<sup>B</sup> 0.83 ± 0.04	<sup>A</sup> 0.12 ± 0.02	<sup>B</sup> 42.51 ± 0.49	<sup>B</sup> 69.63 ± 0.66
Hg	BDL	BDL	BDL	<sup>A</sup> 0.14 ± 0.01	<sup>B</sup> 0.32 ± 0.01	<sup>C</sup> 0.66 ± 0.01
Ni	BDL	<sup>A</sup> 0.65 ± 0.03	<sup>A</sup> 0.58 ± 0.01	<sup>A</sup> 0.18 ± 0.02	<sup>B</sup> 24.75 ± 0.38	<sup>B</sup> 39.94 ± 0.39
Pb	<sup>A</sup> 0.15 ± 0.01	<sup>A</sup> 0.1 ± 0.01	BDL	<sup>A</sup> 0.76 ± 0.03	<sup>B</sup> 15.87 ± 0.22	<sup>C</sup> 29.69 ± 0.20
Se	BDL	BDL	7.22 ± 0.27	<sup>A</sup> 0.45 ± 0.05	<sup>B</sup> 5.37 ± 0.15	<sup>C</sup> 21.18 ± 0.14
Sr	<sup>A</sup> 6.97 ± 0.13	<sup>B</sup> 22.01 ± 0.98	<sup>C</sup> 170.64 ± 3.29	<sup>A</sup> 1.24 ± 0.04	<sup>B</sup> 176.88 ± 3.46	<sup>C</sup> 285.32 ± 5.67
V	<sup>A</sup> 0.29 ± 0.01	<sup>B</sup> 0.54 ± 0.02	<sup>C</sup> 5.24 ± 0.12	<sup>A</sup> 3.41 ± 0.70	<sup>B</sup> 65.56 ± 0.80	<sup>C</sup> 118.69 ± 0.93
Zn	<sup>A</sup> 8.15 ± 0.46	<sup>A</sup> 8.55 ± 0.28	<sup>B</sup> 14.69 ± 0.62	<sup>A</sup> 1.56 ± 0.20	<sup>B</sup> 31.16 ± 0.47	<sup>B</sup> 39.64 ± 0.27

<sup>a</sup> Significant differences indicated by varying letters.<sup>b</sup> Reference sediments were uncontaminated river sand.

concentrations of Cd, Hg, Pb, Se, Sr, and V were significantly different among all three sediment types (in all cases:  $F_{2, 33} > 109.38$ ,  $p < 0.001$ ; Table 2), with levels in ash basin sediments being generally higher than ash plume sediments, which in turn exceeded reference sediment concentrations. Sediment Hg concentrations were 2–4 times higher in both contaminated treatments compared with the reference mesocosms. Selenium was 47 times higher in the ash basin sediments and 12 times higher in the ash plume compared with the reference sediments (Table 2). Concentrations of As, Cr, Pb, V, and Zn were 20–40 times higher, and Ni, Sr, and Cu concentrations were more than 136 times higher in ash basin and ash plume sediments compared with reference sediments (Table 2).

#### 3.4. Elemental concentrations in mesocosm water

Elemental concentrations in mesocosm water differed significantly among the sediment treatments (Pillai's trace = 1.92,  $F_{18, 52} = 67.81$ ,  $p < 0.001$ ). Concentrations that were BDL were excluded from statistical models (Table 2). Chromium concentrations in water samples were similar among sediment treatments. However, water concentrations of As, Sr, and V were 13–24 times higher in ash basin mesocosms and 2–3 times higher in ash plume mesocosms compared to reference mesocosms (Table 2). Water concentrations of Zn were nearly two times higher in ash basin mesocosms compared with ash plume and reference mesocosms (Table 2). Copper concentrations in mesocosm water were 40–50% higher in reference mesocosms compared with ash plume and ash basin (Table 2).

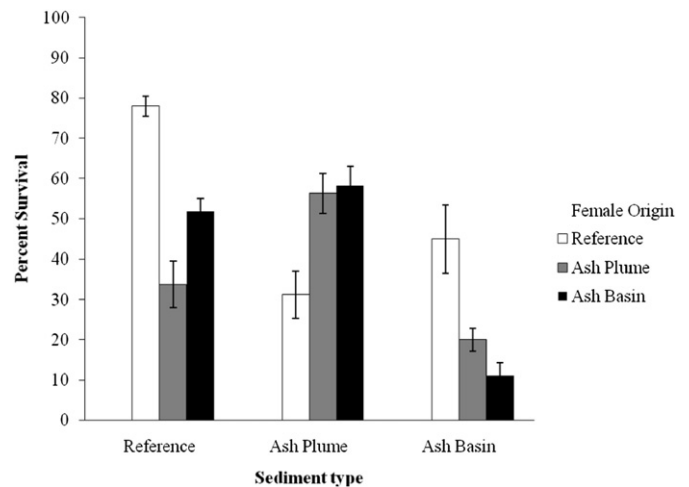
#### 3.5. Elemental concentrations in metamorphs

