CHANGES IN ALUMINUM, CADMIUM, CHROMIUM, COPPER, NICKEL, LEAD, AND ZINC CONCENTRATIONS IN WATER, SEDIMENT, AND FISH TISSUES OVER 2 YEARS FOLLOWING THE 2008 KINGSTON, TN COAL ASH SPILL

A Thesis
by
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Foreword

The thesis will be submitted to and has been prepared in accordance to *Environmental Science & Technology*, a peer-reviewed journal published by the American Chemical Society.

Abstract

CHANGES IN ALUMINUM, CADMIUM, CHROMIUM, COPPER, NICKEL, LEAD, AND ZINC CONCENTRATIONS IN WATER, SEDIMENT, AND FISH TISSUES OVER 2 YEARS FOLLOWING THE 2008 KINGSTON, TN COAL ASH SPILL. (August 2011)

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On December 22, 2008, TVA's sixty-foot wall enclosing several decades of stored wet coal fly ash (CFA) collapsed and released 4.13 million cubic meters of CFA into the Watts Bar Reservoir in Kingston, TN. CFA is known to contain toxic elements such as aluminum, cadmium, chromium, copper, nickel, lead, and zinc which can become bioavailable to the aquatic biota and pose a threat to the ecosystem. In this study, analysis of sediment, water, and fish samples was conducted from the spill and nearby sites for the elements listed above. The fish of interest in this study were the largemouth bass (*Micropterus salmoides*), redear sunfish (*Lepomis microlophus*), gizzard shad (*Dorosoma cepedianum*), and channel catfish (*Ictalurus punctatus*). Elevated levels of these metals were observed in the sediment for two years following the spill except for copper. The aluminum, copper, and lead contents in the water were initially elevated Above the EPA Criterion Continuous Concentration (EPA CCC) after the spill in January 2009, though these values decreased below the EPA CCC by May 2010. Elevations of these metals were observed in a subset of the fish tissues shortly after the spill, though our study suggests that these levels were not sustained and are not of toxicological concern.

Dedication

This thesis is dedicated to my grandfathers, who have both passed away.

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Introduction

The Tennessee Valley Authority (TVA) Kingston Fossil Plant is a coal-fired power plant that generates 10 billion kilowatt-hours of electricity each year providing energy for about 670,000 homes while consuming approximately 4.6 x 10^6 metric tons per year when operating at full capacity (*1*). On December 22, 2008, TVA's 18 meter wall enclosing several decades of stored wet coal fly ash (CFA) collapsed, releasing 4.13 x 10^6 m^3 of CFA into the surrounding land and Emory River in Kingston, TN (*1*). The Emory River joins the Clinch River 3.2 km downstream of the spill and then converges with the Tennessee River 6.4 km further downstream, forming the Watts Bar Reservoir (Figure 1).

CFA is known to contain elevated levels of toxic elements such as As, Cd, Cr, Cu, Ni, Pb, and Se (*2*). Given the appropriate environmental conditions, these elements can become bioavailable and may pose a threat to the aquatic ecosystems in the Emory, Clinch, and Tennessee Rivers. Some of these environmental conditions include: water hardness, pH, and chelating agents present in the system such as dissolved organic matter (*3, 4*). Metals exist in their ionic form under acidic conditions, becoming dissolved in the water column and can be readily transported across biological membranes such as fish gills and intestinal lining (*2, 5, 6*). Ca^{2+} and Mg^{2+} can compete against other metal cations for biotic ligands such as gill epithelial surfaces and intestinal linings, saturating potential binding sites to reduce toxic effects (*7, 8*). Under alkaline conditions, these toxic elements can form soluble or insoluble complexes with organic and inorganic substrates forming carbonates, oxides, chlorides, and hydroxides (*4, 5, 6*). These suspended species are biologically unavailable due to precipitation and thus its removal from mode of action. The purpose of this study was to provide a two-year assessment of the potential accumulation of toxic elements in fish tissue and their associated impacts on the population from the TVA spill. The research focused on measurements of toxic elements in sediments, water, and fish samples in the vicinity of the CFA spill. This study will contribute to the better understanding of toxic element mobilization in a large lotic and lentic aquatic ecosystem, namely the Watts Bar reservoir and the contributing Emory and Clinch River systems.

Analytical Methods and Materials

Fish, sediment, and water samples were collected from the Emory, Clinch, and the Tennessee Rivers in east Tennessee, USA on a quarterly basis. Samplings were conducted at five routine sites in these rivers including: Emory River Mile 4.0 (ERM4.0), ERM2.0, Clinch River Mile 5.5 (CRM5.5), CRM3.5, and the Tennessee River Mile 567 (TRM567) (Figure 1). Samples were collected on January 2009 (17 days after the spill), March 2009 (88 days after the spill), July 2009 (228 days after the spill), January 2010 (379 days after the spill), May 2010 (499 days after the spill), September 2010 (676 days after the spill), and January 2011 (793 days after the spill).

Fish samples were collected with approval from the Appalachian State University Institutional Animal Care Use and Committee (IACUC) protocol #09-6 using an electroshocking boat, identified to species, external pathologies noted, and total lengths recorded. Fish selected for body burden analysis were tagged with site specific colors, stored on ice for transport, and taken to the Appalachian State University (ASU) toxicology lab. The fish selected for analysis were the adult largemouth bass (*Micropterus salmoides*), redear sunfish (*Lepomis microlophus*), gizzard shad (*Dorosoma cepedianum*), and channel catfish (*Ictalurus punctatus*). In the lab, fish weight and length were recorded and dissected for liver (L), muscle (Mu), gonads (testes, T; ovaries, O), stomach (St), gastric caecum (GC), and spleen (Sp). The tissues were individually wrapped in aluminum foil, frozen at -20°C, lyophilized, and digested with 10 mL 70% OmniTrace nitric acid (EMD Inc., North America) and aid of a MARSXpress (CEM, Matthews, NC) following protocol EPA 3051b

(*9*). The minimum sample weight of fish tissue to digest and analyze using the ICP-OES was determined to be 0.5g (dry wt). This reduced the sample size for many of the smaller organs in *L. microlophus* including the gonads, spleen, liver, and stomach. These organs from the same species, site, and day were combined to form composite samples when necessary; however, some samples were too small to measure accurately. Winter sampling also resulted in reduced sample sizes as fish were most likely deeper habitats that were out of reach of electroshocking (>4 meters).

Sediment samples (*n* =1-3 per site) were collected using an Ekman dredge grab sampler, homogenized, and stored in Whirl-Paks® on ice for transport. Sediment samples were frozen at -20°C, lyophilized, split into triplicates, and digested following protocol EPA 3051b (*9*).

Water samples were collected for dissolved metals by using a 0.4 µm pore size syringe filter (Whatman plc., North America) at a depth of 30 cm. Total recoverable metal water samples were obtained using 250mL plastic containers at a depth of 30cm using the grab sampling method. The samples were immediately fixed with 2.5mL of 70% OmniTrace nitric acid and stored on ice for transport. In the lab, the samples were split into 40 mL triplicates and digested following protocol EPA 3015 with 10 mL of 70% OmniTrace nitric acid and aid of a MARSXpress microwave reactor (*10*).

All samples were analyzed for aluminum (Al), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) concentrations by SW-846 EPA method 6010C (*11*).

Gill analyses were conducted on the *M. salmoides*, *L. microlophus*, and *I. punctatus* collected on January 2009. Gills were removed in the field, fixed in 4% paraformaldehyde solution, and stored on ice for transport. In the lab, gills were microtome cut to 7 µm sections, stained with hematoxylin and eosin, and examined using a light microscope with image capture capability.

Due to heteroscedasticity of our data, they were first ranked by the Wilcoxon signed-rank test, followed by a non-parametric analysis of variance using the general linear model (GLM) using SAS 9.2 (SAS Institute INC., Cary, NC). The differences were then determined using Tukey’s Studentized Range test (HSD). Graphs were composed using Minitab 16.1.0 (Minitab Inc., State College, PA).

Results and Discussion

Sediment Contamination. Al concentrations were found at the highest concentrations, ranging from 106±59 to 12287±1257 mg/kg at TRM567 in March 2009 and at CRM3.5 in September 2009, respectively. These values are consistent with the high background values found in the soils (9363±3475 mg/kg) and in the CFA composition (14109±7264 mg/kg) (*12*). In January 2011, low values of Al were seen at ERM4.0 while the highest values of Al were seen at the downstream TRM567, suggesting the downstream movement of the ash-laden sediment and its Al contents. This observed movement of Al may be a result of the natural fluvial movement of the system, re-suspension and subsequent re-entrainment due to dredging and flooding events.

Cd concentrations were between 0.01 mg/kg at ERM4.0 and 0.22±0.01 mg/kg at CRM3.5 in January 2009 (Table 1). In March 2009, an increase in Cd concentration was observed at all sites, ranging from 0.6±0.1 to 12±7 mg/kg at CRM3.5 and ERM4.0, respectively. However, these high values found in March 2009 were not sustained, returning to similar values to those in January 2009 by July 2009. Again in January 2011, the lowest values were found at the upstream ERM4.0 while the highest values of Cd were observed at TRM567.

In January 2009, Cr concentrations were initially below the background levels of soils (11.9±5.3 mg/kg) ranging from 0.5±0.1 to 3.9±0.1 mg/kg at ERM4.0 and ERM2.0, respectively (Table 1). In March 2009 (concurrent with start of dredging activities), these values increased greatly, ranging from 20±20 to 35±7 mg/kg at ERM4.0 and TRM567, respectively. The Cr values never decrease again to those comparable to January 2009 through the two-year sampling. In January 2011, the value at ERM4.0 was 9.1±8.4 mg/kg, about half the concentration observed at the downstream ERM 2.0 (17±4 mg/kg), CRM3.5 (17±1 mg/kg), and TRM567 (18±1 mg/kg). This again may suggest the downstream movement of ash-laden sediment and its associated metals.

Cu levels were initially high in January 2009 around the spill site at ERM4.0 (46±51 mg/kg), ERM2.0 (43±20 mg/kg), and ERM0.5 (47±29 mg/kg), about twice the value observed downstream at CRM3.5 (20±5 mg/kg) and TRM567 (24±14 mg/kg) (Table 1). These values were above the background levels of soil (15.9±35.2 mg/kg), but consistent with the levels in the ash (42.2±16.6 mg/kg). A drastic decrease was observed at ERM4.0 in July 2009 (9±11 mg/kg) and at ERM2.0 (5.9±0.4 mg/kg) in January 2010; this may suggest the downstream movement of Cu or removal by dredging.

Ni showed a trend similar to that observed in Cr; initial concentrations in January 2009 were below the background levels in soil (4.4±4.7 mg/kg), ranging from 0.7±0.4 to 3.8±0.7 mg/kg at ERM4.0 and ERM2.0, respectively (Table 1). There was a statistical increase in March 2009 (concurrent with dredging activities), where values ranged from 19±3 to 46±30 mg/kg at TRM567 and ERM4.0, respectively (see supporting document). These values decreased at ERM4.0 by July 2009, where the values are about half of those seen at ERM2.0 and the downstream sites, suggesting the movement of uncontaminated sediments from upstream.

