Environ Monit Assess (2015) 187: 334
DOI 10.1007/s10661-015-4578-3

Bioaccumulation of metals in three freshwater mussel species exposed in situ during and after dredging at a coal ash spill site (Tennessee Valley Authority Kingston Fossil Plant)

Ryan R. Otter · David McKinney · Bobby Brown · Susan Lainer · William Monroe · Don Hubbs · Bob Read

Received: 26 January 2015 / Accepted: 29 April 2015 / Published online: 9 May 2015

Abstract On December 22, 2008, a dike containing coal fly ash at the Tennessee Valley Authority Kingston Fossil Plant (TN, USA) failed, and within months, dredging operations began to remove ash-contaminated sediments. The purpose of this study was to investigate differences in the bioaccumulation of metals in three mussel species during and after dredging operations. Mussels were caged for approximately 1 year during dredging and after, and then mussel condition index values and As, Cd, Cr, Pb, Ni, Se, Hg, U, Fe, Mg, Al, Sb, Ba, Be, Co, Cu, Mn, Mo, Ag, Sr, Tl, V, and Zn concentrations in soft tissue were determined via inductively coupled plasma–mass spectrometery. Overall, the differences observed in metal bioaccumulation and mussel health suggest that mussels in the immediate downstream area of the dredging site may have been impacted, as evidenced by a significant decrease in mussel condition index values, but that this impact did not result in increased tissue concentrations of metals.

R. R. Otter (✉) · W. Monroe
Department of Biology, Middle Tennessee State University, Box 60, Murfreesboro, TN 37132, USA
e-mail: Ryan.Otter@mtsu.edu

D. McKinney · B. Brown · S. Lainer · D. Hubbs
Tennessee Wildlife Resources Agency, Nashville, TN, USA

B. Read
Division of Laboratory Services, Tennessee Department of Health, Nashville, TN, USA

Keywords Mussels · Coal ash · Condition index · Metals · Dredging · Freshwater

Introduction

On December 22, 2008, the largest coal ash spill in US history occurred, releasing approximately 4.1 million m^3 of coal ash from the Tennessee Valley Authority Kingston Fossil Plant near Kingston, Tennessee, due to a dike failure (Ruhl et al. 2010; TVA 2009). The release impacted terrestrial areas, the adjacent Emory River, and downstream river locations due to ash transport in the river channel (TVA 2009; Bednar et al. 2010).

Long-term toxicological concerns exist due to high concentrations of metals associated with coal fly ash (Yudovich and Ketris 2005; Yudovich and Ketris 2006; Rowe et al. 2002; Ruhl et al. 2009). Previous studies at coal-ash-contaminated sites, including the Kingston spill site, have shown significant bioaccumulation of metals in various organisms including turtles (Nagle et al. 2001), caddisflies (Reash et al. 2006), crayfish (Nagle et al. 2001), tadpoles (Hopkins et al. 1999), largemouth bass (*Micropterus salmoides*) (Baumann and Gillespie 1986), bluegill (*Lepomis macrochirus*) (Reash et al. 2006; Baumann and Gillespie 1986; Lohner et al. 2001), green sunfish (*Lepomis cyanellus*) (Lohner et al. 2001), redear sunfish (*Lepomis microlophus*) (Otter et al. 2012; Sorensen 1988), snakes (Hopkins et al. 1999), and spiders (Otter et al. 2013).

Dredging has been an effective way to remove contaminated sediments at large-scale contaminated sites (NRC 2007) impacted by contaminants such as oil, polychlorinated biphenyls (PCBs), and metals (Bridges et al. 2010). Although dredging allows for the direct removal of contaminated sediments, multiple factors directly influence its overall effectiveness in terms of managing and reducing risk. Difficulties in site characterization, measuring effects, and predicting future transport of contaminated sediments are all challenges in predicting and measuring the effectiveness of dredging (NRC 2007; Gustavson et al. 2008). Another concern associated with dredging is the downstream transport of contaminated sediments caused by physical disruption (Gustavson et al. 2008).

Dredging of the Emory River, in response to the coal ash spill, began in March 2009 and was completed in May 2010, with the removal of more than 2.6 million m^3 of ash and sediment (TVA 2011a; Stanley et al. 2013). Previous research focused on water contamination during dredging showed no significant increases of ash-related constituents downstream of the spill site; however, elevated concentrations were observed in the immediate vicinity of dredging (TVA 2010a). In a study focused on characterizing the metals released from coal ash during dredging, Bednar et al. (2013) concluded that the dredging operations did not release large amounts of bioavailable metals into the river environment indicating that the combination of sedimentation processes and oxidation, sorption, and precipitation processes were effective in removing metals from the system. Post-dredging data suggests little concern for continuing impacts of the ash spill on water quality in the Emory River or other downstream waterways, evidenced by very few exceedences of any water quality standards since dredging was completed (TVA 2011b). Previous research investigating the potential impacts of dredging, utilizing laboratory experiments, showed only minor effects on surface water quality and little potential for toxicity to fish (Ruhl et al. 2010; Stanley et al. 2013).

Mussels are commonly used bioindicators of environmental pollution in marine and freshwater ecosystems (Schintu et al. 2008; Andral et al. 2004; Tsangaris et al. 2011; O'Connor 1996). Previous research has shown mussels to be useful bioindicators at metal-contaminated sites, including those associated with by-products of coal-fired power plants (Wang et al. 2013; Hull et al. 2006; Peltier et al. 2009; Roméo et al. 2005; Lafabrie et al. 2007). The deployment of mussels in cages to monitor aquatic ecosystems through tissue concentrations and integrated physiological measurements (e.g., condition indices) has also been well established (Schintu et al. 2008; Andral et al. 2004; Tsangaris et al. 2011; Damiens et al. 2007).

The purpose of this study was to investigate the effect of dredging on the bioaccumulation of ash-associated metals in caged mussels. Two separate field deployments of mussels were utilized (during dredging and post-dredging) at multiple locations downstream the spill site. The specific objectives were to use metal bioaccumulation and a mussel condition index to determine differences in mussels based on: (1) site location, (2) time (active dredging vs non-dredging), and (3) species.

Material and methods

Mussels

Three mussel species were utilized in this study: black sandshell (*Ligumia recta*), elephant ear (*Elliptio crassidens*), and purple wartyback (*Cyclonaias tuberculata*). Approximately 75 individuals of each species were collected from the lower Tennessee River (river mile 195–197 below Pickwick Dam, near Savannah, TN) and transported to the Cumberland River Aquatic Center (CRAC) (Gallatin, TN) in aerated coolers, while being monitored, to ensure minimal temperature fluctuations (<3 °C). All mussels were submerged in ~35 cm of water at the CRAC for 45 days prior to deployment in flow-through concrete raceways. Raceway water was supplied directly from the adjacent Cumberland River (36.309495, −86.401710) at a flow rate of 454 L/min and was unfiltered. Mussels maintained at the CRAC were not fed. Prior to mussel deployment, each individual was thoroughly scrubbed, weighed, and affixed with a unique tag number.