Elemental concentrations in metamorphs were dependent on sediment type (Pillai's trace = 1.77,  $F_{22, 34} = 12.06$ ,  $p < 0.001$ ), but not female origin (Pillai's trace = 0.87,  $F_{22, 34} = 1.19$ ,  $p = 0.32$ ) or their interaction (sediment\*female interaction; Pillai's trace = 1.68,  $F_{44, 76} = 1.26$ ,  $p = 0.19$ ). Specifically, concentrations of As, Se, Sr, V, and Zn in recent metamorphs differed significantly among sediment types (in all cases:  $F_{2, 26} > 7.12$ ,  $p < 0.003$ ; Table 1). Concentrations of As, Se, and Sr in metamorphs were 15–25 times higher in ash basin and 3–5 times higher in ash plume than reference (Table 1). Vanadium concentrations in ash basin metamorphs were 5 times higher than reference, and Zn concentrations were 57% higher in ash basin compared to reference metamorphs (Table 1). However, V and Zn concentrations were similar between ash plume and reference metamorphs. Although Cu concentrations in metamorphs reared in ash basin mesocosms were twice as high

as those reared in ash plume and reference mesocosms, the differences were not statistically different ( $F_{2, 26} = 2.52$ ,  $p = 0.10$ ; Table 1).

#### 3.6. Effects on larvae

*Bufo terrestris* larvae began to metamorphose on 4 May 2009 and continued until 22 June (36–77 day larval period). We observed a significant interaction between sediment type and female origin on survival to metamorphosis (sediment\*female:  $F_{4, 27} = 2.90$ ,  $p = 0.04$ ; sediment:  $F_{2, 27} = 4.78$ ,  $p = 0.02$ , female origin:  $F_{2, 27} = 1.18$ ,  $p = 0.32$ ). Survival to metamorphosis was dramatically reduced in larvae of ash basin and ash plume females compared to those of reference females, but the effect was dependent on sediment type during the larval period (Fig. 1). Survival to metamorphosis was highest in reference larvae reared on reference sediments and lowest in ash basin larvae reared on ash basin sediments. Survival of larvae from ash basin females reared on reference sediments was reduced by 34%, compared to reference larvae reared on reference sediments ( $F_{1, 27} = 2.29$ ,  $p = 0.14$ ; Fig. 1). Similarly, survival of larvae from ash plume females reared on reference sediments was reduced by 57% compared to reference larvae reared on reference sediments ( $F_{1, 27} = 6.23$ ,  $p = 0.02$ ; Fig. 1). When reference larvae were reared on ash basin and ash plume



**Fig. 1.** Percentage of *Bufo terrestris* larvae that survived to metamorphosis after exposure to sediments from either a reference site (reference), a natural wetland contaminated with coal combustions wastes (ash plume), or a coal ash settling basin (ash basin) in experimental mesocosms. Data are presented as means ± 1 SE.

sediments, survival was reduced by 42–60% compared to reference larvae reared on reference sediments (ash basin:  $F_{1, 27} = 3.50$ ,  $p = 0.07$ , ash plume:  $F_{1, 27} = 6.43$ ,  $p = 0.02$ ; Fig. 1). Additionally, survival of larvae with mothers originating from the ash plume reared on ash plume sediments was similar when reared on reference sediments ( $F_{1, 27} = 1.73$ ,  $p = 0.20$ ; Fig. 1), but was reduced by 65% when reared on ash basin sediments ( $F_{1, 27} = 6.44$ ,  $p = 0.02$ ; Fig. 1). Survival of larvae from ash basin females increased by 427% when reared on ash plume sediments ( $F_{1, 27} = 6.47$ ,  $p = 0.02$ ; Fig. 1), and by 373% when reared on reference sediments ( $F_{1, 27} = 4.39$ ,  $p = 0.05$ ; Fig. 1).

Larval exposure to contaminated sediments had sublethal effects on metamorphs (sediment: Pillai's trace = 0.78,  $F_{8, 46} = 3.86$ ,  $p = 0.001$ ; female origin: Pillai's trace = 0.35,  $F_{8, 46} = 1.25$ ,  $p = 0.29$ ; sediment\*female: Pillai's trace = 0.41,  $F_{16, 104} = 0.74$ ,  $p = 0.75$ ). Larval period length differed significantly among mesocosm sediment types ( $F_{2, 26} = 20.13$ ,  $p < 0.001$ , Fig. 2). After accounting for female origin, larval period duration on ash basin sediments was 27–40% longer than on the other two sediment types (Fig. 2). Mass of metamorphs reared on reference and ash plume sediments was significantly greater (30%) than metamorphs reared on ash basin sediments (female:  $F_{2, 26} = 0.05$ ,  $p = 0.96$ , sediment:  $F_{2, 26} = 5.84$ ,  $p = 0.01$ , sediment\*female:  $F_{4, 26} = 0.73$ ,  $p = 0.58$ ; Fig. 3). However, metamorph body length was statistically similar among treatments (female:  $F_{2, 26} = 0.14$ ,  $p = 0.87$ , sediment:  $F_{2, 26} = 3.0$ ,  $p = 0.07$ , sediment\*female:  $F_{4, 26} = 0.54$ ,  $p = 0.71$ ). After accounting for female origin, growth rate of larvae reared on ash basin sediments was significantly reduced by an average of 0.4 mg/day compared to those reared on reference and ash plume sediments (female:  $F_{2, 26} = 0.83$ ,  $p = 0.45$ ; sediment:  $F_{2, 26} = 13.96$ ,  $p < 0.001$ ; sediment\*female:  $F_{4, 26} = 0.84$ ,  $p = 0.51$ ; Fig. 4).