Pb values were initially between 1.2±0.4 and 4±1 mg/kg in January 2009 at TRM567 and ERM0.5, respectively, well below the background concentration for both nearby soils (16.5±8.4 mg/kg) and ash compositions (19±7 mg/kg) (Table 1). In March 2009, the Pb concentrations were significantly elevated and remained elevated throughout the two-year samplings, ranging from 7±1 mg/kg at ERM2.0 in January 2010 to 20±2 mg/kg at ERM2.0 in July 2009 (Table 1). In January 2011, the highest values are seen at the downstream TRM567 (18±2 mg/kg) while the lowest value was observed at ERM4.0 (9±6 mg/kg), suggesting the downstream movement of the ash-laden sediments and its associated Pb contents.

Zn values were all initially below instrument detection limit and below the background soils (29.7±1.4 mg/kg) at all sites (Table 1). Subsequently, the values at all sites were elevated in March 2009, where values ranged from 38±11 mg/kg to 92±11 mg/kg at ERM4.0 and TRM567, respectively. These values remained elevated throughout the two-year sampling. In January 2011, the highest values were seen at TRM567 (72±11 mg/kg), while the lowest values were observed at ERM4.0 (26±11 mg/kg), again, suggesting the downstream movement of the ash-laden sediment.

During May 2010 and the increased discharge, a common trend can be seen in the sediment load in all elements where a successive increase in concentration was observed from upstream to downstream (Table 1). This supports the movement of metals downstream due to re-suspension and subsequent re-entrainment of sediments during flooding periods.

In conclusion, concentrations of Cd, Cr, Ni, Pb, and Zn increased during the dredging period, which commenced in March 2009. Subsequently in all metals observed, values decreased at the upstream sites, especially at ERM4.0, as uncontaminated sediment moved in from further upstream. A concomitant increase in the downstream sites at CRM3.5 and TRM567 was observed as the ash-laden sediments moved downstream from ERM4.0 and ERM2.0. A common trend was seen in all elements at CRM5.5, where changes in concentration were not observed as expected. CRM5.5 is 3.2 km downstream of the Emory

River spill site and 2.4 km upstream from the confluence of the Clinch and Emory Rivers; very little coal ash made it this far, which was visually confirmed during sampling.

Water Contamination. Water chemistry results indicate that the Clinch River before merging with the Emory River is moderately hard to hard while that of the Emory River is soft to slightly hard (Table 2) (*13*). The Tennessee River has slightly hard to moderately hard water, most likely due to dilution by the Emory River. The pHs of these rivers are similar to each other, ranging from 6.89 to 8.83 (Table 2). Under these conditions, the toxic metals can form soluble and insoluble complexes with substrates which exhibit limited bioavailability due to the formation of less reactive compounds (*5*). Due to the amount of suspended ash in the river, turbidity at ERM2.0 in January 2009 was at 40550 NTU, over 2000 times higher than CRM5.5; turbidity at ERM2.0 subsequently decreased to comparable values to the other collection sites by March 2009 (Table 2). Normal episodes of increased turbidity can be observed (e.g. May 2010), corresponding to the increase in discharge following rainfall events (Table 2) (*14*).

Immediately following the spill, Al concentrations were elevated for both total recoverable and dissolved water samples, exceeding the EPA criterion continuous concentration (EPA CCC) of 87µg/L (Table 3) (*15*). The EPA CCC is the recommended water criterion for a given contaminant for the protection of aquatic life and is based on the dissolved portion of water samples for chronic exposure (*15*). In January 2009, total recoverable Al concentrations ranged from 1662±42 to 4489±62 µg/L and the dissolved values ranged from 1169±24 to 2182±20 µg/L. Subsequent data suggests a decrease which was evident in March 2009 and which continued over the two year period to concentrations below the EPA CCC; by January 2011, the maximum total recoverable Al and dissolved aluminum were 389±49 and 56±2 µg/L, respectively.

Total recoverable Cd concentrations were highest during the July 2009 sampling (concurrent with dredging activities); the highest observed value was 14.1±0.3 µg/L (Table 3). By January 2011, maximum total recoverable Cd values had decreased to 0.4±0.1 µg/L. The dissolved Cd concentrations ranged from 0.14±0.04 to 5.6±0.1 µg/L, above the EPA CCC of 0.25µg/L at some of the sites. Despite the EPA CCC of 0.25µg/L, the bluegill sunfish's (*Lepomis macrochirus*) one-year exposure to 31µg/L dissolved Cd had no effect on survival, while concentrations of 80-239 µg/L reduced survival rates by greater than 50% (*14*). Similarly, 120 day exposure to 80 µg/L dissolved Cd had no effect on *M. salmoides* survival or growth (*16*). These values are well above the observed values during this research and should not be of toxicological concern.

Chromium can become acutely toxic to aquatic fish at dissolved Cr concentrations between 24-72 mg/L depending on the duration and sensitivity of the species of interest (*17*). The EPA CCC for Cr is 74 µg/L (*15*), although the highest dissolved Cr level observed during the two-year study was 1.8±0.1 µg/L (Table 3), well below the toxic threshold of even the most sensitive species (*17*). Total recoverable Cr concentration reached 344±23 µg/L, five days after the spill in December 2008 at ERM2.0, three orders of magnitude greater than the concentration seen in January 2011. However, there is no EPA CCC for total recoverable metals; these values were also not sustained, decreasing to 4±1 µg/L by January 2009 and should not be of concern.

Dissolved Cu concentrations were initially elevated in January 2009 at CRM5.5 and ERM2.0 with values of 28±17 and 32±2 µg/L, respectively, which are both above the EPA

CCC of 9 µg/L (Figure 3). These values were not sustained at either site, decreasing to concentrations of below the instrument detection limit at CRM5.5 and ERM2.0, respectively, by March 2009 and should not be of toxicological concern. Total recoverable Cu value in December 2008 at ERM2.0 was 1025±121 µg/L, three orders of magnitude greater than the concentration seen in January 2011. However, by January 2009 the concentration had decreased to 46±5 µg/L and continued to decrease over the two year period with seasonal spikes.

Total recoverable Ni concentrations ranged from 363±43 to 2.5±0.5 µg/L in December 2008 and January 2011, respectively, decreasing two orders of magnitude over the two-year period. Dissolved Ni concentrations ranged from 0.8±0.6 to 4.9±0.8 µg/L, well below the EPA CCC value of 52µg/L (*15*) and should not be of toxicological concern.

The current EPA CCC for Pb is 2.5µg/L though subject to change due to current investigations (*15*). Our observed values for dissolved Pb exceeded this value in September 2010 with maximum values of 27±15 µg/L at ERM2.0 (Table 3). In the *L. macrochirus*, chronic laboratory flow-through exposure to 775µg/L Pb for 660 days had no effect on survival, growth, or reproduction (*17*). In conclusion, further investigation should be conducted to fully assess the chronic exposure of Pb to fish and narrow down the broad range of thresholds to species specific values.

The dissolved Zn concentrations measured in September 2010 showed elevated levels (92±29 µg/L) close to the EPA CCC (120 µg/L), though the subsequent values in January 2011 show lower levels (Table 3) (*15*). Elevated levels were not sustained and did not exceed the EPA CCC over time so it should not be of toxicological concern.

Fish Tissue Results. ***Dorosoma cepedianum*****.** Whole *D. cepedianum* samples were only processed for May 2010 and September 2010 collections. At ERM4.0, significant differences were seen in Cr, Ni, and Pb concentrations. Mean Cr values increased from 0.9 to 6.1 mg/kg, mean Ni values increased from 0.4 to 3.6 mg/kg, and Pb values increased from 1.2 to 3.2 mg/kg (see supporting documents). At ERM2.0, significant differences were observed in Cr, Cu, and Ni concentrations. Mean Cr values increased from 1 to 14 mg/kg, mean Cu concentrations increased from 6.8 to 11.5 mg/kg, and Ni concentrations increased from 0.2 to 7.4 mg/kg (see supporting documents). No significant differences in whole body burdens were observed at CRM3.5. At TRM567, significant differences in whole body burdens were observed again in Cr, Cu, Ni, and Pb. Mean Cr concentrations increased from 0.7 to 11.2 mg/kg, mean Cu concentrations increased from 6.7 to 9.3 mg/kg, mean Ni concentrations increased from 0.8 to 5.4 mg/kg, and mean Pb concentrations increased from 1.4 to 4.7 mg/kg (see supporting documents).

At ERM2.0, *D. cepedianum* muscle samples were available in July 2009 and May 2010; during this time significant differences were observed in Cr, Cu, Pb, and Zn. Mean Cr concentrations increased from 0.4 to 1.4 mg/kg, mean Cu concentrations increased from 1 to 4 mg/kg, mean Pb concentrations increased from 0.2 to 1.8 mg/kg, and mean Zn concentrations increased from 16 to 36 mg/kg (see supporting documents). At CRM3.5, significant differences in muscle burdens were observed in Cr, Cu, Ni, Pb, and Zn. Mean Cr concentrations increased from 0.3 to 1.2 mg/kg, mean Cu concentrations increased from 1.7 to 4.3 mg/kg, mean Ni concentrations increased from 0.2 to 0.9 mg/kg, mean Pb concentrations increased from 0.3 to 0.9 mg/kg, and mean Zn concentrations increased from 0 to 30 mg/kg (see supporting documents).

Cr values at ERM2.0 and CRM3.5 in the muscle tissue had mean values of 1.4 and 1.2 mg/kg, respectively, in January 2010. Muscle Cr burdens of 1.61 mg/kg in the sensitive Rainbow trout (*Oncorhynchus mykiss*) had no effect on survival and should not be of concern for the local fish mortality (*18*). Mean Cu muscle burdens reached values of 3.7±0.3 and 4.3±2.3 mg/kg at ERM2.0 and CRM3.5, respectively. Cu concentrations of 3.4 mg/kg had no effect on survival, growth, or reproduction in the *O. mykiss* and should not be of concern (*19*). Mean Ni muscle burdens reached values of 0.37±0.04 and 0.9±0.6 mg/kg at ERM2.0 and CRM3.5, respectively. Ni concentrations of 0.82 mg/kg had no effect on survival in the *O. Mykiss* (*18*); in the common carp (*Cyprinus carpio*), muscle burdens of 46.7 mg/kg showed no effect on survival and should not be of concern (*20*). Mean Pb muscle values reached values of 1.8 mg/kg and 0.9 mg/kg at ERM2.0 and CRM3.5, respectively. Mean Zn concentrations in the muscles ranged from 36±7 to 31±10 mg/kg at ERM2.0 and CRM3.5 respectively. In the *M. salmoides*, 100 mg/kg is the known tolerance limit for human consumption and should not be of concern (*21*).

In conclusion Cr, Cu, Ni, and Pb accumulated in the whole *D. cepedianum* samples since the spill. Accumulation of these elements was also seen in the muscle samples, suggesting that the muscles may be the site of sequestration for these metals.

Ictalurus punctatus**.** Histological gill sections of the *I. punctatus* caught during the January 2009 sampling revealed edema, vasodilation, epithelial proliferation, lamellar epithelium lifting, and lamellar fusion at ERM2.0 (Figure 2). This may be a result of the overall increase in suspended particles and dissolved metals in the water column at ERM2.0 during this time. The *I. punctatus* had the highest concentrations of Al in ovaries, liver, and spleen compared to the other species, though differences are not statistically significant (see supporting documents). This may be associated with their benthic habitat and exposure through direct contact with the ash laden sediment which has been shown in our study to contain high levels of Al (Table 1).