Site locations

Mussels were deployed at four sites in the vicinity of the ash spill (Fig. 1). During the dredging deployment, mussels were deployed at three locations: (1) in the Emory River (Emory) immediately downstream of the spill location, approximately 1 mi upstream of the

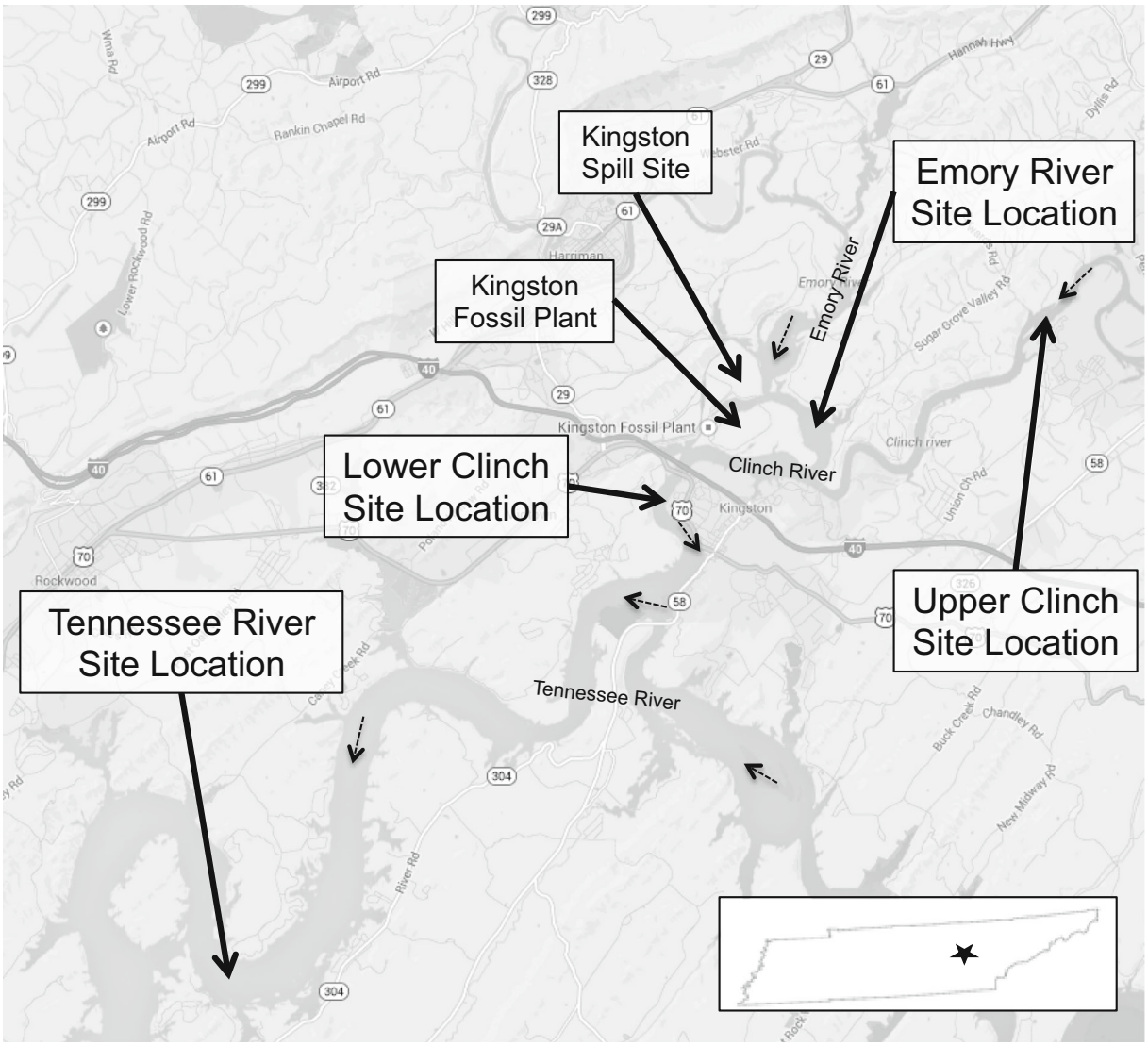

Fig. 1 Map of spill and sampling locations. *Dashed arrows* indicate water flow direction. Base map provided by Google maps

Clinch River confluence; (2) in the lower Clinch River (Lower Clinch), approximately 2 mi downstream of the Emory River confluence; and (3) in the Emory River approximately 4 mi upstream of the spill location. During the post-dredging deployment, mussels were deployed at four locations: (1) in the Emory River immediately downstream of the spill location (same location as dredging deployment); (2) in the Lower Clinch (same location as dredging deployment); (3) at an additional site on the Clinch River, approximately 6 mi upstream of the Emory River confluence (Upper Clinch); and (4) in the Tennessee River approximately 5 mi downstream of the Clinch River confluence, near Thief Neck Island (Tennessee). Additionally, "lab-reference" mussels (10 individuals of each species) were kept in concrete raceways at the CRAC under the same conditions described above for the 45-day holding period.

During the dredging deployment, mussel enclosures were deployed in Emory on June 20, 2009 and retrieved on June 14, 2010, and deployed in Lower Clinch on October 21, 2009 and retrieved on August 31, 2010. During the post-dredging deployment, all mussels were deployed on October 28, 2010 and retrieved on October 3, 2011. Mussels held at the CRAC were first introduced on May 5, 2009 and processed between July 20 and August 10, 2010.

Deployment enclosures

All deployed mussels were secured in custom deployment enclosures constructed of 5-cm polyvinyl chloride (PVC) pipe in a 1.2×1.2 m square frame design with 2.5 cm squared high-density polyethylene (HDPE) plastic mesh covering the interior of the frame. Anchor supports (1 m long; made of 5-cm PVC) with custom pointed ends (for attachment into the river bottom) were attached to each corner of the frame.

In situ mussel deployment

Mussels were transported from the CRAC in aerated coolers, first by truck, then by boat, to each site location. Enclosures were loaded with mussels (~15 total mussels per enclosure, ~4 mussels of each species, and ~4 enclosures per site) and delivered to the river bottom by SCUBA divers. Enclosures were driven into the river bottom at each site until the mussels were approximately 30 cm above the river bottom. Once enclosures were in place, 10-kg block anchors were affixed to each corner of the enclosure using anchor chains. Additionally, a single metal fence post was driven into the river bottom and affixed to one corner of each enclosure.

Post-deployment processing

After retrieval, mussels were transported in aerated coolers and monitored to ensure minimal temperature fluctuations (<3 °C) until post-deployment processing occurred. All mussels were held between 36 and 48 h to ensure the purging of gut contents before processing. Additionally, each mussel received light scrubbing, immediately prior to processing, to remove debris from the shell.

Condition index and weight gain

After depuration, each mussel was weighed (total weight) and then the soft tissue was carefully removed and weighed (tissue wet weight). Filling one half of the shell with water then transferring that volume to a graduated cylinder and multiplying that volume by two determined total shell volume were done. Prior to the study, individual mussels of each species were tested to ensure equal shell volumes existed within each half of an individual. The mussel condition index was calculated for each mussel using the following formula: condition index = tissue wet weight / shell volume (Davenport and Chen 1987). Weight gain for each individual mussel deployed was calculated using the following formula: ((total weight after deployment − total weight before deployment) / total weight before deployment) * 100

Metal analyses

After removal of sections of foot, mantle, and gill for use in other assays (data not shown), the remaining tissue was frozen and held at −20 °C until laboratory analysis. Each sample was thawed at room temperature, homogenized, then oven-dried at 106 °C. Percent solid information was collected, and each sample was then digested and analyzed for total concentrations of iron, magnesium, aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel, selenium, silver, strontium, thallium, uranium, vanadium, and zinc using EPA methods USEPA 6020 and 1631.