After accounting for body length, performance of metamorphs was similar among treatments (sediment: Pillai's trace = 0.33,  $F_{4, 50} = 2.45$ ,  $p = 0.06$ ; female: Pillai's trace = 0.02,  $F_{4, 50} = 0.13$ ,  $p = 0.97$ ; sediment\*female: Pillai's trace = 0.36,  $F_{8, 50} = 1.36$ ,  $p = 0.24$ ; covariate (SVL): Pillai's trace = 0.71,  $F_{2, 24} = 29.68$ ,  $p < 0.001$ ). Although not statistically significant, overall average hopping speed of metamorphs exposed to ash plume sediments was 15–17% greater than those exposed to ash basin and reference sediments (sediment:  $F_{2, 25} = 3.48$ ,  $p = 0.05$ ; female:  $F_{2, 25} = 0.05$ ,  $p = 0.95$ ; sediment\*female:  $F_{4, 25} = 1.90$ ,  $p = 0.14$ ; covariate (SVL):  $F_{1, 25} = 52.67$ ,  $p < 0.001$ ; Fig. 5). Similarly, endurance of metamorphs

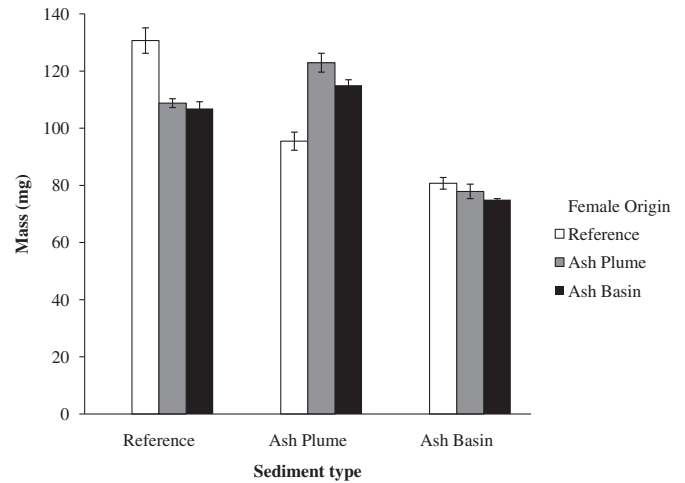


Fig. 3. Mass (mg) of recently metamorphosed *Bufo terrestris* exposed in experimental mesocosms to sediments from either a reference site (reference), a natural wetland contaminated with coal combustions wastes (ash plume), or a coal ash settling basin (ash basin). Data are presented as means  $\pm$  1 SE.

exposed to ash plume sediments was 18–22% greater than those exposed to the other sediments (sediment:  $F_{2, 25} = 4.03$ ,  $p = 0.05$ ; female:  $F_{2, 25} = 0.095$ ,  $p = 0.91$ ; sediment\*female:  $F_{4, 25} = 1.62$ ,  $p = 0.20$ ; covariate (SVL):  $F_{1, 25} = 57.77$ ,  $p < 0.001$ ; Fig. 6).

#### 4. Discussion

Our study is the first to document a latent effect of previous maternal exposure to CCW in amphibians. Larval exposure to CCW contaminated sediments reduced survival to metamorphosis, but also had sublethal effects on survivors. Moreover, previous maternal exposure and larval exposure to CCWs interacted to substantially reduce survival to metamorphosis. Taken together, our results suggest that CCW disposal basins may be ecological traps that contribute to amphibian population declines.

Comparison of concentrations of As, Se, Sr, V, and Zn in metamorphs from our factorial experiment revealed that bioaccumulation during early development was primarily attributable to larvae

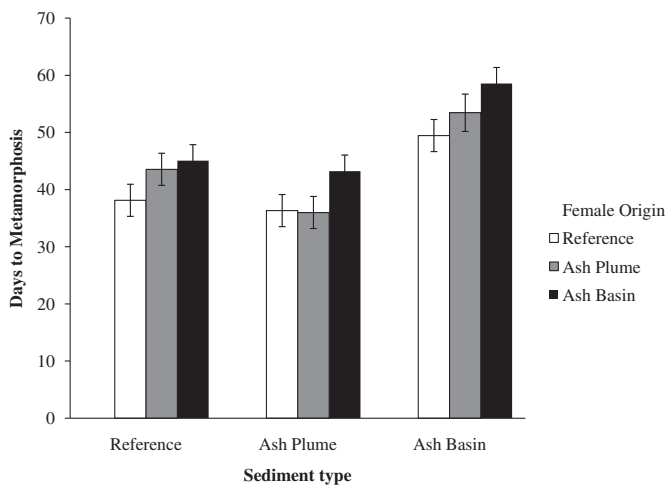


Fig. 2. Number of days to metamorphosis of *Bufo terrestris* larvae exposed in experimental mesocosms to sediments from either a reference site (reference), a natural wetland contaminated with coal combustions wastes (ash plume), or a coal ash settling basin (ash basin). Data are presented as means  $\pm$  1 SE.