***Lepomis microlophus*.** Cd and Cu concentrations were significantly higher in the liver compared to the other organs (Figure 3). Cd accumulated in the tissues of interest in the following hierarchy: L > GC > Sp > St > T > O > Mu. Cu accumulated in the tissues in the following order: L > St > GC > Sp > T > O > Mu. Cd and Cu are known to accumulate in the liver tissue as a mechanism to maintain their homeostasis; their accumulation stimulates the production of metallothionein (MT) which sequester Cd and Cu to prevent its toxicity (*22, 23, 24*). However, despite the significant increase in the liver, the hepatic concentrations of Cd were low ranging from 0.15 to 1.54 mg/kg. These values are well below the acute toxicityLC_{50} in the closely related *L. macrochirus* of 55 mg/kg and may not be of toxicological concern (*25*). In contrast, Cu exhibited relatively high values ranging from 6.9 to 95 mg/kg. Hepatic concentrations of 96 mg/kg in *L. macrochirus* have shown reductions in survival, growth, and reproduction (*17*), posing a potential threat to fish such as those collected at CRM5.5 where values reached 95±12 mg/kg in January 2010. However, these values subsequently decreased to 63±16 mg/kg in September 2010 and 69±41 mg/kg in January 2011, escaping toxic threshold levels. Interestingly, CRM5.5 should have been minimally impacted by the CFA spill due to its location (Figure 1) which is supported by the copper content of the sediment there; therefore, it is possible migration of downstream populations up to this site is contributing to the high values observed in the *L. microlophus* liver. No other significant changes were observed in the *L. microlophus* liver at ERM4.0, ERM2.0, CRM3.5, or TRM567.

The *L. microlophus* feed on benthic organisms including microcrustaceans, larvae, snails, and filter-feeding fingernail clams (*26*). It is intuitive that their benthic feeding strategy may also result in the direct consumption of sediments, further exposing themselves to harmful contaminants. Though statistically indifferent, the *L. microlophus* had the highest concentrations of all metals examined in the GC compared to the other species of interest (see supporting documents). This may indicate the importance of the GC to this species in maintaining metal homeostasis.

In the *L. microlophus* muscle tissues, significant differences in toxic metal burdens were not observed at ERM2.0, CRM5.5, or TRM567 during the two-year sampling. At ERM4.0, significant differences were seen in muscle aluminum concentrations between the January 2009, May 2010, and September 2010 collections; these values decreased from 64 mg/kg, to 30 mg/kg, to 9 mg/kg, respectively (Figure 4). These numbers correspond to decreasing dissolved and total Al concentrations in the water (Table 1) and the observed trend could have resulted from decreased exposure or from immigration of previously unexposed fish due to the return of natural habitats. During winter, the Watts Bar Reservoir lowers the water level by approximately 2 m for flood control purposes, resulting in the loss of littoral habitats and the forced migration into the deeper sections of the system (*27*). Water levels rise again in May, allowing the fish to migrate into various sites. The *L. microlophus* muscle and liver Al burdens were regressed against the sediment Al concentrations because they are thought to have the highest site tenure among the species of interest. The linear correlation coefficient resulted in an R^2= 0.01 for muscle and R^2=0.00008 for liver, indicating that body burdens of lead had little dependence on local sediment concentrations and that the migration of fish may be responsible for the decrease in muscle Al burdens observed along with decreased exposure (see supporting documents). At CRM3.5, a significant difference in Al muscle concentrations was observed between January and September 2010; the values increased from 5±2 to 18±9 mg/kg and remained at these levels through January 2011, representing a 3.6 fold increase. There was also an increase in total Al concentration in the water at CRM3.5 in May 2010, between January 2010 and September 2010. The increase observed in the muscle tissues at CRM3.5 likely resulted from the increase in ambient exposure, leading to bioconcentration [bioconcentration factor (BCF) of 11x] and sequestration into the muscle tissues. Alternatively and/or in combination with the increase in ambient exposure, contaminated food availability may have contributed to this observed trend. Food availability was likely low in the winter season prior to the collection in January 2010, resulting in decreased exposure through lower consumption rates. During the spring and summer season preceding the collection in September 2010, the fish had ample opportunity to forage, which potentially lead to the increase in Al bioconcentration (*28*).

***Micropterus salmoides*.** Similar to the *L. microlophus*, Cd and Cu concentrations were the highest in the liver compared to the other organs of interest (Figure 3). The accumulation into the various organs for Cd was in the following hierarchy: L > GC > Sp > T > O > St > Mu. The accumulation into organs for Cu was in the following order: L > O > St > GC > T > Sp > Mu.

M. salmoides did not exhibit significant differences in tissue metal levels or levels that raise toxicological concerns except for muscle Al concentrations at ERM4.0 and CRM5.5. A significant decrease in Al muscle burdens were seen at ERM4.0 where values dropped from 34±3 to 7±3 mg/kg between January 2009 and May 2010 (Figure 4). Again, these numbers correspond to decreasing dissolved and total Al concentrations in the water

(Table 1) and the observed trend could have resulted from decreased exposure or from immigration of previously unexposed fish due to the return of natural habitats. CRM5.5 showed an increasing trend with concentrations peaking in September 2010 with an average of 14±8 mg/kg (see supporting documents). Again, this site is minimally impacted by the CFA spill and except for ERM4.0 had consistently had the lowest sediment levels of Al, so fish migration from areas of higher exposure may be responsible for the levels of fish contamination at this site.

Comparing a collection at ERM14.0, 20 km upstream of the spill to ERM2.0 at the spill site in March 2009, a difference in bioconcentration factor was observed for Cr and Zn based on available dissolved water and muscle samples. A bioconcentration factor of 4321 and 4242 were observed at ERM14.0 for Cr and Zn, respectively. A bioconcentration factor of 182 and 586 were observed at ERM2.0 for Cr and Zn, respectively, despite the higher concentration of the two metals dissolved in the water column at ERM2.0 and similar water hardness. This suggests increased competition for uptake by the biotic ligands at ERM2.0 where higher levels of metals were present.

The inability to detect differences in concentration over time may be due to the extensive home range occupied by *M. salmoides*. In systems with abundant course woody habitats (CWH), *M. salmoides* will generally maintain small home ranges in the littoral regions and exhibit a sit-and-wait strategy to conserve energy (*27*). In systems like the Watts Bar reservoir, human influences (e.g. removal of woody debis and winter draw down of water levels) have greatly altered the littoral regions for lakeshore residential development and recreational uses and thereby reduced the CWH in the littoral regions. In areas with low

CWH, the *M. salmoides* will shift their foraging strategy to an active searching method and hence increase their home range (*27*).

Conclusion. In January 2011, two years following the spill, a common trend can be seen in the sediments among all metals; the upstream sites have lower values than the downstream ones, suggesting the importance of fluvial movement of the sediments and associated metals. Dissolved metal concentrations in the water were initially high in January 2009, exceeding the EPA CCC for Al, Cu, and Pb, though by our March 2009 sampling all metals examined were below the EPA CCC. In the *D. cepedianum*, Cr, Cu, Ni, Pb, and Zn body burdens were elevated at the sites proximal to the spill. *I. punctatus* gill histopathology showed alterations in gill morphology consistent with toxic metal exposure in the proximity to the spill. An episode of increased hepatic Cu burdens were observed at CRM5.5 in the *L. microlophus* indicating that they may have migrated away from the spill impacted zone. The *M. salmoides* caught at ERM14.0 had a higher BCF compared to those caught at ERM2.0 for Cr and Zn, suggesting increased competition for uptake by the biotic ligands at ERM2.0 where higher levels of metals were present. The long term consequences on development and reproduction are uncertain given the complexity of the possible interactions of the metals released into this system and should be further examined.

The Watts Bar reservoir is an extensive system that encompasses 1,162 km of shoreline and over 156,192 km^2 of water surface (*29*). Despite the never-before-seen amount of CFA entering the system, the sheer size of this system in tandem with the discharge from the three contributing rivers (20,350 cfs) and migration of fishes may dilute the effects of the contamination to prevent adverse long-term consequences (*24*, *28*). The constant migrations of fish populations have repopulated initial losses of communities following the spill (see supporting documents).

Though the TVA spill was catastrophic, it created a large scale field study that will ultimately lead to the better understanding of the effects of large scale CFA releases and its associated toxic elements on open aquatic ecosystems.

TABLE 1. Average Metal Concentrations (mg/kg) in Sediments Associated with the TVA Coal Ash Spill Area in Kingston, TN.

		Days Since Spill							background****		IDL
		17	88**	228	379	499***	676	793	soils	ash	µg/L
Al*	CRM5.5	NA	124.1±3.4	NA	6030±1685	6737±620	9012±1904	6008±1695			
	ERM4.0	208±28	106±59	NA	4733±4036	6731±2507	3887±1765	4715±4010			
	ERM2.0	1453±27	193±84	NA	9191±931	8383±508	11588±27	9167±969	9363 ±3485	14109 ±7264	
	ERM0.5	NA	NA	NA	NA	N A	NA	NA			100
	CRM3.5	1508±40	167±25	5204±4934	8830±341	11323±673	12287±1257	8807±345			
	TRM567	1224±49	160±20	9577±710	10114±599	NA	11299±600	10122±522			
Cd	CRM5.5	NA	0.71±0.02	0.6±0.4	1.3±0.1	1.2±0.1	0.52±0.03	0.3±1			
	ERM4.0	0.01	12±7	0.2±0.3	NA	0.6±0.2	0.3±0.1	0.2±0.1			
	ERM2.0	0.11±0.04	0.6±0.06	0.66±0.03	0.76±0.04	0.6±0.2	0.5±0.1	0.4±0.1	0.01 ±0.21	0.03 ±0.08	
	ERM0.5	0.07±0.06	NA	0.6±0.5	1.5±0.1	NA	0.5±3	NA			0.2
	CRM3.5	0.22±0.01	0.6±0.1	0.61±0.06	1.4±0.1	1.2±0.1	0.5	0.37±0.04			
	TRM567	0.1	16±8	0.6±0.1	1.5±0.1	1.5±0.3	0.6±0.1	0.5±0.1			
Cr	CRM5.5	NA	18.6±0.3	17	13.7	14	16	15			
	ERM4.0	0.5±0.1	20±20	7.7±8.1	NA	10±8	14±9	9.1±8.4			
	ERM2.0	3.9±0.1	30±15	36±2	8.7±0.2	11±5	23±6	17±4	11.9 ±5.3	24.8 ±7.6	
	ERM0.5	3.3±0.1	NA	18±3	19±2	NA	21.9±0.1	NA			0.4
	CRM3.5	3.3±0.1	24±2	25±3	17±2	20±1.6	22±2	17±1			
	TRM567	2.5±0.2	35±7	18±4	20±3	22±3	20±2	18±1			
Cu	CRM5.5	NA	NA	16±4	14±1	14±1	16±4	15±5			
	ERM4.0	46±31	NA	9±11	NA	15±15	21±11	13±16			
	ERM2.0	43±20	46±30	52±4	5.9±0.4	17±7	34±11	21±5	15.9 ±35.2	46.2 ±16.6	
	ERM0.5	47±29	NA	26±2	26±1	NA	30.2±0.2	NA			4.4
	CRM3.5	20±5	39±4	33±2	26±1	30±5	37±2	31±5			
	TRM567	24±14	35±7	25±10	24±1	34±13	32±2	31±2			
Ni	CRM5.5	NA	18±1	15±1	13±1	14±1	17±4	11±1			
	ERM4.0	0.7±0.4	46±30	10±11	NA	10±5	15±8	10±7			
	ERM2.0	3.8±0.7	27±8	26±3	9.1±0.3	12±6	22±4	19±3	4.4 ±4.7	23 ±8	
	ERM0.5	3.1±0.2	NA	19±1	19±2	NA	23.1±0.2	NA			0.6
	CRM3.5	2.9±0.2	23±1	22±1	17.6±0.4	19±1	21±3	17±1			
	TRM567	2.01±0.28	19±3	15±4	16±1	30±25	22±2	19±1			
Pb	CRM5.5	NA	17.7±0.1	15±1	12.9±0.1	15±1	17±1	14±3			
	ERM4.0	2±1	9±5	8±7	NA	8±4	10±5	9±6			
	ERM2.0	3±1	19±6	20±2	7±1	9±4	15±4	13±2	16.5 ±8.4	19 ±7	
	ERM0.5	4±1	NA	13±2	13.4±0.2	NA	17.3±0.2	NA			4.5
	CRM3.5	2±1	18±2	14±1	13.2±0.4	14±1	18±3	15±2			
	TRM567	1.2±0.4	18±2	17±1	14±3	31±37	17±1	18±2			
Zn	CRM5.5	NA	73±2	71±5	53±1	64±7	62±7	52±7			
	ERM4.0	IDL	38±11	33±30	NA	27±10	43±16	26±11			
	ERM2.0	IDL	69±41	54±4	31±2	34±15	56±12	53±10	29.7 ±1.4	40.4 ±12.2	2.9
	ERM0.5	IDL	NA	57±11	56±2	NA	65±1	NA			
	CRM3.5	IDL	79±9	80±48	50±2	58±3	69±8	56±5			
	TRM567	IDL	92±11	73±11	61±2	92±28	76±9	72±11			