For digestions of all metals, except mercury, approximately 1.0 g of homogenized tissue was added to 2.5 mL of concentrated nitric acid, allowed to sit overnight, and then placed on a heating block at 95 °C for 10 min. After a 10-min cooling period, 1 mL of concentrated nitric acid was added to each sample, and it was returned to the block for an additional 10 min. In addition, hydrogen peroxide and more nitric acid were added to each sample until fumes were no longer visible, indicating samples were free of organic material. Each sample was then brought to a final volume of 50 mL with reagent grade water.

For mercury digestions, approximately 1.0 g of homogenized sample was added to 2 mL of sulfuric acid and 2 mL of nitric acid and allowed to sit overnight. Each sample was then placed on a heating block at 95 °C for 45 min. Reagent grade water (30 mL) and 5 % $KMnO_4$ (5 mL) were then added to each sample, and samples were returned to the heating block for at least 30 min. Samples were cooled for 10 min, and 1.5 mL of a solution containing 12 % sodium chloride and 12 % hydroxylamine sulfate was added to each sample. Each sample was then brought up to a final volume of 50 mL with reagent grade water.

Analysis of all metals, except mercury, was performed on a Perkin Elmer ELAN 9000 inductively coupled plasma–mass spectrometer (ICP-MS), or on a Perkin Elmer Optima 3000XL inductively coupled

plasma–optical emission spectrophotometer (ICP-OES). Mercury analysis was performed on a Perkin Elmer FIMS 400 Mercury Analyzer.

Quality control measures during all analysis included laboratory reagent blanks, laboratory fortified blanks and matrices, calibration blanks, and spiked standards. Spiked standards were considered acceptable if within ±10 % when using ICP-OES or the FIMS 400 Mercury Analyzer and ±15 % for ICP-MS. Fortified blanks were considered acceptable if within ±15 % of the expected concentration.

Contaminant advisory levels

Mussel advisory concentrations outlined by the Food and Drug Administration for arsenic (86 mg/kg) (FDA 1993), cadmium (4.0 mg/kg) (FDA 1993), chromium (13 mg/kg) (FDA 1993), lead (1.7 mg/kg) (FDA 1993), nickel (80 mg/kg) (FDA 1993), and mercury (1.0 mg/kg) (FDA 1996) were used for comparison to average deployed tissue concentrations. Likewise, a concentration of 7.9 mg/kg of selenium (USEPA 2004), based on the chronic exposure aquatic life criteria for whole-body fish, was used for comparison.

Statistics

Analyses of metal concentrations and condition index for each species were investigated for differences between each site and the lab-reference data using one-way ANOVAs with Tukey's post hoc tests. One-way ANOVAs were also used for comparison between years for each species at the same site with Tukey's post hoc tests. All analyses were performed using JMP Pro 9 software (Cary, NC). Significant differences for all analyses were defined by p values <0.05.

Results

Unfortunately, during the dredging deployment, all mussel enclosures deployed in the Emory River site upstream of the spill location were lost. Therefore, as an alternative to field reference data, comparisons to lab-reference data were performed. With the exception of the upstream Emory River site, survival in all other enclosures, during all deployments was greater than 90 %.

Bioaccumulation

Bioaccumulation concentrations of iron, magnesium, aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel, selenium, silver, strontium, thallium, uranium, vanadium, and zinc in black sandshell, purple wartyback, and elephant ear mussels are shown in Tables 1, 2, and 3, respectively. Comparisons to both lab-reference tissue concentrations and contaminant advisory levels showed that no single mean mussel concentration exceeded either one of these comparison values in mussels deployed in the Emory River throughout the entire study. Likewise, no exceedences of these two values (lab-reference tissue concentrations and contaminant advisory level) were observed in any mean mussel concentration during the dredging-deployment in any species at any site. However, during the post-dredging deployment, significantly elevated concentrations, above both lab-reference tissue concentrations and contaminant advisory levels, were observed. In purple wartybacks, concentrations of uranium, antimony, beryllium, and thallium were found at concentrations significantly higher than lab-reference tissue concentrations at both the Upper and Lower Clinch sites. These same four elements were also found in significantly higher concentrations in elephant ear in the Upper Clinch. Throughout the entire study, only two mean concentrations exceeded their respective contaminant advisory level. Both exceedences were concentrations of lead (advisory level, 1.7 mg/kg) and both occurred during the post-dredging deployment (purple wartyback, Lower Clinch, 3.2 mg/kg; and elephant ear, Upper Clinch, 1.9 mg/kg). Also noteworthy was that copper concentrations in lab-reference mussels were significantly higher ($p<0.05$) in the all species, at all sites, during all deployments, with the exception of purple wartyback from the dredge-deployment in the Lower Clinch ($p=0.98$).

Significant differences in tissue concentrations between dredging-deployed mussels and post-dredging-deployed mussels were observed less often in the Emory River compared to the Lower Clinch. In the Emory River, only two significant increases, thallium (black sandshell) and mercury (purple wartyback), and two significant decreases, thallium (purple wartyback) and zinc (elephant ear), were observed. In contrast, in the Lower Clinch, significant increases of eight metals

Table 1 Bioaccumulation of metals (mg/kg dry weight) in black sandshell (*Ligumia recta*) (mean±SE)

	Advisory level	Emory River		Lower Clinch		Upper Clinch	Tennessee	Lab-reference
	(ppm)	Dredge	Post-dredge	Dredge	Post-dredge	Post-dredge	Post-dredge	–
n		16	12	12	9	9	10	10
Arsenic	86.0	6.8±0.9	6.0±0.4	4.2±0.4	6.5±0.8	3.7±0.5	3.7±0.4	5.2±0.6
Cadmium	4.0	0.8±0.2	1.2±0.3	0.6±0.1	1.4±0.4	0.9±0.3	1.5±0.2	1.0±0.2
Chromium	13.0	3.1±0.6	3.0±0.4	2.5±0.3	4.0±0.6	2.8±0.6	2.5±0.6	3.1±0.3
Lead	1.7	0.5±0.1	0.8±0.3	0.6±0.1	1.2±0.3	0.4±0.2	0.3±0.1	0.6±0.2
Nickel	80.0	3.7±0.8	2.7±0.3	2.8±0.3	3.3±0.4	2.4±0.2	2.4±0.5	4.4±0.5
Selenium	7.9	3.9±0.5	3.2±0.3	2.3±0.2	3.3±0.4	2.3±0.3	2.1±0.1	2.6±0.3
Mercury	1.0	0.1±0.01	0.1±0.01	0.1±0.02	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01
Uranium	–	0.4±0.1	41.0±17.4	0.3±0.0	48.0±23.9	13.5±13.3	0.2±0.02	0.5±0.04
Iron	–	1913.0±237.0	1901.7±272.4	1870.0±301.0	2633.3±269.3	2188.9±311.6	2122.0±348.7	2430.0±350.0
Magnesium	–	1812.0±168.0	1808.3±130.5	1925.0±168.0	2422.2±195.6	2466.7±132.2	1600.0±118.3	2220.0±100.9
Aluminum	–	151.8±44.3	25.5±6.5	269.0±44.3#	61.1±51.2#	32.0±8.6	36.0±13.9	223.0±48.6
Antimony	–	0.03±0.0	0.6±0.3	0.02±0.01	0.7±0.4	0.2±0.2	0.02±0.01	0.1±0.1
Barium	–	1050.0±149.0	1000.8±128.8	1183.0±418.0	1551.1±241.3	1424.4±171.4	980.0±141.4	870.0±0.1
Beryllium	–	0.1±0.0	0.6±0.2	0.1±0.01	0.8±0.4	0.2±0.2	0.05±0.01	0.1±0.02
Cobalt	–	1.4±0.2	1.5±0.3	1.1±0.1	2.1±0.4	1.7±0.6	0.8±0.1	1.9±0.4
Copper	–	9.2±1.4*	10.8±2.3*	35.3±2.7*	16.8±1.8*	7.5±1.0*	6.1±0.4*	62.4±5.5
Manganese	–	11,537.5±1734.1	9716.7±1369.2	23,033.3±12,506.2	16,011.1±2236.1	13,700.0±1729.0	10,830.0±2341.1	9990.0±817.6
Molybdenum	–	0.6±0.1	1.2±0.3	0.4±0.0	1.3±0.4	0.7±0.2	0.4±0.1	0.7±0.1
Silver	–	0.3±0.1	0.3±0.1	0.2±0.02	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.03
Strontium	–	209.4±27.2	128.0±19.7*	185.5±23.9	226.7±28.4	194.7±32.9	129.7±15.3*	300.0±21.8
Thallium	–	0.1±0.01#	0.7±0.27#	0.05±0.01#	0.9±0.37#	0.2±0.2	0.02±0.01	0.01±0.01
Vanadium	–	1.1±0.3	1.2±0.3	1.0±0.1	1.6±0.4	0.8±0.2	0.3±0.1	1.7±0.5
Zinc	–	604.4±86.4	451.2±48.9	769.2±103.5	831.1±138.2	807.8±87.3	457.1±107.9	538.0±74.3