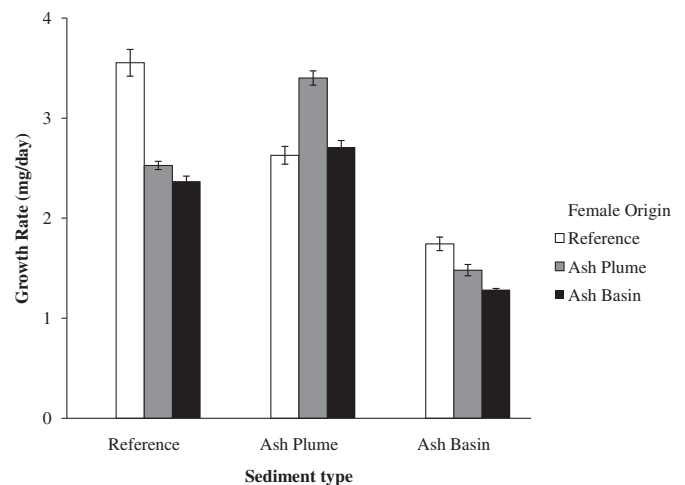
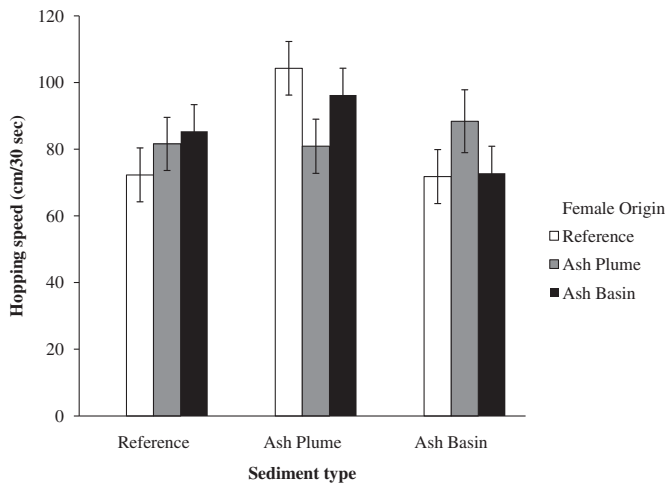


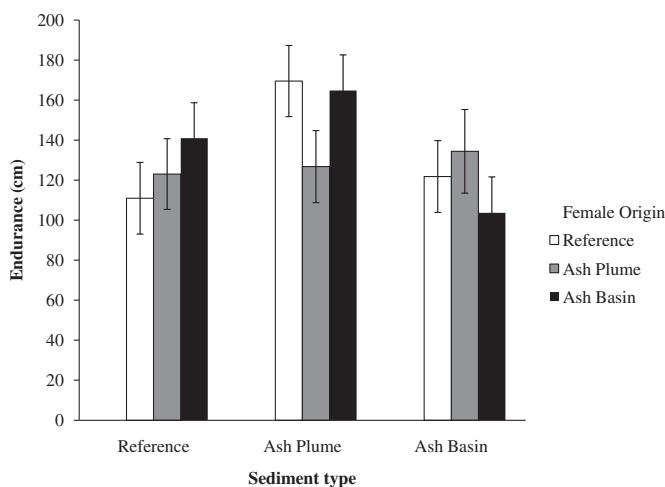
Fig. 4. Growth rate (mg/day) of *Bufo terrestris* larvae exposed in experimental mesocosms to sediments from either a reference site (reference), a natural wetland contaminated with coal combustions wastes (ash plume), or a coal ash settling basin (ash basin). Data are presented as means  $\pm$  1 SE.