Concentrations are reported in mg/kg dry weight and are reported as mean value ± standard deviation. Elements are indicated as aluminum (Al), cadmium, (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). IDL refers to the instrument detection limit reported in µg/L and NA refers to samples that were not analyzed. Collections dates are indicated as 17 days since spill (January 2009), 88 days since spill (March 2009), 228 days since spill (July 2009), 379 days since spill (January 2010), 499 days since spill (May 2010), 676 days since spill (September 2010), and 793 days since spill (January 2011). * Indicates elements that had values above the highest standard and were extrapolated from the slope; ** indicates when dredging commenced, *** indicates when dredging was terminated; and **** indicates background levels of the nearby soils and coal ash composition that were collected and analyzed by the Tennessee Department of Environment and Conservation and the Tennessee Department of Health.

TABLE 2. Water Chemistry Results at Sites Associated with the TVA Coal Ash Spill Area in Kingston, TN.

		Days Since Spill						
		17	88**	228	379	499***	676	793
CRM5.5	total hardness (mg/L)	NA	110±4	98±1	193±2	97±2	123±4	51±1
	dissolved hardness (mg/L)	NA	140±2	NA	NA	92±1	103±2	64±1
	pH	7.45	8.08	7.63	7	7.87	8.42	7.8
	temperature (°C)	9.2	12.5	27.6	7.3	23.1	23.7	7.54
	turbidity (NTU)	20	13	6	5.17	47.3	4.5	10.6
	conductivity (µS/cm)	NA	306	232.1	259.4	213.7	242.2	277
	discharge (cfs)*	10900	3560	925	1970	8010	384	2670
ERM4.0	total hardness (mg/L)	NA	NA	40±1	37±2	NA	102±3	27±2
	dissolved hardness(mg/L)	NA	NA	NA	NA	25±1	88±2	28.2±0.2
	pH	6.93	NA	7.64	7.68	7.09	8.15	6.89
	temperature (°C)	8.4	NA	28	2.69	21.3	24.4	4.69
	turbidity (NTU)	21	NA	11	15.4	13.8	NA	7
	conductivity (µS/cm)	54.4	NA	104.8	65.1	48.3	199	55.6
	discharge (cfs)*	4420	1970	503	1130	2660	21	1220
ERM2.0	total hardness (mg/L)	NA	22±7	54.1±0.4	43.8±0.3	34±1	117±10	26±2
	dissolved hardness (mg/L)	NA	21±1	NA	NA	23±2	111±3	28.7±0.2
	pH	6.72	7.98	8.15	7.33	6.82	8.37	6.9
	temperature (°C)	9.3	14.1	28.8	3.2	19.2	24.2	5.01
	turbidity (NTU)	40550	13.5	12	13.4	58	3	8.2
	conductivity (µS/cm)	289	63.25	134.7	73.5	49.6	245.3	55.7
	discharge (cfs)*	4420	1970	503	1130	2660	21	1220
CRM3.5	total hardness (mg/L)	NA	101±16	91±2	185±96	51±1.4	121±1	51±1
	dissolved hardness (mg/L)	NA	123±17	NA	NA	47±5.2	104.8±0.4	63.8±0.4
	pH	7.41	8.44	7.64	7.28	7.81	8.2	7.86
	temperature (°C)	9	12.3	29.5	6.9	20	25.7	7.5
	turbidity (NTU)	NA	14.83	7	5.63	59.6	4.5	6.7
	conductivity (µS/cm)	188	261.73	218.3	244.1	177.1	241	278
	discharge (cfs)*	15320	5530	1428	3100	10670	405	3890
TRM567	total hardness (mg/L)	NA	NA	80±2	71±1	65±1	99±14	110±4
	dissolved hardness (mg/L)	NA	NA	NA	NA	65±1	75±3	52.8±0.2
	pH	7.42	NA	8.11	6.98	8.83	7.81	7.7
	temperature (°C)	9.6	NA	26	5.3	21.6	24.1	7.93
	turbidity (NTU)	NA	NA	7	15.4	17.5	5	12.9
	conductivity (µS/cm)	157.2	162.4	186	159.7	138.2	160.1	211
	discharge (cfs)*	NA	NA	NA	NA	NA	NA	NA
ERM14.0	total hardness (mg/L)	NA	20±2	NA	NA	NA	NA	NA
	dissolved hardness (mg/L)	NA	19.5±0.1	NA	NA	NA	NA	NA
	pH	NA	8.02	NA	NA	NA	NA	NA
	temperature (°C)	NA	11.3	NA	NA	NA	NA	NA
	turbidity (NTU)	NA	7	NA	NA	NA	NA	NA
	conductivity (µS/cm)	NA	60.7	NA	NA	NA	NA	NA
	discharge (cfs)*	4420	1970	503	1130	2660	21	1220

Total hardness and dissolved hardness are reported in mg/L, as mean value ± standard deviation, and were calculated using [Ca (mg/L)*2.417] + [Mg (mg/L)*4.116]. Temperatures are reported in degrees Celsius (°C), turbidity measurements are reported in Nephelometric Turbidity Units (NTU), conductivities are reported as microsiemens per cm, and discharges were reported as cubic feet per second. Collections dates are indicated as 17 days since spill (January 2009), 88 days since spill (March 2009), 228 days since spill (July 2009), 379 days since spill (January 2010), 499 days since spill (May 2010), 676 days since spill (September 2010), and 793 days since spill (January 2011). * Indicates discharge data that was provided by the United States Geological Survey National Water Information System (*30*), ** indicates when dredging commenced, and *** indicates when dredging was terminated.

TABLE 3. Average Metal Concentrations (µg/L) in Total Recoverable and Dissolved Metals in Water Samples at Sites Associated with the TVA Coal Ash Spill Area in Kingston, TN.