*Different from lab-reference concentration: one-way ANOVA with Tukey's, $P<0.05$

#Different between dredge and post-dredge for same species and same element one-way ANOVA with Tukey's, $p<0.05$

Table 2 Bioaccumulation of metals (mg/kg dry weight) in purple wartyback (*Cyclonaias tuberculata*) (mean±SE)

	Advisory Level	Emory River		Lower Clinch		Upper Clinch	Tennessee	Lab-reference
	(ppm)	Dredge 15	Post-dredge 7	Dredge 8	Post-dredge 9	Post-dredge 10	Post-dredge 10	– 10
Arsenic	86.0	4.4±0.3	3.2±0.2	4.7±0.3	6.6±0.3	2.8±0.3	2.3±0.2	2.9±0.5
Cadmium	4.0	1.8±0.2	1.0±0.1	2.1±0.3#	3.6±0.2#	2.0±0.3	1.3±0.1	1.7±0.2
Chromium	13.0	4.2±0.4	3.5±0.7	4.6±0.6	7.2±0.4	4.4±0.5	4.1±0.5	4.4±0.9
Lead	1.7	0.3±0.03	0.3±0.02	0.9±0.05#	3.2±0.09*	1.0±0.3	0.3±0.04	0.4±0.04
Nickel	80.0	2.0±0.2	2.1±0.2	1.9±0.1	4.3±0.2	2.6±0.3	1.9±0.2	1.8±0.3
Selenium	7.9	5.6±1.0	2.7±1.5	3.2±1.3	5.0±1.3	3.2±1.2	1.9±1.2	3.0±1.2
Mercury	1.0	0.1±0.01#	0.2±0.04#	0.2±0.03	0.2±0.02	0.2±0.01	0.1±0.01	0.2±0.01
Uranium	–	0.2±0.02	0.2±0.02	0.2±0.01#	144.4±8.2*#	50.1±7.8*	0.2±0.02	0.2±0.1
Iron	–	3333.0±658.0	2542.9±287.7	2400.0±395.0	3077.8±249.9	3020.0±376.2	2810.0±260.5	2192.0±438.0
Magnesium	–	1184.0±66.0	1472.9±141.6	1320.0±90.0	1422.2±64.1	1580.0±134.0	1395.0±133.4	1518.0±223.0
Aluminum	–	54.0±32.4	53.6±11.2	280.0±43.0#	106.0±25.4#	47.3±9.4	37.3±8.2	156.2±39.7
Antimony	–	0.02±0.01	0.02±0.01	0.02±0.01#	2.2±0.04*#	0.8±0.29*	0.02±0.01	0.02±0.01
Barium	–	649.0±90.0	921.4±130.7	686.0±94.0	725.6±92.3	639.0±100.8	790.0±125.3	540.0±98.6
Beryllium	–	0.1±0.01	0.05±0.01	0.1±0.01#	2.1±0.07*#	0.8±0.31*	0.1±0.01	0.02±0.01
Cobalt	–	1.2±0.1	1.3±0.1	1.2±0.1#	3.8±0.1*#	1.7±0.3	1.2±0.1	1.2±0.2
Copper	–	17.9±1.2*	12.7±0.9*	38.8±8.8	19.7±1.1*	12.0±0.8*	12.0±0.7*	42.3±7.0
Manganese	–	4948.8±795.0	7400.0±1034.9	5575.0±844.5	7711.1±798.7	5850.0±723.1	10,340.0±1303.1*	4580.0±949.4
Molybdenum	–	0.7±0.1	0.5±0.05	0.6±0.10#	2.8±0.06*#	1.2±0.3	0.4±0.03*	0.7±0.1
Silver	–	0.3±0.1	0.1±0.03	0.3±0.2	0.5±0.04	0.2±0.05	0.2±0.05	0.2±0.0
Strontium	–	118.6±15.1	116.3±15.6	160.6±23.3	128.1±10.6	107.9±10.9	111.0±13.3	132.6±29.1
Thallium	–	0.1±0.01#	0.03±0.01#	0.1±0.01#	2.4±0.04*#	0.8±0.3*	0.03±0.01	0.1±0.1
Vanadium	–	0.4±0.03*	0.5±0.04*	1.1±0.10#	3.1±0.2*#	1.2±0.3	0.3±0.04*	1.7±0.3
Zinc	–	288.7±30.2*	430.0±99.9	641.3±73.3	416.7±65.8	365.0±88.8	507.0±84.6	735.0±105.2

*Different from lab-reference concentration: one-way ANOVA with Tukey's, $P<0.05$

#Different between dredge and post-dredge for same species and same element one-way ANOVA with Tukey's, $p<0.05$

Table 3 Bioaccumulation of metals (mg/kg dry weight) in elephant ear (*Elliptio crassidens*) (mean±SE)