**Fig. 5.** Hopping speed (cm/30 s) of recently metamorphosed *Bufo terrestris* after larval exposure to sediments from either a reference site (reference), a natural wetland contaminated with coal combustions wastes (ash plume), or a coal ash settling basin (ash basin) in experimental mesocosms. Data are presented as LS means (corrected for SVL)  $\pm$  1 SE.

grazing contaminated sediments and not from maternal exposure. Tissue concentrations in our study were consistent with previous mesocosm experiments and other research conducted at our field sites. For instance, mole salamanders (*Ambystoma talpoideum*) reared to metamorphosis in mesocosms containing sediments from the ash basin had mean Se and Sr concentrations of 35 and 250  $\mu\text{g/g}$  (Roe et al., 2006), while male *B. terrestris* had mean concentrations of 17 and 387  $\mu\text{g/g}$ , respectively (Hopkins et al., 1998). Adult *B. terrestris* and southern leopard frogs (*Rana (Lithobates) sphenoccephala*) collected from the ash plume wetland had Se and Sr concentrations of 7 and 325  $\mu\text{g/g}$  (Roe et al., 2005), and *B. terrestris* metamorphs had mean concentrations of 46 and 225  $\mu\text{g/g}$ , respectively (Roe et al., 2005). Collectively these studies demonstrate the propensity of amphibians to accumulate trace elements as a result of exposure to CCW, making subsequent transfer of contaminants to terrestrial food webs likely (Snodgrass et al., 2003; Unrine et al., 2007).

We documented a latent effect of previous maternal exposure to CCW on larval survival to metamorphosis. Specifically, when



**Fig. 6.** Endurance (cm) of recently metamorphosed *Bufo terrestris* after larval exposure to sediments from either a reference site (reference), a natural wetland contaminated with coal combustions wastes (ash plume), or a coal ash settling basin (ash basin) in experimental mesocosms. Data are presented as LS means (corrected for SVL)  $\pm$  1 SE.

*B. terrestris* larvae of ash basin and ash plume females were reared on reference sediments, maternal exposure to contaminants reduced survival to metamorphosis by 34–57% compared to larvae from reference females raised on reference sediments. In other vertebrate groups, maternal exposure to contaminants can also reduce post-hatching survival of offspring. For example, maternal exposure of snapping turtles (*Chelydra serpentina*) to polychlorinated biphenyls (PCBs) reduced survival of their offspring during the first 14 months of life (Eisenreich et al., 2009). Our findings highlight the importance of tracking the effects of maternal exposure to contaminants beyond embryonic development because important responses may manifest later in ontogeny.

Larval exposure to contaminated sediments also reduced survival to metamorphosis. In our study, survival was highest in reference larvae reared on reference sediments, but exposure of reference larvae to ash basin and ash plume sediments reduced their survival by 42 and 60%, respectively. Larvae of ash plume females reared on ash basin sediments experienced a 65% reduction in survival to metamorphosis compared to those reared on ash plume sediments. In contrast, larvae of ash basin females reared on reference or ash plume sediments had improved survival compared to those reared on ash basin sediments, demonstrating that the combined effect of maternal and environmental exposure to trace elements was much greater than maternal exposure alone. Together, our results suggest that female toads from uncontaminated sites that are attracted to CCW contaminated wetlands to breed can experience a reduction in reproductive success. In contrast, females from ash basins that migrate to less contaminated wetlands to breed may experience improved reproductive success relative to their success when breeding in ash basins.

Our study demonstrated an interaction between previous maternal exposure and subsequent larval environmental exposure to CCW contaminated sediments, resulting in very low recruitment. The combination of maternal exposure and larval exposure to fresh CCW reduced survival to metamorphosis by 85% relative to reference conditions. In a laboratory study, Bergeron et al. (2011b) found that in *B. americanus* previous maternal Hg exposure and larval dietary Hg exposure acted synergistically to reduce survival by 50% compared to reference larvae. In combination, these two studies suggest that the interaction of these two common exposure routes warrant further attention because such dramatic reductions in recruitment could ultimately contribute to amphibian population declines. In addition, because of their high abundance (Burton and Likens, 1975a), diverse roles in food webs (consumers, predators, and prey), and importance for the transfer of energy and nutrients through food webs and across the landscape (Beard et al., 2002; Burton and Likens, 1975b; Gibbons et al., 2006; Ranvestel et al., 2004; Regester et al., 2006; Wyman, 1998), reductions in amphibian recruitment in populations associated with contaminated wetlands may have important ecological ramifications (Rowe et al., 2001).

We found sublethal effects (e.g., size and time to metamorphosis) on larvae exposed to sediments with the highest levels of contaminants. We found that ash basin sediments prolonged the larval period by 11–15 days compared to reference and ash plume sediments, respectively. Considering the hot and dry conditions that cause annual pond drying in the southeastern U.S., a two week extension of larval development could limit the number of individuals reaching metamorphosis and reduce the frequency of years with successful recruitment (Pechmann et al., 1989). For example, previous work has shown that exposure to CCW resulted in large numbers of *A. talpoideum* extending their larval period and failing to metamorphose before ponds completely dried (Roe et al., 2006). In our study, larvae reared in mesocosms containing ash basin sediments also grew more slowly and were 30% smaller at

metamorphosis than those reared in ash plume or reference mesocosms. Such a considerable reduction in size may negatively influence survival and future reproduction of individuals metamorphosing from ash basins (Berven and Gill, 1983). For instance, Semlitsch et al. (1988) found that size at metamorphosis can influence reproductive success of *A. talpoideum*, although this pattern has not been confirmed for *B. terrestris* (Beck and Congdon, 1999, 2000). Taken together, the sublethal effects on growth and development may further reduce amphibian recruitment from sites contaminated with CCWs under unpredictable environmental conditions (Rowe and Hopkins, 2003).

