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Influence of metal(loid) bioaccumulation and maternal transfer on embryo-larval development in fish exposed to a major coal ash spill☆

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ABSTRACT

In December 2008, an earthen retaining wall at the Tennessee Valley Authority (TVA) Kingston Fossil Fuel Plant failed and released 4.1 million m^3 of coal ash to rivers flowing into Watts Bar Reservoir in east Tennessee, United States (U.S.). As part of a comprehensive effort to evaluate the risks to aquatic resources from this spill – the largest in U.S. history – we compared bioaccumulation and maternal transfer of selenium (Se), arsenic (As), and mercury (Hg) in adult redear sunfish (*Lepomis macrolophus*), collected two years after the spill from both coal-ash exposed and non-exposed areas of the Emory and Clinch Rivers, with the success of embryo-larval development in their offspring. Whole body and ovary concentrations of Se in female sunfish at three study sites downstream of the spill were significantly elevated (site means = 4.9–5.3 and 6.7–9.0 mg/kg d.w. whole body and ovary concentrations, respectively) compared with concentrations in fish from reference sites upstream of the spill site (2.2–3.2 mg/kg d.w. for whole bodies and 3.6–4.8 mg/kg d.w. for ovaries). However, Se concentrations in coal ash-exposed areas remain below proposed U.S. Environmental Protection Agency (USEPA) criteria for the protection of aquatic life. Site-to-site variation in fish concentrations of As and Hg were not well-correlated with ash-exposure, reflecting the multiple sources of these metal(loid)s in the affected watersheds. In 7-day laboratory tests of embryos and larvae derived from in vitro crosses of eggs and sperm from these field-collected sunfish, fertilization success, hatching success, embryo-larval survival, and incidences of developmental abnormalities did not differ significantly between ash-exposed and non-exposed fish. Furthermore, these developmental endpoints were not correlated with whole body or ovary concentrations of Se, As, or Hg in the maternal fish, or with fish size, ovary weight, or gonadal-somatic indices. Results from this and related studies associated with the Kingston coal ash spill are consistent with proposed USEPA fish-based water quality criteria for Se, and to date continue to suggest that long-term exposures to sediment containing residual ash may not present a significant chronic risk to fish populations exposed to this major coal ash release.

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1. Introduction

Coal combustion waste from coal-fired electrical power generating plants – often referred to as coal ash – constitutes one of the largest waste streams produced in the United States (U.S.). Approximately 40% of this ash is currently re-used for beneficial purposes, including incorporation into a variety of building materials. The remainder is typically stored or disposed in surface impoundments or landfills, many of which are unlined. A complex mixture of combustion byproducts, coal ash is enriched in potentially toxic substances such as selenium (Se), arsenic (As), and mercury (Hg) that can bioaccumulate or biomagnify in aquatic organisms and wildlife (Yudovich and Ketris, 2005a,b; Yudovich and Ketris, 2006; Reash, 2012; Otter et al., 2012). Releases of coal ash or ash leachates to the environment can pose significant risks

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to the health of aquatic organisms and terrestrial wildlife, as well as to human health (Baumann and Gillespie, 1986; Sorensen, 1988; Birge and Westerman, 1996; Rowe et al., 2002; Dorman et al., 2010; Lemly and Skorupa, 2012; Ruhl et al., 2012; Mayfield et al., 2013; Rice et al., 2014).

Environmental releases of coal ash or ash constituents can occur through a variety of routes, including: (1) air-borne contamination from open surface storage impoundments; (2) wastewater discharges from coal-fired power plants; (3) runoff from ash stilling ponds or surface impoundments; (4) leaching from landfills, impoundments, or ash ponds to surface waters or groundwater; and (5) the failure of retaining structures (USEPA (U.S. Environmental Protection Agency), 2014). An extreme example of the latter occurred in December 2008 with the collapse of an earthen retention wall at the Tennessee Valley Authority's (TVA) Kingston Fossil Fuel Plant in east Tennessee, U.S. The resulting spill – the largest in U.S. history – released approximately 4.1 million m^3 of wet coal fly ash and bottom ash to surrounding land and adjacent waterways, including the Emory, Clinch, and Tennessee Rivers, that flow into the TVA Watts Bar Reservoir (ARCADIS, 2012; Walls et al., 2015).

The environmental risks associated with chronic releases of coal ash or coal ash leachates from coal-fired plants or coal ash storage impoundments are well-known (Baumann and Gillespie, 1986; Besser et al., 1996; Lemly, 1993, 1996; Hamilton, 2004; Young et al., 2010). For instance, fish populations in the vicinity of coal-fired power plants often have elevated body burdens of Se compared with fish at locations further removed from power plants (Young et al., 2010). In some well-characterized cases such as Hyco and Belews Lakes in North Carolina, Se contamination has been associated with reproductive impairments in fish populations, significant increases in the incidences of developmental abnormalities in fish larvae, and even the local extinction of sensitive fish species (Cumbie and Van Horn, 1978; Lemly, 2002). In light of such well-documented adverse outcomes to fish populations attributed to chronic Se exposures, often from coal ash-related contamination, understandable concerns were raised by the massive Kingston spill as to the potential for significant impacts to fish communities in the affected river systems and Watts Bar Reservoir (Babyak et al., 2009; Ruhl et al., 2009; Lisenby et al., 2009). To systematically evaluate the ecological and human health risks of the ash spill, and provide data for the formulation of a long-term strategy for managing the affected resources, TVA and the USEPA initiated a diverse environmental assessment program in the immediate aftermath of the spill (Jacobs Engineering, 2010, 2012).