	Advisory Level	Emory River		Lower Clinch		Upper Clinch	Tennessee	Lab-reference
	(ppm)	Dredge 15	Post-dredge 7	Dredge 8	Post-dredge 9	Post-dredge 10	Post-dredge 10	– 10
Arsenic	86.0	5.0±0.3	4.6±0.4	4.1±0.2	5.1±0.5	4.3±0.5	3.2±0.4	2.7±0.3
Cadmium	4.0	1.8±0.3	0.9±0.1	1.7±0.2	1.6±0.2	3.1±0.4*	1.3±0.1	1.5±0.1
Chromium	13.0	8.3±2.6	6.6±1.8	8.0±2.2	11.4±2.8	7.5±1.6	10.4±1.8	4.0±0.8
Lead	1.7	0.4±0.08	0.4±0.08	0.6±0.09	0.6±0.05	1.9±0.1*	0.4±0.10	0.5±0.07
Nickel	80.0	2.1±0.4	1.8±0.2	1.6±0.2	2.2±0.3	4.3±0.3*	2.5±0.3	2.2±0.2
Selenium	7.9	3.3±0.3	3.3±0.1	2.9±0.2	3.3±0.2	4.0±0.4	2.4±0.1	2.0±0.1
Mercury	1.0	0.21±0.03	0.15±0.01	0.21±0.03	0.19±0.01	0.17±0.01	0.10±0.01	0.24±0.02
Uranium	–	0.3±0.05	0.1±0.02	0.2±0.02	0.2±0.04	91.1±7.23*	0.3±0.04	0.3±0.05
Iron	–	3254.0±730.0	3026.4±417.9	3249.0±716.0	4617.0±745.0	4690.9±744.4	4340.0±522.0	2860.0±453.4
Magnesium	–	1220.0±131.0	1317.3±144.6	1141.0±90.0	1597.0±162.7	1954.5±158.3*	1747.0±162.7*	1280.0±96.9
Aluminum	–	46.0±58.9*	92.1±56.0	215.3±48.0	75.3±61.9	141.2±58.8	44.6±58.8*	310.1±58.8
Antimony	–	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	1.34±0.30*	0.02±0.01	0.04±0.02
Barium	–	841.0±230.0	938.2±177.6	866.5±165.0	1197.0±205.1	1212.7±198.3	1245.0±190.3	491.8±140.6
Beryllium	–	0.07±0.02	0.11±0.05	0.08±0.02	0.08±0.02	1.35±0.29*	0.08±0.01	0.03±0.01
Cobalt	–	1.4±0.3	0.8±0.1	1.0±0.2	1.4±0.2	2.7±0.4*	1.2±0.1	1.0±0.1
Copper	–	5.2±1.8*	5.9±1.7*	13.6±1.5*	8.1±1.9*	7.3±1.8*	6.1±1.8*	23.4±1.8
Manganese	–	7086.9±2172.3	6200.0±956.4	6319.3±1275.7	9220.0±1481.5	9063.6±1547.3	12,100.0±1908.6*	4940.0±727.7
Molybdenum	–	0.7±0.11	0.4±0.04	0.6±0.06	0.7±0.06	2.1±0.41*	0.4±0.05	0.6±0.08
Silver	–	0.1±0.03	0.1±0.01	0.1±0.01	0.1±0.02	0.4±0.05*	0.1±0.02	0.1±0.01
Strontium	–	122.5±25.2	92.9±15.3	105.6±22.6	139.2±20.5	140.2±25.8	157.7±19.7	119.2±17.0
Thallium	–	0.05±0.01	0.03±0.01	0.05±0.01	0.03±0.01	1.34±0.33*	0.02±0.01	0.13±0.11
Vanadium	–	0.3±0.14*	0.5±0.04*	0.8±0.17	0.6±0.13	1.9±0.32	0.2±0.03*	1.3±1.00
Zinc	–	120.4±18.5#	73.1±3.8#	56.1±5.9	85.2±2.6	91.0±10.9	88.0±6.9	93.6±10.2

*Different from lab-reference concentration: one-way ANOVA with Tukey's, $P<0.05$

#Different between dredge and post-dredge for same species and same element one-way ANOVA with Tukey's, $p<0.05$

(all in purple wartyback), thallium, cadmium, uranium, antimony, beryllium, molybdenum, and vanadium, and significant decreases of two metals, aluminum (black sandshell and purple wartyback) and copper (purple wartyback), were observed.

Condition index and weight gain

Lab-reference condition index values were significantly lower at all sites, during both the dredging and post-dredging deployments, for black sandshell and purple wartback (Table 4). Significant increases in condition index values at the Emory River were observed for all three species during the post-dredging deployment compared to the dredging deployment. No deployment-based differences were observed at Lower Clinch.

Average weight gain analysis for deployed mussels showed no significant differences in weight gain between sites or deployments for black sandshell ($F_{(4,53)}=0.18$, $p=0.94$), purple wartyback ($F_{(4,48)}=0.82$, $p=0.51$), or elephant ear ($F_{(4,54)}=1.45$, $p=0.23$). On average, each mussel species, at each site, gained weight during the deployment, with the exception of black sandshell (dredging deployment), which lost an average 0.29 % of its body weight.

Discussion

The spill at the Kingston Fossil Plant was one of the largest ash spill in US history (Ruhl et al. 2009; Greeley et al. 2014). Previous research investigating the effects of coal-ash-associated metals on aquatic organisms have

Table 4 Condition index and weight gain of three mussel species (mean±SE)

Species	Site	Year	Number	Condition index	Weight gain (%)
Black sandshell					
	Emory River	Dredge	16	0.71±0.04#	−0.29±0.71
		Post-dredge	12	1.08±0.03*#	2.97±3.39
	Lower Clinch	Dredge	12	1.03±0.03*	2.28±0.64
		Post-dredge	9	1.09±0.04*	2.42±6.88
	Upper Clinch	Post-dredge	10	0.98±0.04*	0.76±0.33
	Tennessee	Post-dredge	10	1.12±0.06*	–
		Lab-reference	–	0.55±0.05	–
Purple wartyback					
	Emory River	Dredge	15	0.58±0.04#	1.92±0.30
		Post-dredge	7	0.92±0.05*#	1.61±0.25
	Lower Clinch	Dredge	8	0.85±0.01*	1.95±0.30
		Post-dredge	9	0.79±0.03*	6.03±4.85
	Upper Clinch	Post-dredge	10	0.86±0.03*	0.89±0.22
	Tennessee	Post-dredge	10	0.86±0.02*	–
	Reference tissue	–	10	0.54±0.07	–
Elephant war					
	Emory River	Dredge	13	0.60±0.05#	3.77±1.79
		Post-dredge	11	0.86±0.06#	7.80±4.80
	Lower Clinch	Dredge	15	0.84±0.02	1.46±0.21
		Post-dredge	10	0.94±0.04	1.72±0.43
	Upper Clinch	Post-dredge	10	0.88±0.02	0.62±0.49
	Tennessee	Post-dredge	10	0.91±0.02*	–
	Reference tissue	–	10	0.67±0.08	–

*Different from lab-reference concentration: one-way ANOVA with Tukey's, $P<0.05$

#Different between dredge and post-dredge for same species and same element one-way ANOVA with Tukey's, $p<0.05$

focused primarily on areas receiving coal ash effluent (Reash et al. 2006; Baumann and Gillespie 1986; Lohner et al. 2001) and not from an environmental spill, as was the case in this study. As unfortunate as the spill was, it provided an opportunity to directly study the impact of coal fly ash in a large lotic system where the amount of downstream transport of ash was also being investigated and monitored (TVA 2010b).

Previous research on the Kingston spill has provided insight on the contaminants of concern from multiple perspectives including sediment biogeochemistry, metal speciation, and metal bioaccumulation across multiple organisms (Ruhl et al. 2010; Bednar et al. 2010; Ruhl et al. 2009; Otter et al. 2013; Deonarine et al. 2013; Bartov et al. 2012; Liu et al. 2013; Beck et al. 2013; Souza et al. 2013). Otter et al. (2012) showed elevated concentrations of arsenic and selenium in various tissues of multiple fish species at ash-associated sites compared to reference sites. Laboratory experiments showed elevated concentrations of arsenic, selenium, and mercury in sediment samples, but no effects on larval or juvenile fathead minnows (*Pimephales promelas*) when exposed to ash-amended sediment or elutriates (Stanley et al. 2013; Greeley et al. 2014). When the current body of research on the Kingston spill is viewed collectively, a couple of major observations can be made: (1) The major contaminants of concern appear to be metals, such as arsenic and selenium, and (2) metals associated with ash appear to be largely sediment-bound.