The reduced size and extended larval periods we observed in larvae reared on ash basin sediments may partially be attributable to lower resource abundance (periphyton biomass) in ash basin mesocosms compared to reference and ash plume mesocosms. The decrease in food resources (i.e., periphyton biomass) we observed in the ash basin mesocosms over the duration of the experiment is consistent with our observations of slower growth, reduced mass at metamorphosis, and prolonged development of larval toads. Similarly, higher resource abundance in ash plume mesocosms may explain the increased growth rate and size of larvae reared on ash plume sediments. Two recent studies suggest that resource abundance may influence the effects of exposure to contaminants on anuran larvae. For instance, under low resource abundance conditions, previous maternal Hg exposure and larval dietary Hg exposure each negatively affected offspring health, but together acted synergistically to cause high mortality (Bergeron et al., 2011b). However, under higher resource abundance conditions, maternal Hg exposure negatively affected offspring, but no effect of dietary exposure was found (Todd et al., 2011).

Our use of river sand low in organic content as reference sediments may have resulted in conservative estimates of the negative effects exposure to contaminants can have on larval growth and size. In our study, periphyton from ash basin and ash plume mesocosms contained greater proportions of organic content than reference mesocosms. Thus, the growth rate and size of larvae in reference mesocosms were likely lower than larvae from natural uncontaminated wetlands where resources are considerably more abundant due to increased nutrient availability. Thus, differences in growth and size between reference and ash plume larvae would likely be greater (Figs. 2–4) if natural wetland sediments were used.

We predicted reduced bioavailability of contaminants in aged ash would ameliorate the effects of CCW exposure on larval amphibians. After 35 years of natural succession, the ash plume area has become revegetated. Elemental concentrations in the sediments were presumably reduced by plant uptake and sequestration, flood events that transported dissolved elements offsite, and other biogeochemical processes that lead to attenuation and/or downward migration of elements in the sediments (Sandhu et al., 1993). Indeed, most trace element concentrations in sediments were lower in ash plume mesocosms compared to ash basin mesocosms. As a result, many of the trace element concentrations bioaccumulated by metamorphs reared on ash plume sediments were lower than those of metamorphs raised on ash basin sediments. In addition, larval period length, mass, and growth rate were negatively influenced by ash basin sediments but not by ash plume sediments. Moreover, survival of larvae from ash basin and ash plume females was similar when reared on reference and ash plume sediments, but was significantly reduced when reared on ash basin sediments. Still, survival to metamorphosis was significantly reduced in reference larvae reared in the ash plume mesocosms compared to reference conditions, suggesting that sediment aging alone does not entirely alleviate the health risks associated with CCW.

## 5. Conclusions

Our study demonstrated that larval exposure to CCW contaminated sediments had sublethal and lethal effects, and previous maternal exposure to CCWs significantly reduced survival to metamorphosis. The maternal exposure effects on offspring health we observed are particularly important because they occurred during larval development, long after the embryonic period which has been the predominant focus of most studies on maternal transfer. Our findings suggest that longitudinal studies are needed to fully appreciate the influence of maternal transfer on reproductive success and offspring viability. Importantly, we also demonstrated that the interaction of previous maternal exposure and subsequent larval exposure reduced survival to metamorphosis up to 85% relative to reference conditions. Such a dramatic reduction in recruitment may ultimately result in local amphibian population declines, and suggests that CCW contaminated basins and wetlands may be ecological traps for amphibians.

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## References

- Beard, K.H., Vogt, K.A., Kulmatiski, A., 2002. Top-down effects of a terrestrial frog on forest nutrient dynamics. *Oecologia* 133, 583–593.
- Beck, C.W., Congdon, J.D., 1999. Effects of individual variation in age and size at metamorphosis on growth and survivorship of southern toad (*Bufo terrestris*) metamorphs. *Canadian Journal of Zoology* 77, 944–951.
- Beck, C.W., Congdon, J.D., 2000. Effects of age and size at metamorphosis on performance and metabolic rates of southern toad, *Bufo terrestris*, metamorphs. *Functional Ecology* 14, 32–38.
- Bergeron, C.M., Bodinof, C.M., Unrine, J.M., Hopkins, W.A., 2010. Bioaccumulation and maternal transfer of mercury and selenium in amphibians. *Environmental Toxicology and Chemistry* 29, 989–997.
- Bergeron, C.M., Hopkins, W.A., Bodinof, C.M., Budischak, S.A., Wada, H., Unrine, J.M., 2011a. Counterbalancing effects of maternal mercury exposure during different stages of early ontogeny. *Science of the Total Environment* 409, 4746–4752.
- Bergeron, C.M., Hopkins, W.A., Todd, B.D., Hepner, M.J., Unrine, J.M., 2011b. Interactive effects of maternal and dietary mercury exposure have latent and lethal consequences for amphibian larvae. *Environmental Science and Technology* 45, 3781–3787.
- Berven, K.A., Gill, D.E., 1983. Interpreting geographic variation in life-history traits. *American Zoologist* 23, 85–97.
- Berven, K.A., 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* 71, 1599–1608.
- Bogert, C.M., 1947. A field study of homing in the Carolina toad. *American Museum Novitates* 1355, 24.
- Breden, F., 1987. The effect of post-metamorphic dispersal on the population genetic structure of Fowler's toad, *Bufo woodhousei fowleri*. *Copeia* 2, 386–395.
- Burton, T.M., Likens, G.E., 1975a. Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. *Copeia* 3, 541–546.
- Burton, T.M., Likens, G.E., 1975b. Energy flow and nutrient cycling in salamander populations in the Hubbard Brook experimental forest, New Hampshire. *Ecology* 56, 1068–1080.
- Cochran, W.G., 1957. Analysis of covariance: its nature and uses. *Biometrics* 13, 261–281.
- Collins, J.P., Storfer, A., 2003. Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* 9, 89–98.