		Total Recoverable Metals in Water Samples								Dissolved Metals in Water Samples						
		Days Since Spill								Days Since Spill						
		5	17	88**	228	379	499***	676	793	17	88**	499***	676	793	IDL	EPACCC
Al *	CRM5.5	NA	NA	169±3	265±89	181±37	497±52	230±22	198±42	1793±51	177	56±9	224±1	27±2	100	87
	ERM4.0	NA	1662±42	NA	256±6.5	575±16	NA	177±13	345±35	1422±24	NA	62±3	102±34	41±1		
	ERM2.0	3300172±354303	4489±62	188±64	853±28	575±16	1608±187	180±20	261±46	1978±26	94	41±17	110±45	56±2		
	ERM0.1	480304±164205	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
	CRM3.5	NA	3091±805	210±72	191±7	217±33	859±134	289±36	336±24	1169±24	101	69±15	106±28	15±1		
	TRM567	NA	4516±78	NA	253±14	232±12	483±12	192±51	383±49	2182±20	NA	49±7	46±3	32±2		
Cd	CRM5.5	NA	0.51±0.04	0.4±0.1	0.01	1.1±0.2	0.7±0.3	7.1±0.1	0.3±0.1	IDL	0.21±0.02	0.3±0.1	5.6±0.1	0.2±0.1	0.2	0.25
	ERM4.0	NA	0.5±0.1	NA	14.1±0.3	0.8±0.1	NA	7.5±0.2	0.4±0.1	IDL	IDL	0.17±0.04	1.4±0.1	0.3±0.1		
	ERM2.0	8.3±0.5	0.5±0.2	0.3±0.1	10±3	0.9±0.1	0.8±0.1	7.1±0.2	0.2±0.1	0.15±0.06	IDL	0.14±0.04	1.3±0.1	0.33±0.04		
	ERM0.1	1.3±0.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
	CRM3.5	NA	0.481±0.004	0.4±0.1	13.5±0.2	0.7±0.1	0.8±0.1	8±0.4	0.4±0.1	IDL	IDL	0.21±0.02	3.1±0.1	0.26±0.04		
	TRM567	NA	0.5±0.1	NA	12.3±0.3	1.6±1.2	0.8±0.2	8.1±0.1	0.4±0.1	0.2±0.1	IDL	0.2±0.1	1.7±0.3	0.3±0.1		
Cr	CRM5.5	NA	0.88±0.08	0.8±0.2	9±1	3.8±1.1	3.3±0.3	8.3±0.2	2.1±0.3	1.9±0.1	0.9±0.3	1.5±0.4	2.2±0.2	0.41±0.04	0.4	74
	ERM4.0	NA	0.68±0.04	NA	9.7±0.7	2.8±0.5	NA	8.6±0.5	3.2±2.2	0.58±0.07	NA	1.1±0.2	4.1±2.8	0.3±0.1		
	ERM2.0	344±23	4±1	0.6±0.1	6.3±2.4	4±1	5.4±0.7	10±1	2.9±0.4	1.8±0.1	2±1	0.8±0.4	5.9±2.3	0.2±0.1		
	ERM0.1	49±14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
	CRM3.5	NA	0.89±0.16	1.1±0.3	9.8±0.4	4.1±1.5	4.1±0.6	9.6±0.9	3.2±2.2	0.48±0.05	1.6±0.9	1.4±0.6	2.2±0.2	0.26±0.03		
	TRM567	NA	1.5±0.3	NA	5.7±0.9	4.6±2.1	4±1	9±1	2.7±0.4	0.64±0.03	NA	0.6±0.1	1.7±0.3	0.5±0.1		
Cu	CRM5.5	NA	33±42	3.7±2.1	16±4	7.7±1.8	9.5±2.8	6.9±0.3	2.2±0.2	28±17	IDL	NA	7.8±0.5	1.2±0.1	4.4	9
	ERM4.0	NA	IDL	NA	18±2	10±3	NA	4.6±0.3	2.4±0.2	IDL	NA	NA	4.1±0.5	1.2±0.5		
	ERM2.0	1025±121	46±5	5.7±1.3	14±4	8.7±2.6	12±4	6.7±0.2	2.6±1.5	32±2	IDL	NA	5±1	1.6±0.3		
	ERM0.1	95±19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
	CRM3.5	NA	23±11	4±2	16±1	10±2	8.5±1.6	8.3±1.5	3.2±2.2	IDL	IDL	NA	5.74±0.01	1.4±0.1		
	TRM567	NA	18±11	NA	12±2	7.9±2.6	10±3	5±2	45±2.4	IDL	NA	NA	2.7±0.3	1.3±0.2		
Ni	CRM5.5	NA	2.7±0.2	2.2±0.4	4.5±0.3	4.2±1.3	4±1	6.7±0.1	2.8±0.4	2.9±0.2	1.5±0.5	NA	2.8±0.1	1.7±0.1	0.6	52
	ERM4.0	NA	2.7±0.8	NA	4.3±0.5	5.1±0.6	5±1	7±1	2.5±0.5	1.99±0.28	NA	2.5±0.4	1.7±0.4	1.2		
	ERM2.0	363±43	4±1	3.3±0.5	4±1	3.8±0.7	6±1	6.7±0.1	2.6±0.4	4.1±0.4	0.8±0.6	3.1±0.5	4.9±0.8	1.6±0.3		
	ERM0.1	46±12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
	CRM3.5	NA	3.2±0.4	2.1±0.6	5±1	2.9±0.3	5±1	6.7±0.2	2.5±0.5	2.4±0.4	2±2	3.2±0.5	2.3±0.4	1.4±0.1		
	TRM567	NA	3.7±0.1	NA	2.1±0.3	2.8±0.6	4.7±1.7	6.6±0.9	2.5±1.2	2.3±0.2	NA	2.8	2.2±0.8	1.3±0.2		
Pb	CRM5.5	NA	IDL	IDL	3±1	8.5±0.9	2.7±1.3	9±2	7.9±3.9	2.9±0.2	IDL	NA	2.7±1.5	1.3±0.4	4.5	2.5
	ERM4.0	NA	IDL	NA	4.3±2.2	1.7	NA	7.4±1.4	15±7	IDL	NA	5.8±3.8	11±4.8	0.6±0.1		
	ERM2.0	313±44	6.8±2.3	IDL	6.6±0.7	9.2±7.2	5.2±3.2	22±5	12±4	IDL	IDL	4.1±1.8	27±15	0.6		
	ERM0.1	29±6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
	CRM3.5	NA	5.5±2.8	5.4±2.8	8±2	10±8	1.8±0.9	17±6	19±13	IDL	IDL	1.82	8.2±1.6	1.3±1.1		
	TRM567	NA	IDL	NA	13	4±3	8.3±2.3	10±3	17±4	IDL	NA	4.2±1.9	4.3±2.5	0.8		
Zn	CRM5.5	NA	67±25	69±19	306±199	45±5	46±3	59±14	43±22	28.1±0.2	18±6	NA	NA	15	2.9	120
	ERM4.0	NA	93±55	NA	64±5	37±8	NA	59±10	87±17	29.7±0.2	NA	70±4	NA	1.6±1.5		
	ERM2.0	619±22	53.4±2.7	170±84	51±10	41±3	60±13	65±11	40±15	22.4±0.3	40±25	57±6	NA	4.4±0.9		
	ERM0.1	164±45	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
	CRM3.5	NA	63±24	107±35	50±37	40±9	52±9	64±13	37±22	25±5	29±18	37	NA	IDL		
	TRM567	NA	107±81	NA	670±66	84±41	73±15	121±69	24±6	32.5±0.1	NA	73±10	92±29	1.1±0.3		

Concentrations are reported in µg/L and are reported as mean value ± standard deviation. Elements are indicated as aluminum (Al), cadmium, (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). IDL refers to the instrument detection limit reported in µg/L; EPA CCC refers to the Environmental Protection Agency Criterion Continuous Concentration for chronic exposure, and NA refers to samples that were not analyzed. Collections dates are indicated as 5 days since spill (December 2008), 17 days since spill (January 2009), 88 days since spill (March 2009), 228 days since spill (July 2009), 379 days since spill (January 2010), 499 days since spill (May 2010), 676 days since spill (September 2010), and 793 days since spill (January 2011). * Indicates samples that were above the highest standard and were extrapolated from the slope, ** indicates when dredging activities commenced, and *** indicates when dredging was terminated.

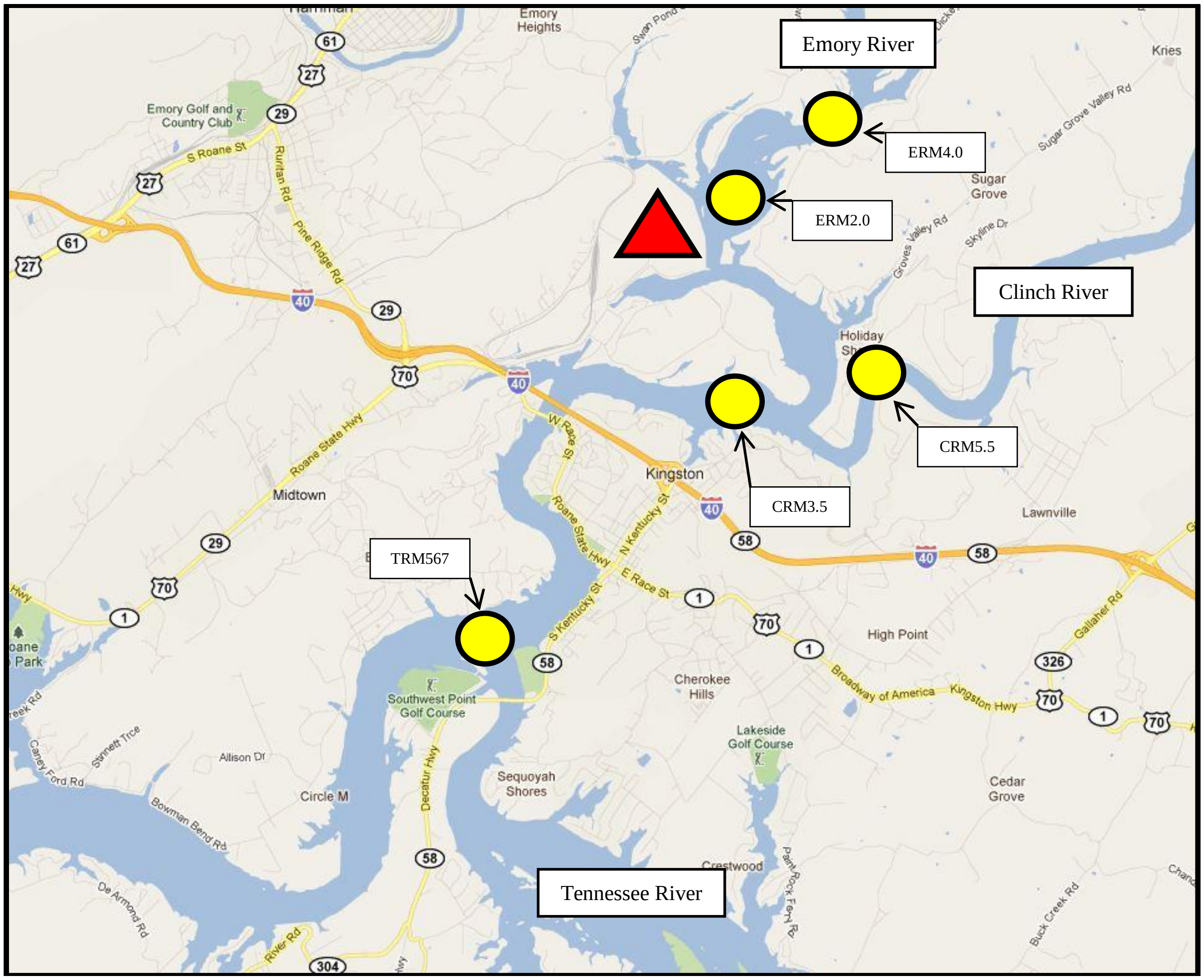

FIGURE 1. Map of the Collection Sites Associated with the TVA CFA Spill Area in Kingston, TN. The spill site is indicated by the triangle and the collection sites are indicated by the circles. Two collection sites are located on the Emory River, Emory River Mile 2.0 and 4.0 (ERM2.0 and ERM4.0); two collection sites are located on the Clinch River, Clinch River Mile 3.5 and 5.5 (CRM3.5 and CRM5.5), and one collection site is located on the Tennessee River, Tennessee River Mile 567 (TRM567). The map was provided by Google Maps (http://maps.google.com).