Dredging has been used as a remedial option for sediments contaminated with oil, PCBs, and metals (Bridges et al. 2010). However, the removal of contaminated sediments also comes with significant challenges, including the re-suspension of potential contaminants, measuring effects, and potential changes in metal speciation (Bednar et al. 2010; Rowe et al. 2002; Gustavson et al. 2008). Given the volume of the ash spilled (4.1 million m^3), the volume of contaminated sediments dredged (more than 2.6 million m^3), and the fluctuations in Emory River discharge during dredging (50–70,000 cfs), issues related to the physical disruption of ash-associated sediments were a concern at the Kingston Fossil spill site. Stanley et al. (2013) used an integrated weight of evidence laboratory approach (water chemistry, metal speciation, toxicity, and bioaccumulation) with ash-associated sediments from the Kingston site and concluded that little potential for toxicity to fish existed due to fly ash dredging activities. In the present study, transplanted mussels were used in situ and provided spatially and temporally integrated data (Gunther et al. 1999) on the bioaccumulation of metals and overall mussel health (using a mussel health index and weight gain endpoints) at the Kingston site, utilizing pre- and post-dredging deployments.

Transport and deposition of ash immediately following the 2008 spill was identified as far as 15 river miles downstream of the Kingston Fossil spill location (TVA 2010b). This highlights the potential for re-suspended ash from dredging to impact not only the dredging location itself but also sites downstream as well. In the present study, the farthest downstream site (Tennessee) was located in the vicinity of where ash had already been reported from this spill (TVA 2010b). No clear spatial patterns of mussel tissue accumulation for any of the 23 metals tested in the study were apparent for any of the three species (Tables 1, 2, and 3). If significant bioaccumulation of ash-associated metals were to occur, increased concentrations at the Emory River site (closest to the dredging) would likely have been observed with decreasing concentrations as downstream distance from the dredging site increased. Data from the present study showed no tissue concentrations above contaminant advisory levels, for any metal, and no significantly elevated concentrations of any metal above lab-reference concentrations throughout the two deployments at the Emory River. Previous research on the Kingston spill showed similar results for raccoons, with no spatial pattern of metal concentrations observed (Souza et al. 2013). However, multiple other studies have observed significant increases, mainly in selenium and arsenic concentrations, at ash-associated sites compared to references locations in fish (Otter et al. 2012) and spiders (Otter et al. 2013).

Post-dredging deployment data from the present study showed elevated concentrations of multiple metals, typically not associated with coal fly ash, particularly in the Clinch River (Tables 1, 2, and 3). Concentrations of uranium, antimony, beryllium, and thallium were observed at significantly higher levels than lab-reference concentrations at both the Upper Clinch and Lower Clinch sites in purple wartyback mussels. Part of the Clinch River, upstream of the sites used in the present study, is a Superfund Site with known historic releases of hazardous substances, including radionuclides, uranium, and coal ash (Cook et al. 1999). The presence of each of these metals, which have been shown to be a concern to either public health, the environment, or both (Simon et al. 2011a, 2011b; Lourenço et al. 2010; DeBoeck et al. 2003; Strupp and Beryllium metal II 2011;

Rodriguez-Mercado and Altamirano-Lozano 2013), warrants further investigation.

The condition index of mussels can provide important information about the overall health of individual organisms (Mubiana et al. 2006; Zhong et al. 2013; Pampanin et al. 2005). In the present study, condition index measurements showed relatively constant values for each individual species, with two consistent exceptions: (1) Emory River mussels during the dredge-deployment, and (2) lab-reference mussels. Mussels deployed in the Emory River while dredging was occurring showed significantly decreased condition index values in all three species compared to post-dredging-deployed mussels in the same location. Lab-reference mussels had the lowest observed values (typically significantly lower) in all species compared to all deployment data, with only one exception (elephant ear in the Emory River during the dredge-deployment). Although physiological assays (e.g., scope for growth) were not performed in the present study, so direct cause cannot be ascertained, these two exceptions, which both resulted in decreased index values, were likely the result of two different factors. Considering the consistency of the condition index values observed in all deployed mussels, except those in the immediate downstream location while dredging was occurring, it is plausible that the decreases observed may be linked to changes in environmental factors associated with the dredging operations, such as decreased feeding ability and/or decreased food availability Saurel et al. 2013; Aldridge et al. 1987; Osterling et al. 2007; Gascho Landis et al. 2012; Henley et al. 2000). Decreases in lab-reference index values, however, were most likely due to inherent water parameter differences between mussels deployed in the Tennessee River system (Emory River, Clinch River, and Tennessee River) and those held at the CRAC, which is part of the Cumberland River system. This difference is highlighted by the results observed for the essential metal copper, which showed significantly higher concentrations in lab-reference mussels compared to nearly all mussel deployments in the Tennessee River system. The difference between these two river systems is important to take into account when comparing both absolute and relative tissue concentrations. Unfortunately, a comparison to upstream deployed mussels was not possible due to the loss of all upstream reference mussels; therefore, lab-reference mussels were chosen as the best alternative for reference comparisons.

The use of three native mussel species in the present study proved to be a valuable asset in the interpretation of the possible impacts of this spill on mussels. Having the capability to analyze data independently for each mussel species, then to compare similarities and differences among species, allowed for a more confident interpretation than would have been possible with a single species.

It is important to note that the factors that control the bioaccumulation of metals are complex (e.g., pH, redox, and mercury-selenium interactions). This complexity combined with the inherent high variability in lotic systems highlights why these results must be viewed with some degree of caution. As long as residual ash exists in the Emory River (or downstream), the potential for changes in metal binding and/or changes in speciation leading to differing leaching dynamics exists. Previous work, including studies focused on the Kingston spill site, have shown that river conditions play a major role influencing the bioaccumulation of metals known to be present in coal ash (Ruhl et al. 2010; Bednar et al. 2010; Ruhl et al. 2009; Deonarine et al. 2013; Liu et al. 2013). Therefore, it is recommended that a holistic long-term monitoring plan be utilized to fully understand the long-term impacts of this spill and minimize risk.

Conclusions

Overall, the differences observed in metal bioaccumulation and mussel health suggest that mussels in the immediate downstream area of the dredging site may have been impacted, as evidenced by a significant decrease in mussel condition index values, but that this impact did not result in increased tissue concentrations of metals.

Acknowledgments For technical assistance, we would like to thank David Simms, Shannon Otter, Chris Bowman, and Frank Bailey. Funding for this study was provided by the Tennessee Wildlife Resources Agency, Division of Environmental Services.

References

Aldridge, D. W., Payne, B. S., & Miller, A. C. (1987). The effects of intermittent exposure to suspended solids and turbulence on 3 species of freshwater mussels. *Environmental Pollution, 45*, 17–28.

Andral, B., Stanisiere, J. Y., Sauzade, D., Damier, E., Thebault, H., Galgani, F., & Boissery, P. (2004). Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. *Marine Pollution Bulletin, 49*(9), 704–712.

Bartov, G., Deonarine, A., Johnson, T. M., Ruhl, L., Vengosh, A., & Hsu-Kim, H. (2012). Environmental impacts of the Tennessee Valley Authority Kingston coal ash spill. 1. Source apportionment using mercury stable isotopes. *Environmental Science Technology, 47*(4), 2092–2099.