- Eisenreich, K.M., Kelly, S.M., Rowe, C.L., 2009. Latent mortality of juvenile snapping turtles from the upper Hudson River, New York, exposed maternally and via the diet to polychlorinated biphenyls (PCBs). *Environmental Science and Technology* 43, 6052–6057.
- Gibbons, J.W., Winne, C.T., Scott, D.E., Willson, J.D., Glaudas, X., Andrews, K.M., Todd, B.D., Fedewa, L.A., Wilkinson, L., Tsaliagos, R.N., Harper, S.J., Greene, J.L., Tuberville, T.D., Metts, B.S., Dorcas, M.E., Nestor, J.P., Young, C.A., Akre, T., Reed, R.N., Buhlmann, K.A., Norman, J., Croshaw, D.A., Hagen, C., Rothermel, B.B., 2006. Remarkable amphibian biomass and abundance in an isolated wetland: implications for wetland conservation. *Conservation Biology* 20, 1457–1465.
- Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T.M., Butchart, S.H.M., Carpenter, K.E., 2010. The impact of conservation on the status of the World's vertebrates. *Science* 330, 1503–1509.
- Hopkins, W.A., Mendonca, M.T., Rowe, C.L., 1998. Elevated trace element concentrations in southern toads, *Bufo terrestris*, exposed to coal combustion waste. *Archives of Environmental Contamination and Toxicology* 35, 325–329.
- Hopkins, W.A., Congdon, J.D., Ray, J.K., 2000. Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal-burning power plant. *Environmental Toxicology Chemistry* 19, 862–868.
- Hopkins, W.A., Staub, B.P., Snodgrass, J.W., Taylor, B.E., DeBiase, A.E., Roe, J.H., Jackson, B.P., Congdon, J.D., 2004. Responses of benthic fish exposed to contaminants in outdoor microcosms – examining the ecological relevance of previous laboratory toxicity tests. *Aquatic Toxicology* 68, 1–12.
- Hopkins, W.A., DuRant, S.E., Staub, B.P., Rowe, C.L., Jackson, B.P., 2006. Reproduction, embryonic development, and maternal transfer of contaminants in the amphibian *Gastrophryne carolinensis*. *Environmental Health Perspectives* 114, 661–666.
- Jensen, J.B., 2008. Southern toad *Bufo (Anaxyrus) terrestris*. In: Jensen, J.B., Camp, C.D., Gibbons, J.W., Elliott, M.J. (Eds.), *Amphibians and Reptiles of Georgia*. University of Georgia Press, Athens, GA, pp. 44–46.
- Kadokami, K., Takeishi, M., Kuramoto, M., Ono, Y., 2004. Maternal transfer of organochlorine pesticides, polychlorinated dibenzo-p-dioxins, dibenzofurans, and coplanar polychlorinated biphenyls in frogs to their eggs. *Chemosphere* 57, 383–389.
- Kotyzova, D., Sundeman, F.W., 1998. Maternal exposure to Cd (II) causes malformations of *Xenopus laevis* embryos. *Annals Clinical Laboratory Science* 28, 224–235.
- Linder, G., Grillitsch, B., 2000. Ecotoxicology of metals. In: Sparling, D.W., Linder, G., Bishop, C.A. (Eds.), *Ecotoxicology of Amphibians and Reptiles*. SETAC Press, Pensacola, FL, pp. 325–459.
- Parris, M.J., Semlitsch, R.D., 1998. Assymmetric competition in larval amphibian communities: conservation implications for the northern crawfish frog, *Rana areolata circulosa*. *Oecologia* 116, 219–226.
- Pechmann, J.H.K., Scott, D.E., Gibbons, J.W., Semlitsch, R.D., 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. *Wetlands Ecology and Management* 1, 3–11.
- Raimondo, S.M., Rowe, C.L., 1998. Exposure to coal ash impacts swimming performance and predator avoidance in larval bullfrogs (*Rana catesbeiana*). *Journal of Herpetology* 32, 289–292.
- Ranvestel, A.W., Lips, K.R., Pringle, C.W., Whiles, M.R., Bixby, R.J., 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. *Freshwater Biology* 49, 274–285.
- Regester, K.J., Lips, K.R., Whiles, M.R., 2006. Energy flow and subsidies associated with the complex life cycle of ambystomatid salamanders in ponds and adjacent forest in southern Illinois. *Oecologia* 147, 303–314.
- Roe, J.H., Hopkins, W.A., Jackson, B.P., 2005. Species- and stage-specific differences in trace element tissue concentrations in amphibians: implications for the disposal of coal-combustion wastes. *Environmental Pollution* 136, 353–363.