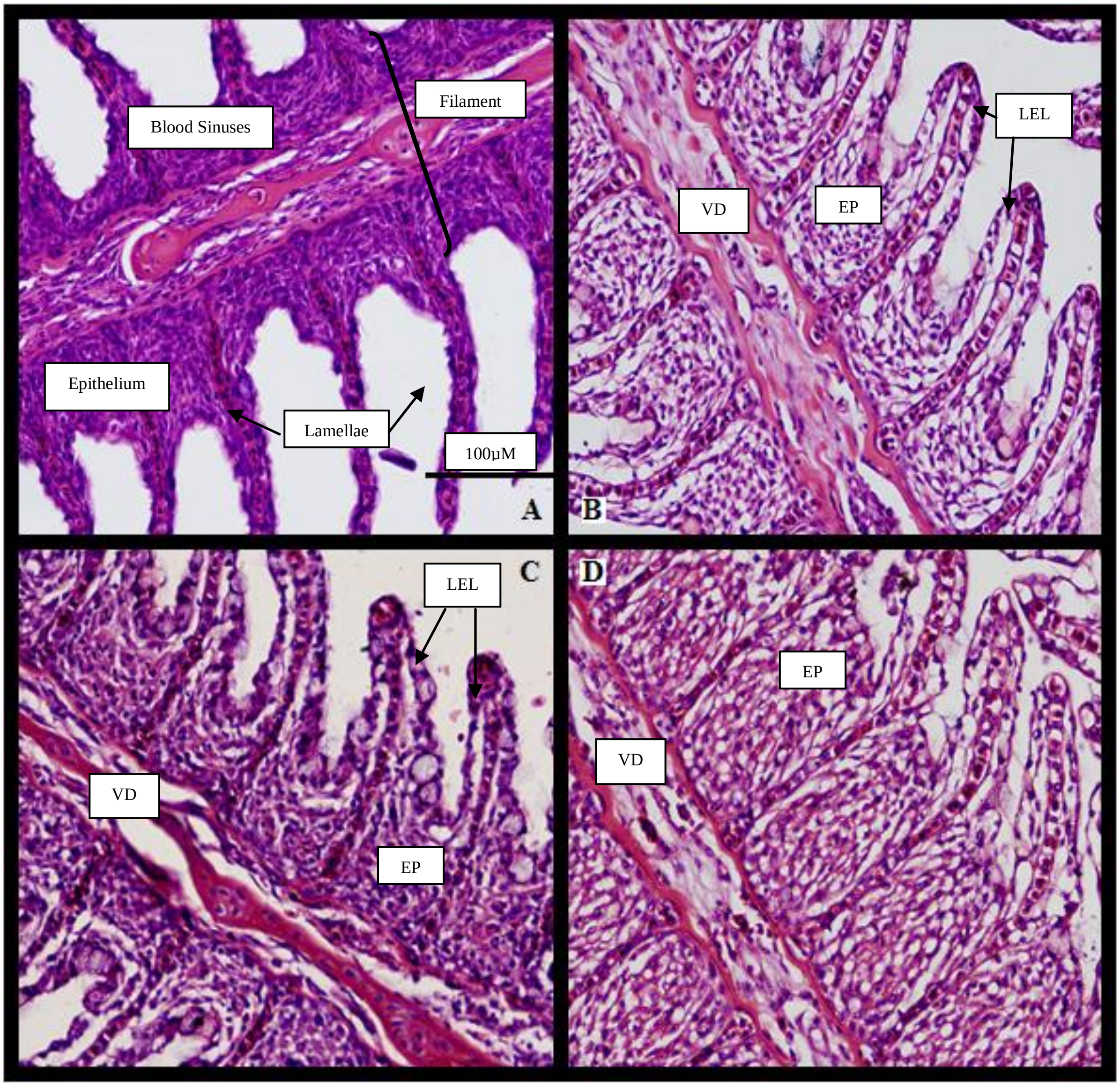

FIGURE 2. Histological Gill Sections at 10x magnification of *I. punctatus* from Sites Associated with the TVA Coal Ash Spill Area in Kingston, TN. (A) *I. punctatus* gill section from Tennessee River Mile 567 (TRM567) and (B-D) *I. puctatus* gill sections from the spill site at Emory River Mile 2.0 (ERM 2.0). (A) shows normal tissue arrangement, while the gills from the 3 ash exposed *I. punctatus* (B-D) all express pathology consistent with toxic metal exposure including edema, vasodilation (VD), epithelial proliferation (EP), lamellar epithelium lifting (LEL), and lamellar fusion (LF).

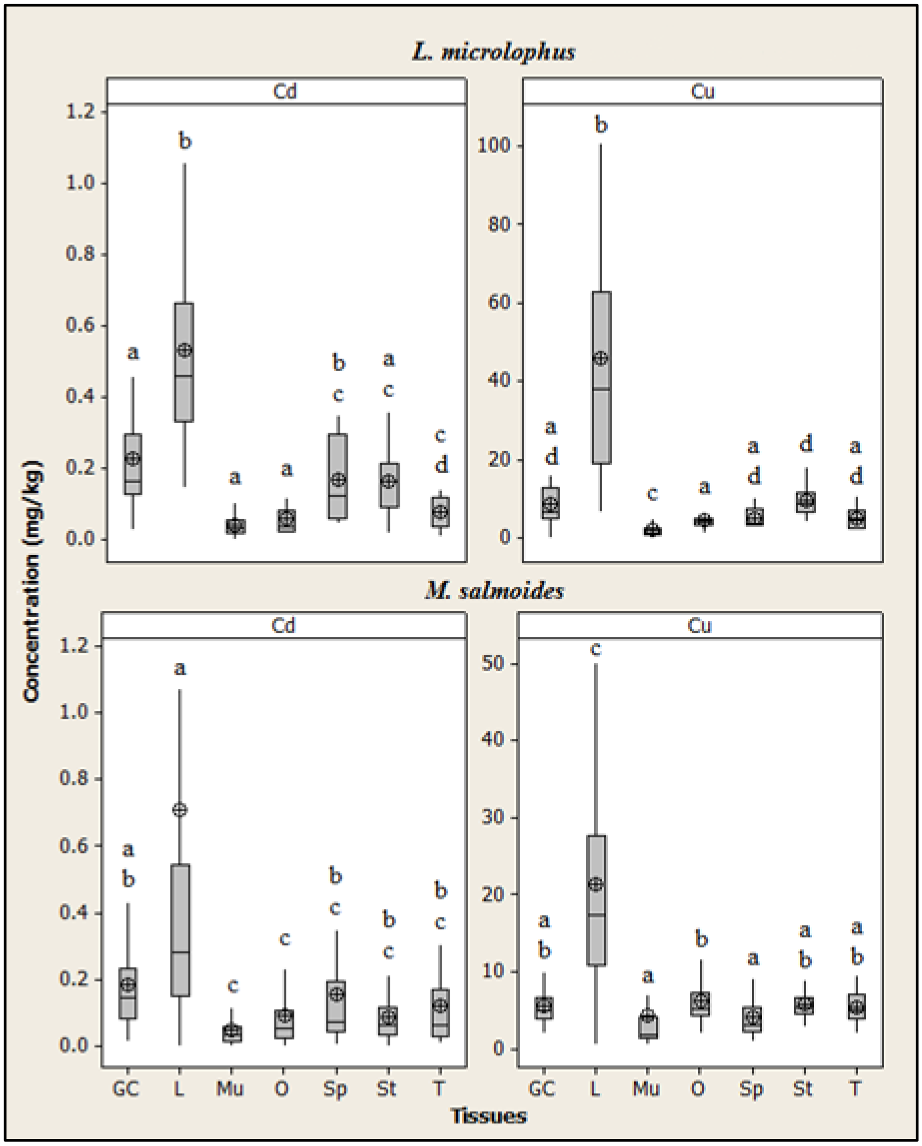

FIGURE 3. ***L. microlophus* and *M. salmoides* Cadmium and Copper Organ Burdens (mg/kg) Including all Collection Dates and Sites Associated with the TVA Coal Ash Spill Area in Kingston, TN.** Elements are indicated as cadmium (Cd) and copper (Cu) and all concentrations are reported in mg/kg dry weight. Organs are indicated as gastric caecum (GC), liver (L), muscle (Mu), ovaries (O), spleen (Sp), stomach (St), and testes (T). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means. Organs with different letters (a,b,c,d) indicate statistical differences among groups at $p < 0.05$.

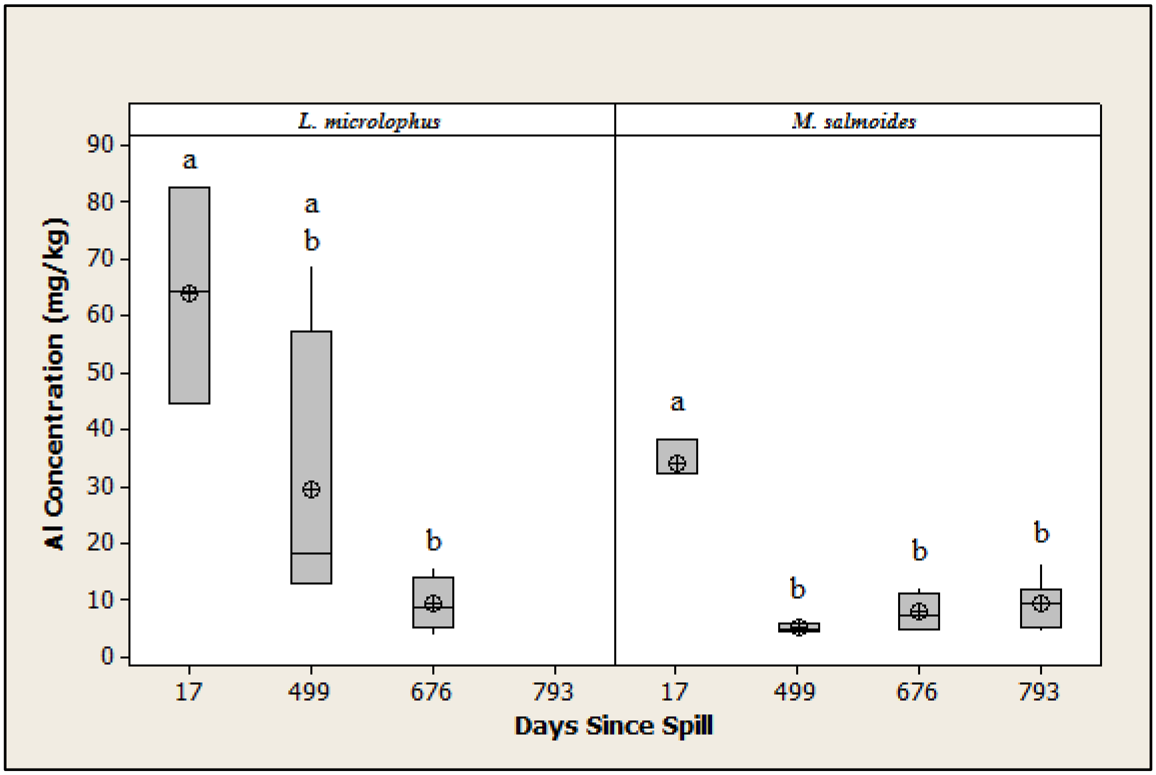

FIGURE 4. *L. microlophus* and *M. salmoides* Muscle Aluminum Concentrations (mg/kg) Over the Two Year Period at Emory River Mile 4.0 (ERM4.0) Associated with the TVA Coal Ash Spill Area in Kingston, TN. Aluminum is indicated as Al and all concentrations are reported in mg/kg dry weight. Collections dates are indicated as 17 days since spill (January 2009), 499 days since spill (May 2010), 676 days since spill (September 2010), and 793 days since spill (January 2011). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means. Days with different letters (a,b) indicate statistical differences among groups at $p < 0.05$.