Baumann, P. C., & Gillespie, R. B. (1986). Selenium bioaccumulation in gonads of largemouth bass and bluegill from three power plant cooling reservoirs. *Environmental Toxicology Chemistry, 5*(7), 695–701.

Beck, M. L., Hopkins, W. A., & Jackson, B. P. (2013). Spatial and temporal variation in the diet of tree swallows: implications for trace-element exposure after habitat remediation. *Archives Environmental Contamination and Toxicology, 65*(3), 575–587.

Bednar, A. J., Chappell, M. A., Seiter, J. M., Stanley, J. K., Averett, D. E., Jones, W. T., Pettway, B. A., Kennedy, A. J., Hendrix, J. A., & Steevens, J. A. (2010). Geochemical investigations of metals release from submerged coal fly ash using extended elutriate tests. *Chemosphere, 81*(11), 1393–1400.

Bednar, A. J., Averett, D. E., Seiter, J. M., Lafferty, B., Jones, W. T., Hayes, C. A., Chappell, M. A., Clarke, J. U., & Steevens, J. A. (2013). Characterization of metals released from coal fly ash during dredging at the Kingston ash recovery project. *Chemosphere, 92*, 1563–1570.

Bridges, T. S., Gustavson, K. E., Schroeder, P., Ells, S. J., Hayes, D., Nadeau, S. C., Palermo, M. R., & Patmont, C. (2010). Dredging processes and remedy effectiveness: relationship to the 4 Rs of environmental dredging. *Integrated Environmental Assessment and Management, 6*(4), 619–630.

Cook, R. B., Suter, G. W., & Sain, E. R. (1999). Ecological risk assessement in a large-river reservoir: 1. Introduction and background. *Environmental Toxicology Chemistry, 18*(4), 581–588.

Damiens, G., Gnassia-Barelli, M., Loquès, F., Roméo, M., & Salbert, V. (2007). Integrated biomarker response index as a useful tool for environmental assessment evaluated using transplanted mussels. *Chemosphere, 66*(3), 574–583.

Davenport, J., & Chen, X. (1987). A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis L.*). *Journal of Molluscan Studies, 53*, 293–297.

DeBoeck, M., Kirsch-Volders, M., & Lison, D. (2003). Cobalt and antimony: genotoxicity and carconogenicity. *Mutation Research, 533*, 135–152.

Deonarine, A., Bartov, G., Johnson, T. M., Ruhl, L., Vengosh, A., & Hsu-Kim, H. (2013). Environmental impacts of the Tennessee Valley Authority Kingston coal ash spill. 2. Effect of coal ash on methylmercury in historically contaminated river sediments. *Environmental Science & Technology, 47*(4), 2100–2108.

FDA. (1993). *Guidance document for arsenic (or cadmium, / chromium/lead/nickel) in shellfish*. Washington: Department of Health and Human Services, Public Health Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood.

FDA. (1996). *Fish, shellfish, crustaceans and other aquatic animals – fresh, frozen, or processed – methyl mercury (CPG 7108.07). Sec. 540.600 (rev 3/95). Compliance Policy Guides, August 1996. 237*. Washington: Department of health and Human Services, Food and Drug Administration.

Gascho Landis, A. M., Haag, W. R., & Stoeckel, J. A. (2012). High suspended solids as a factor in reproductive failure of a freshwater mussel. *Freshwater Science, 32*(1), 70–81.

Greeley, M. S., Jr., Elmore, L. R., McCracken, M. K., & Sherrard, R. M. (2014). Effects of sediment containing coal Ash from the Kingston Ash release on embryo-larval development in the fathead minnow, *Pimephales promelas* (Rafinesque, 1820). *Bulletin of Environmental Contamination and Toxicology, 92*(2), 154–159.

Gunther, A. J., Davis, J. A., Hardin, D. D., Gold, J., Bell, D., Crick, J. R., & Stephenson, M. (1999). Long-term bioaccumulation monitoring with transplanted bivalves in the San Francisco Estuary. *Marine Pollution Bulletin, 38*(3), 170–181.

Gustavson, K. E., Burton, G. A., Francingues, N. R., Jr., Reible, D. D., Vorhees, D. J., & Wolfe, J. R. (2008). Evaluating the effectiveness of contaminated-sediment dredging. *Environmental Science & Technology, 42*(14), 5042.

Henley, W. F., Patterson, M. A., Neves, R. J., & Lemly, A. D. (2000). Effects of sedimentation and turbidity on lotic food webs: a concise review for natural resource managers. *Reviews in Fisheries Science, 8*(2), 125–139.

Hopkins, W. A., Rowe, C. L., & Congdon, J. D. (1999). Elevated trace element concentrations and standard metabolic rate in banded water snakes (*Nerodia fasciata*) exposed to coal combustion wastes. *Environmental Toxicology and Chemistry, 18*(6), 1258–1263.

Hull, M. S., Cherry, D. S., & Neves, R. J. (2006). Use of bivalve metrics to quantify influences of coal-related activities in the Clinch River watershed, Virginia. *Hydrobiologia, 556*(1), 341–355.

Lafabrie, C., Pergent, G., Kantin, R., Pergent-Martini, C., & Gonzalez, J. L. (2007). Trace metals assessment in water, sediment, mussel and seagrass species–Validation of the use of *Posidonia oceanica* as a metal biomonitor. *Chemosphere, 68*(11), 2033–2039.

Liu, Y. T., Chen, T. Y., Mackebee, W. G., Ruhl, L., Vengosh, A., & Hsu-Kim, H. (2013). Selenium speciation in coal Ash spilled at the Tennessee Valley Authority Kingston Site. *Environmental Science & Technology, 47*(24), 14001–14009.

Lohner, T. W., Reash, R. J., Willet, V. E., & Rose, L. A. (2001). Assessment of tolerant sunfish populations (*Lepomis sp.*) inhabiting selenium-laden coal Ash effluents. *Ecotoxicology and Environmental Safety, 50*(3), 203–216.

Lourenço, J., Castro, B. B., Machado, R., Nunes, B., Mendo, S., Gonçalves, F., & Pereira, R. (2010). Genetic, biochemical, and individual responses of the teleost fish *Carassius auratus* to uranium. *Archives of Environmental Contamination and Toxicology, 58*(4), 1023–1031.

Mubiana, V. K., Vercauteren, K., & Blust, R. (2006). Influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in *Mytilus edulis*. *Environmental Pollution, 144*(1), 272–279.

Nagle, R. D., Rowe, C. L., & Congdon, J. D. (2001). Accumulation and selective maternal transfer of contaminants in the turtle *Trachemys scripta* associated with coal ash deposition. *Archives of Environmental Contamination and Toxicology, 40*(4), 531–536.

National Research Council (US) (2007) Committee on Sediment Dredging at Superfund Megasites. *Sediment dredging at Superfund megasites: assessing the effectiveness*. National Academies Press

O'Connor, T. P. (1996). Trends in chemical concentrations in mussels and oysters collected along the US coast from

1986 to 1993. *Marine Environmental Research, 41*(2), 183–200.

Osterling, M. E., Bergman, E., Greenberg, L. A., Baldwin, B. S., & Mills, E. L. (2007). Turbidity-mediated interactions between invasive filter-feeding mussels and native bioturbating mayflies. *Freshwater Biology, 52*(8), 1602–1610.