- Roe, J.H., Hopkins, W.A., DuRant, S.E., Unrine, J.M., 2006. Effects of competition and coal-combustion wastes on recruitment and life history characteristics of salamanders in temporary wetlands. *Aquatic Toxicology* 79, 176–184.
- Rowe, C.L., Hopkins, W.A., 2003. 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: Linder, G., Krest, S., Sparling, D. (Eds.), *Proceedings from the Global Decline of Amphibian Populations: An Integrated Analysis of Multiple Stressor Effect*. Society of Environmental Toxicology and Chemistry, Racine, Wisconsin, pp. 271–282.
- Rowe, C.L., Kinney, O., Fiori, A., Congdon, J.D., 1996. Oral deformities in tadpoles (*Rana catesbeiana*) associated with coal ash deposition: effects on grazing ability and growth. *Freshwater Biology* 36, 723–730.
- Rowe, C.L., Hopkins, W.A., Coffman, V.R., 2001. 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. *Archives Environmental Contamination and Toxicology* 40, 399–405.
- Rowe, C.L., Hopkins, W.A., Congdon, J.D., 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: a review. *Environmental Monitoring and Assessment* 80, 207–276.
- Sandhu, S.S., Mills, G.L., Sajwan, K.S., 1993. Chapter 8. Leachability of Ni, Cd, Cr, and As from coal ash impoundments of different ages on the Savannah River site. In: Keefer, R.F., Sajwan, K.S. (Eds.), *Trace Elements in Coal and Coal Combustions Residues*. Lewis Publishers Inc, Boca Raton, FL, pp. 165–182.
- Semlitsch, R.D., Scott, D.E., Pechmann, J.H.K., 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology* 69, 184–192.
- Sharitz, R.R., Gibbons, J.W., 1982. *The Ecology of Southeastern Shrub Bogs (Pocosins) and Carolina Bays: A Community Profile*. U.S. Fish and Wildlife Service, Division of Biological Services.
- Sharitz, R.R., 2003. Carolina bay wetlands: unique habitats of the Southeastern United States. *Wetlands* 23, 550–562.
- Snodgrass, J.W., Hopkins, W.A., 2005. Influence of larval period on responses of overwintering green frog (*Rana clamitans*) larvae exposed to contaminated sediments. *Environmental Toxicology and Chemistry* 24, 1508–1514.
- Snodgrass, J.W., Hopkins, W.A., Roe, J.H., 2003. Relationships among developmental stage, metamorphic timing, and concentrations of elements in bullfrogs (*Rana catesbeiana*). *Environmental Toxicology and Chemistry* 22, 1597–1604.
- Snodgrass, J.W., Hopkins, W.A., Broughton, J., Gwinn, D., Baionno, J.A., Burger, J., 2004. Species-specific responses of developing anurans to coal combustion wastes. *Aquatic Toxicology* 66, 171–182.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786.
- Todd, B.D., Bergeron, C.M., Hepner, M.J., Hopkins, W.A., 2011. Aquatic and terrestrial stressors in amphibians: a test of the double jeopardy hypothesis based on maternally and trophically derived contaminants. *Environmental Toxicology and Chemistry* 30, 2277–2284.
- Travis, J., 1983. Variation in growth and survival of *Hyla gratiosa* larvae in experimental enclosures. *Copeia* 1983, 232–237.
- U.S. Department of Energy, 2005. Energy Information Administration. EIA-767 Data Files: Annual Steam-Electric Plant Operation and Design Report. Washington, DC.
- Unrine, J.M., Hopkins, W.A., Romanek, C.S., 2007. Bioaccumulation of trace elements in omnivorous amphibian larvae: implications for amphibian health and contaminant transport. *Environmental Pollution* 149, 182–192.
- Walton, M., 1988. Relationships among metabolic, locomotory, and field measures of organismal performance in the Fowler's toad (*Bufo woodhousii fowleri*). *Physiological Zoology* 61, 107–118.
- Wilbur, H.M., 1997. Experimental ecology of food webs: complex systems in temporary ponds. *Ecology* 68, 1437–1452.
- Wyman, R.L., 1998. Experimental assessment of salamanders as predators of detrital food webs: effects on invertebrates, decomposition and the carbon cycle. *Biodiversity and Conservation* 7, 641–650.