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Supporting Documents

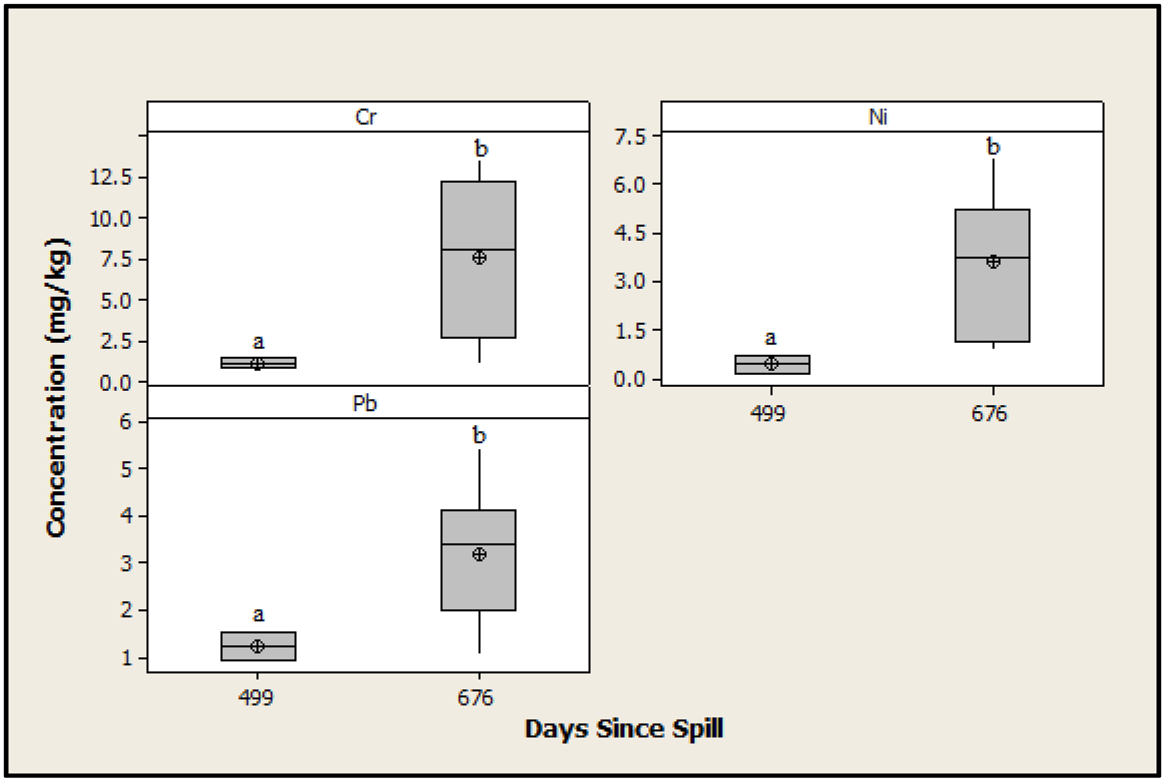

FIGURE S1. ***D. cepedianum*** **Chromium, Nickel, and Lead Whole Body Burdens (mg/kg) at Emory River Mile 4.0 (ERM4.0) Associated with the TVA Coal Ash Spill Area in Kingston, TN.** Elements are indicated as chromium (Cr), nickel (Ni), and lead (Pb) and all concentrations are reported in mg/kg dry weight basis. Collection dates are indicated as 499 days since spill (May 2010) and 676 days since spill (September 2010). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means. Days with different letters (a,b) indicate statistical differences among groups at $p < 0.05$.

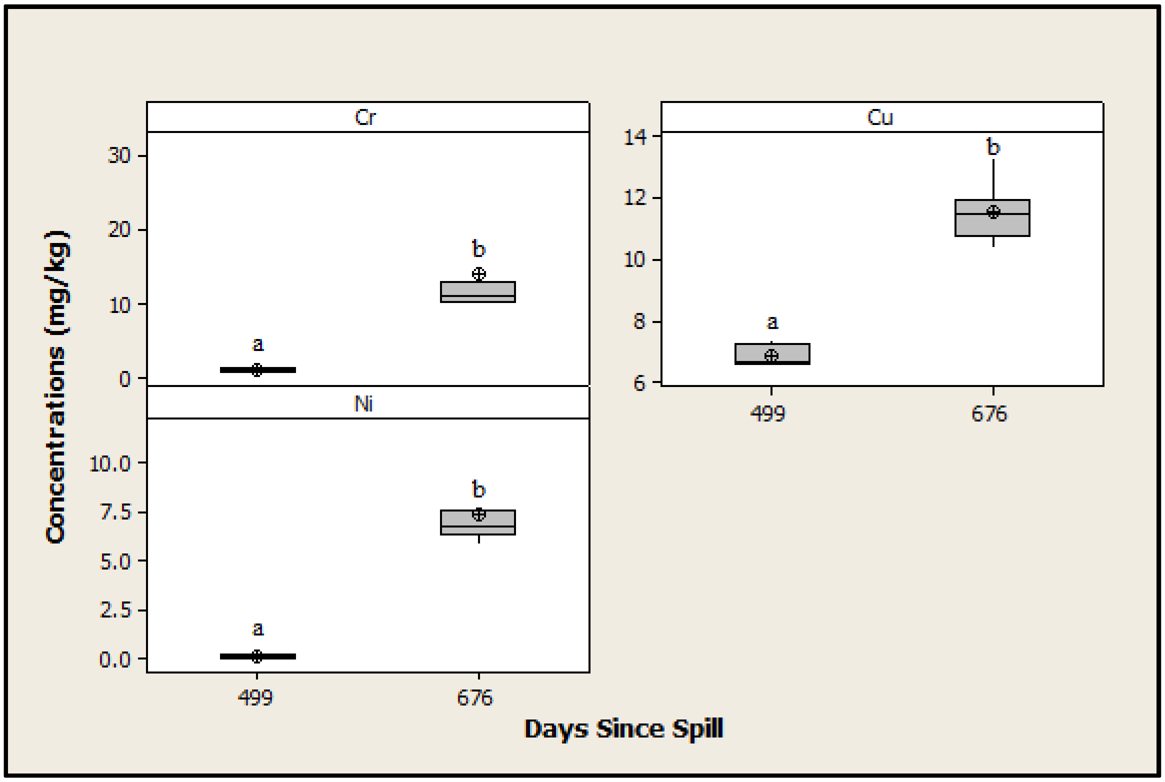

FIGURE S2. ***D. cepedianum*** **Chromium, Copper, and Nickel Whole Body Burdens (mg/kg) at Emory River Mile 2.0 (ERM2.0) Associated with the TVA Coal Ash Spill Area in Kingston, TN.** Elements are indicated as chromium (Cr), copper (Cu), and nickel (Ni) and all concentrations are reported in mg/kg dry weight basis. Collection dates are indicated as 499 days since spill (May 2010) and 676 days since spill (September 2010). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means. Days with different letters (a,b) indicate statistical differences among groups at $p < 0.05$.

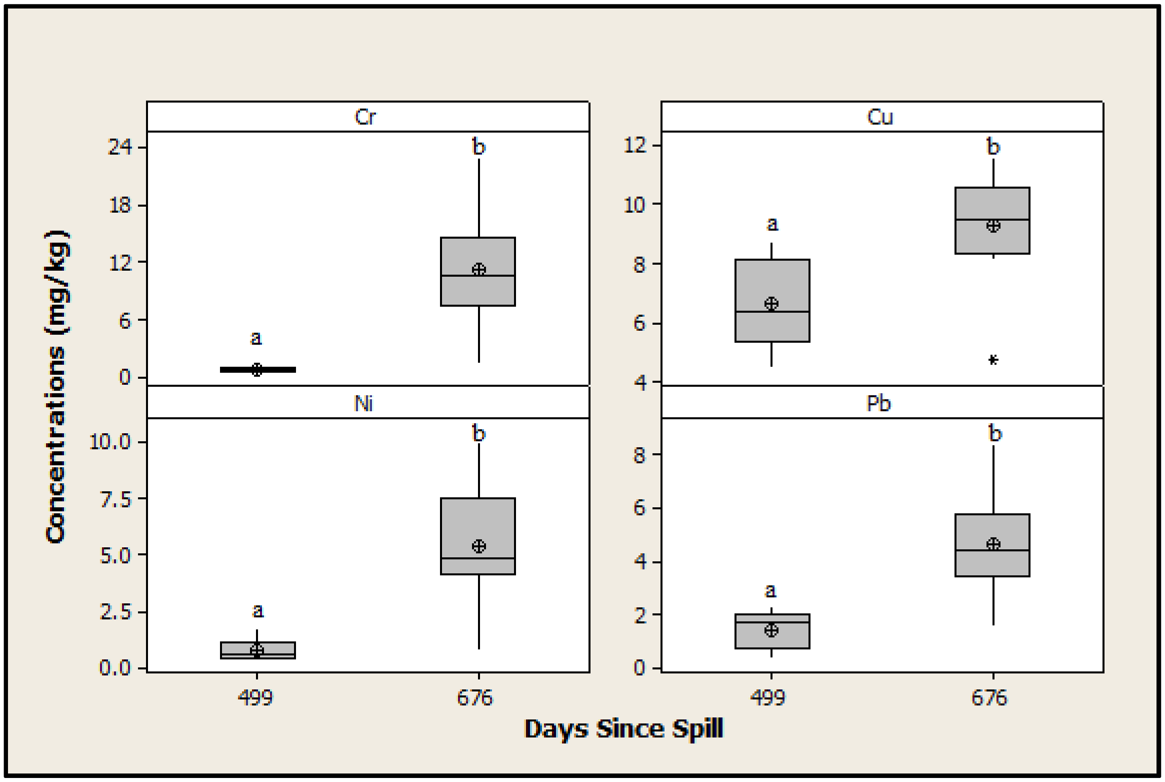

FIGURE S3. *D. cepedianum* Chromium, Copper, Nickel, and Lead Whole Body Burdens (mg/kg) at Tennessee River Mile 567 (TRM567) Associated with the TVA Coal Ash Spill Area in Kingston, TN. Elements are indicated as chromium (Cr), copper (Cu) nickel (Ni), and lead (Pb) and all concentrations are reported in mg/kg dry weight basis. Collection dates are indicated as 499 days since spill (May 2010) and 676 days since spill (September 2010). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means. Days with different letters (a,b) indicate statistical differences among groups at $p < 0.05$.

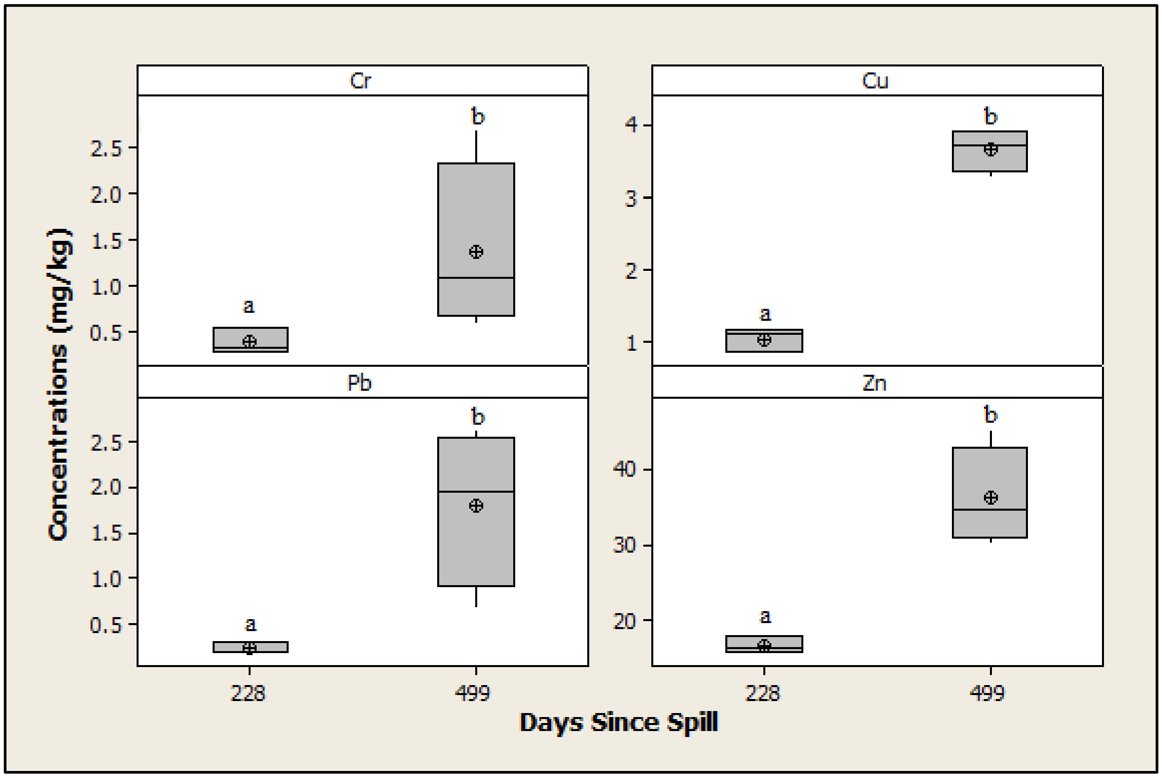

FIGURE S4. *D. cepedianum* Chromium, Copper, Lead and Zinc Muscle Burdens (mg/kg) at Emory River Mile 2.0 (ERM2.0) Associated with the TVA Coal Ash Spill Area in Kingston, TN. Elements are indicated as chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) and all concentrations are reported in mg/kg dry weight basis. Collection dates are indicated as 228 days since the spill (July 2009) and 499 days since spill (May 2010). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means. Days with different letters (a,b) indicate statistical differences among groups at $p < 0.05$.