Otter, R. R., Bailey, F. C., Fortner, A. M., & Adams, S. M. (2012). Trophic status and metal bioaccumulation differences in multiple fish species exposed to coal ash-associated metals. *Ecotoxicology and Environmental Safety, 85*, 30–36.

Otter, R. R., Hayden, M., Mathews, T., Fortner, A., & Bailey, F. C. (2013). The use of tetragnathid spiders as bioindicators of metal exposure at a coal ash spill site. *Environmental Toxicology and Chemistry, 32*(9), 2065–2068.

Pampanin, D. M., Volpato, E., Marangon, I., & Nasci, C. (2005). Physiological measurements from native and transplanted mussel (Mytilus galloprovincialis) in the canals of Venice. Survival in air and condition index. *Comparative Biochemistry and Physiology A, 140*(1), 41–52.

Peltier, G. L., Wright, M. S., Hopkins, W. A., & Meyer, J. L. (2009). Accumulation of trace elements and growth responses in *Corbicula fluminea* downstream of a coal-fired power plant. *Ecotoxicology and Environmental Safety, 72*(5), 1384–1391.

Reash, R. J., Lohner, T. W., & Wood, K. V. (2006). Selenium and other trace metals in fish inhabiting a fly ash stream: implications for regulatory tissue thresholds. *Environmental Pollution, 142*(3), 397–408.

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*(3), 207–276.

Rodriguez-Mercado, J. J., & Altamirano-Lozano, M. A. (2013). Genetic toxicology of thallium: a review. *Drug and Chemical Toxicology, 36*(3), 369–383.

Roméo, M., Frasila, C., Gnassia-Barelli, M., Damiens, G., Micu, D., & Mustata, G. (2005). Biomonitoring of trace metals in the Black Sea (Romania) using mussels *Mytilus galloprovincialis. Water Research, 39*(4), 596–604.

Ruhl, L., Vengosh, A., Dwyer, G. S., Hsu-Kim, H., Deonarine, A., Bergin, M., & Kravchenko, J. (2009). Survey of the potential environmental and health impacts in the immediate aftermath of the coal ash spill in Kingston, Tennessee. *Environmental Science & Technology, 43*(16), 6326–6333.

Ruhl, L., Vengosh, A., Dwyer, G. S., Hsu-Kim, H., & Deonarine, A. (2010). Environmental impacts of the coal ash spill in Kingston, Tennessee: an 18-month survey. *Environmental Science & Technology, 44*(24), 9272–9278.

Saurel, C., Petersen, J. K., Wiles, P. J., & Kaiser, M. J. (2013). Turbulent mixing limits mussel feeding: direct estimates of feeding rate and vertical diffusivity. *Marine Ecology Progress Series, 485*, 105–121.

Schintu, M., Durante, L., Maccioni, A., Meloni, P., Degetto, S., & Contu, A. (2008). Measurement of environmental trace-metal levels in Mediterranean coastal areas with transplanted mussels and DGT techniques. *Marine Pollution Bulletin, 57*(6), 832–837.

Simon, O., Mottin, E., Geffroy, B., & Hinton, T. (2011a). Effects of dietary uranium on reproductive endpoints—fecundity, survival, reproductive success—of the fish *Danio rerio. Environmental Toxicology and Chemistry, 30*(1), 220–225.

Simon, O., Floriani, M., Cavalie, I., Camilleri, V., Adam, C., Gilbin, R., & Garnier-Laplace, J. (2011b). Internal distribution of uranium and associated genotoxic damages in the chronically exposed bivalve *Corbicula fluminea. Journal of Environmental Radioactivity, 102*(8), 766–773.

Sorensen, E. M. (1988). Selenium accumulation, reproductive status, and histopathological changes in environmentally exposed redear sunfish. *Archives of Toxicology, 61*(4), 324–329.

Souza, M. J., Ramsay, E. C., & Donnell, R. L. (2013). Metal accumulation and health effects in raccoons (*Procyon lotor*) associated with coal fly ash exposure. *Archives of Environmental Contamination and Toxicology, 64*(4), 529–536.

Stanley, J. K., Kennedy, A. J., Bednar, A. J., Chappell, M. A., Seiter, J. M., Averett, D. E., & Steevens, J. A. (2013). Impact assessment of dredging to remove coal fly ash at the Tennessee Valley Authority Kingston Fossil plant using fathead minnow elutriate exposures. *Environmental Toxicology and Chemistry, 32*(4), 822–830.

Strupp, C., & Beryllium metal II. (2011). A review of the available toxicity data. *Annals of Occuppational Hygiene, 55*(1), 43–56.

Tennessee Valley Authority (TVA) (2009) Action Memorandum: Request for Removal Action at the TVA Kingston Fossil Fuel Plant Release Site, Roane County, Tennessee. <http://www.tva.gov/kingston/memo.htm> (accessed 03.09.13)

Tennessee Valley Authority (TVA) (2010a) Regulatory Submittal for Kingston Fossil Plant: Summary of changes to surface water monitoring plan: surface water monitoring plan for the Emory, Clinch and Tennessee Rivers (non-time-critical removal action). <http://www.tva.gov/kingston/admin_record/pdf/NTC/NTC10.pdf>, (accessed 12.10.2014).

Tennessee Valley Authority (TVA) (2010b) Time critical removal action completion report for the river system phase II nature and extent of ash investigation. <http://www.epakingstontva.com/Work%20Plan%20Approvals/Nature%20and%20Extent%20Investigations/Time%20Critical%20Removal%20Action%20PhaseII%20Nature%20and%20Extent.pdf> (accessed 03.15.14)

Tennessee Valley Authority (TVA) (2011a) Fact Sheet, Kingston Ash Recovery Project, December 7, 2011a. < http://www.tva.gov/kingston/pdf/KIF_Fact_Sheet.pdf> (accessed 03.09.13)

Tennessee Valley Authority (TVA) (2011b) Regulatory Submittal for Kingston Fossil Plant: Non-time-critical removal action surface water monitoring plan EPA-AL-038. < http://www.tva.gov/kingston/admin_record/pdf/NTC/NTC27.pdf>, (accessed 12.10.2014)

Tsangaris, C., Hatzianestis, I., Catsiki, V. A., Kormas, K. A., Strogyloudi, E., Neofitou, C., & Galgani, F. (2011). Active biomonitoring in Greek coastal waters: application of the integrated biomarker response index in relation to contaminant levels in caged mussels. *Science of the Total Environment, 412*, 359–365.

United States Environmental Protection Agency (USEPA) (2004) Draft for selenium. Office of Water. EPA-822-D-04-001.

Wang, N., Ingersoll, C. G., Kunz, J. L., Brumbaugh, W. G., Kane, C. M., Evans, R. B., & Bakaletz, S. (2013). Toxicity of sediments potentially contaminated by coal mining and natural gas extraction to unionid mussels and commonly tested benthic invertebrates. *Environmental Toxicology and Chemistry, 32*(1), 207–221.

Yudovich, Y. E., & Ketris, M. P. (2005). Arsenic in coal: a review. *International Journal of Coal Geology, 61*(3), 141–196.

Yudovich, Y. E., & Ketris, M. P. (2006). Selenium in coal: a review. *International Journal of Coal Geology, 67*(1), 112–126.

Zhong, H., Kraemer, L., & Evans, D. (2013). Influence of body size on Cu bioaccumulation in zebra mussels *Dreissena polymorpha* exposed to different sources of particle-associated Cu. *Journal of Hazardous Material, 261*, 746–752.