In a series of related studies associated with this comprehensive assessment and monitoring program, Se was demonstrated to be significantly elevated in fish in areas of the Emory and Clinch Rivers affected by coal ash from the Kingston spill, with highest concentrations observed in redear sunfish (*Lepomis macrolophus*) (Adams et al., 2012; Otter et al., 2012; Mathews et al., 2014). Arsenic and Hg were also elevated in fish affected by the ash spill (Rigg et al., 2015), although site-to-site variation in these metal(loid)s appeared to reflect multiple sources of these metal(loid)s in the ash-affected watersheds including the Department of Energy (DOE) facilities in Oak Ridge, TN (Turner et al., 1985; Campbell et al., 1997). To date, few if any adverse effects on fish health (Adams and Fortner, 2012; Bevelhimer et al., 2014), fish reproduction and embryo-larval development (Greeley et al., 2012, 2014b,c Stanley et al., 2013; Rigg et al., 2015), or fish population and community structures (Rigg et al., 2015) have been conclusively linked in either field- or laboratory-based studies to the Kingston coal ash spill or to the increased metal(loid) concentrations in fish.

Concerns remain, however, that elevated Se in particular – accumulated by adult fish through their diet and maternally transferred to their offspring – might still be capable of impacting fish populations in the affected bodies of water as Se and other ash-related metal(loid)s continue to work through the aquatic food web (Mathews et al., 2014). Although remaining below proposed draft water quality criteria, Se concentrations in fish from the vicinity of the Kingston ash spill site (Adams and Fortner, 2012; Otter et al., 2012; Mathews et al., 2014) do exceed concentrations associated in some studies with adverse effects on fish reproductive or developmental processes (USEPA, 2014). To address such concerns, we examined through a combined field and laboratory study the potential relationships between: (1) the bioaccumulation of Se, As, and Hg by adult redear sunfish exposed in situ to ash from the Kingston spill; (2) the maternal transfer of these metal(loid)s to their offspring during oogenesis; and (3) potential adverse outcomes to the survival and early development of offspring from continuing exposures of fish to residual coal ash from the spill

The primary objective of this investigation was to determine if redear sunfish affected by the Kingston coal ash spill transferred ash-related contaminants to their eggs in sufficient quantities and chemical forms to affect fertilization success or induce teratogenic abnormalities in F1-generation embryos and larvae. A secondary objective was to evaluate the potential direct or synergistic toxic contributions of water from ash-exposed study sites to the developing embryos and larvae. As reproduction and development provide key linkages between the effects of environmental stressors on individual organisms and adverse outcomes manifested at higher levels of biological organization (Rose et al., 1999), the results of this study are expected to assist in assessing the risks to fish populations from residual coal ash remaining in the Watts Bar Reservoir system following remediation. The results are also expected to further a general understanding of the toxicity and risks of coal ash and coal ash-associated contaminants such as Se, As, and Hg to aquatic organisms under real-life exposure conditions, and examine the protectiveness of proposed fish-based water quality criteria for Se. A preliminary report concerning these results was previously submitted to TVA for purposes of the Kingston spill investigation (Greeley et al., 2014a).

2. Materials and methods

2.1. Setting and study sites

The Kingston Fossil Fuel Plant is located on a peninsula formed by the confluence of the Emory and Clinch Rivers, approximately 6 km upstream of the confluence of the Clinch and Tennessee Rivers in the TVA Watts Bar Reservoir (Fig. 1). Upon the catastrophic failure of a retaining wall, coal ash stored on-site since the opening of the facility in 1955 was released into the Emory River in the vicinity of Emory River mile (ERM) 2.2. The ash front surged both upstream and downstream in the Emory River and then further downstream into the Clinch River, with additional downstream movement into the Tennessee River and Watts Bar reservoir proper during subsequent high-water events. More detailed descriptions of the ash spill and subsequent ash movements can be found in Jacobs Engineering (2010, 2011), ARCADIS (2012), and Walls et al. (2015).

For the purposes of this investigation, several ash-exposed study sites and unexposed reference sites in the Emory and Clinch Rivers were selected to represent various levels of fish exposure to coal ash (Fig. 1). Fish and water sampling locations included upstream reference sites at ERM 8.0 and Clinch River mile (CRM) 8.0, along with ash-exposed sites at ERM 3.0 (in the immediate vicinity of the ash release), ERM 0.9 (in the lower reaches of the Emory River downstream of the spill site), and CRM 1.5 (downstream of the spill site and the confluence of the Emory and Clinch Rivers). Neither of the upstream reference sites can be considered pristine, as the ERM 8.0 reference site is affected by urban and rural runoff and

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