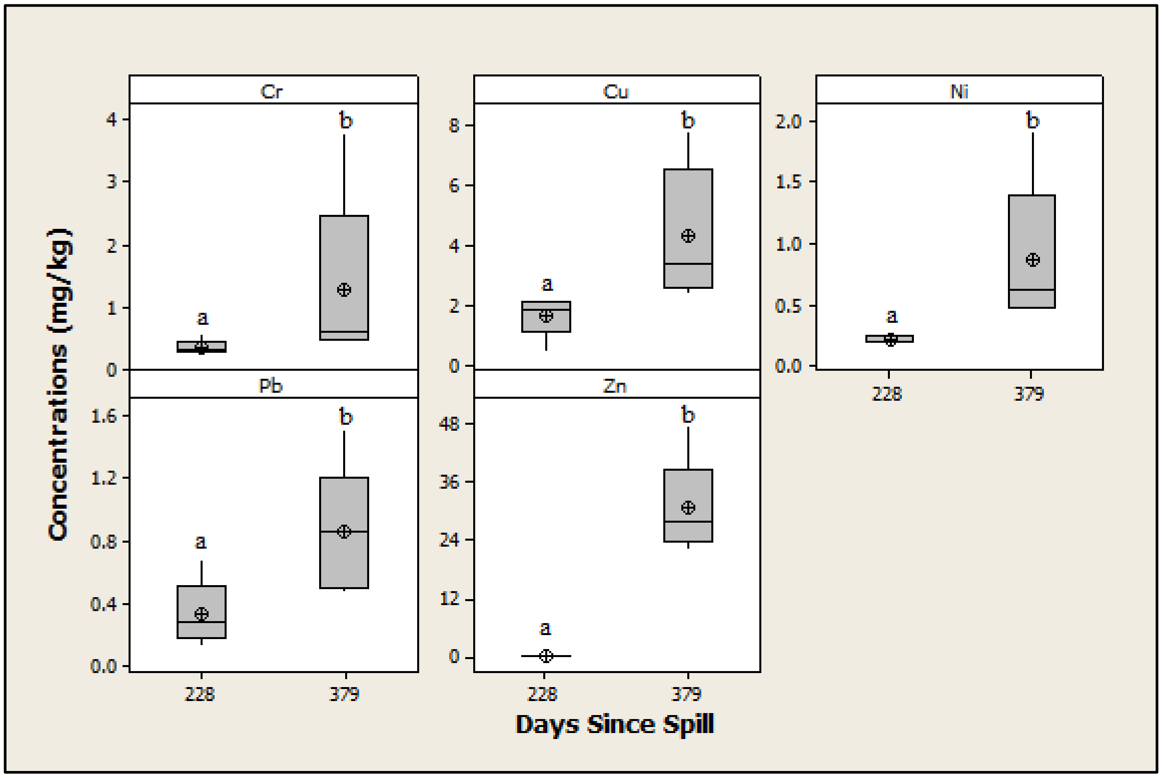

FIGURE S5. ***D. cepedianum*** **Chromium, Copper, Nickel, Lead, and Zinc Muscle Burdens (mg/kg) at Clinch River Mile 3.5 (CRM3.5) Associated with the TVA Coal Ash Spill Area in Kingston, TN.** Elements are indicated as chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) and all concentrations are reported in mg/kg dry weight basis. Collection dates are indicated as 228 days since spill (July 2009) and 379 days since spill (January 2010). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means. Days with different letters (a,b) indicate statistical differences among groups at *p* <0.05.

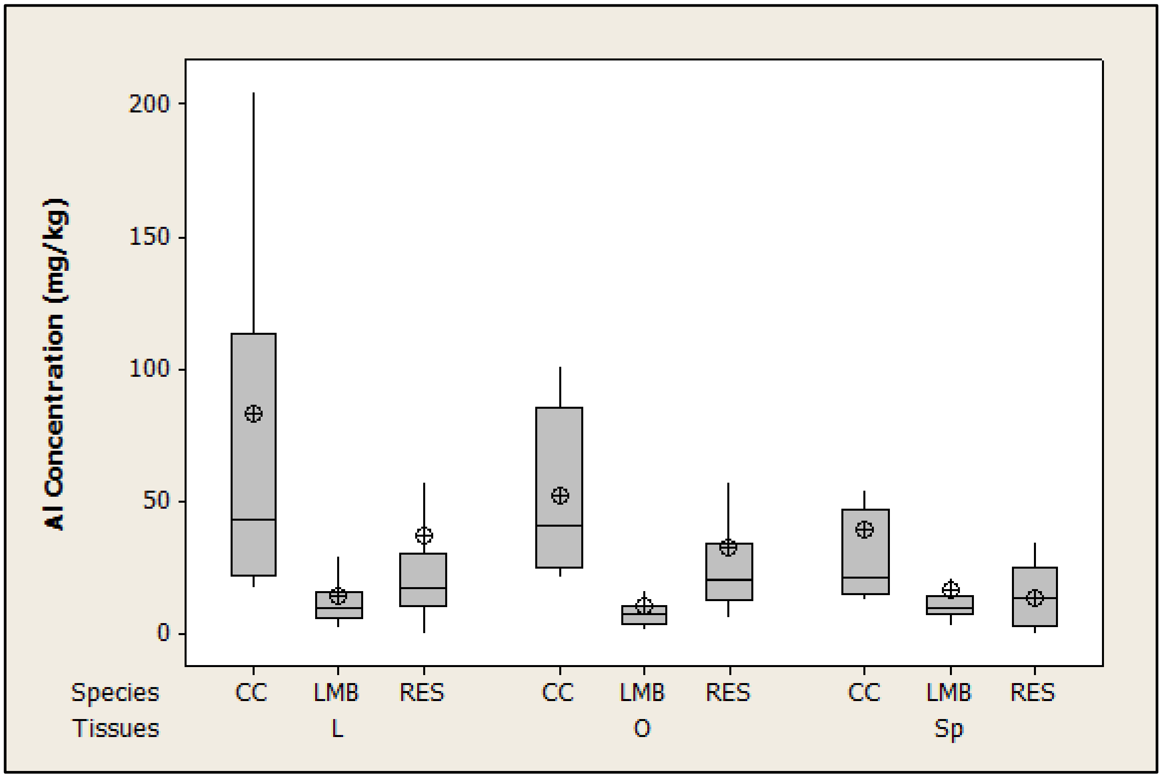

FIGURE S6. Comparison of Aluminum Concentrations in Liver, Ovaries, and Spleen among *I. punctatus*, *M. salmoides*, and *L. microlophus* Including all Collection Dates and Sites Associated with the TVA Coal Ash Spill Area in Kingston, TN. Aluminum is indicated as Al and all concentrations are reported in mg/kg dry weight basis. Fish species are indicated as *I. punctatus* (CC), *M. salmoides* (LMB), and *L. microlophus* (RES). Organs are indicated as liver (L), ovaries (O), and spleen (Sp). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means.

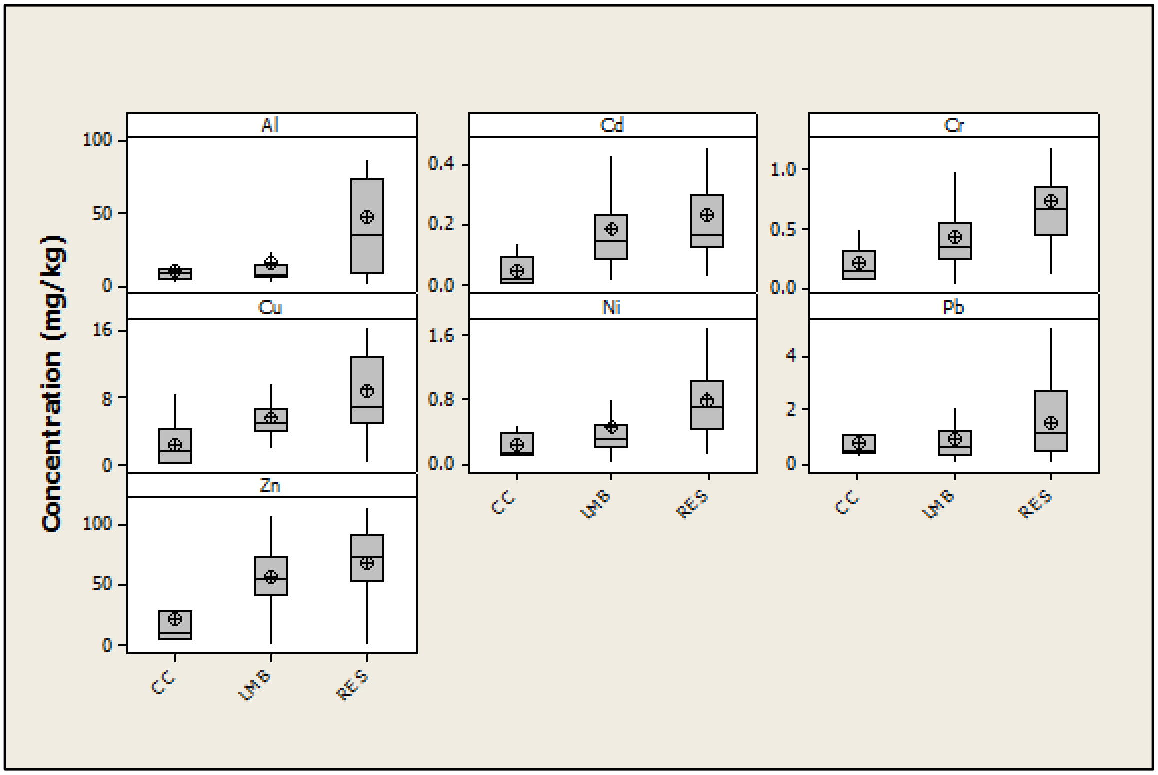

FIGURE S7. Comparison of Aluminum, Cadmium, Chromium, Copper, Nickel, Lead, and Zinc Concentrations in the Gastric Caeca among *I. punctatus*, *M. salmoides*, and *L. microlophus* Including all Collection Dates and Sites Associated with the TVA Coal Ash Spill Area in Kingston, TN. Elements are indicated as aluminum (Al), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) and all concentrations are reported in mg/kg dry weight basis. Fish species are indicated as *I. punctatus* (CC), *M. salmoides* (LMB), and *L. microlophus* (RES). Organs are indicated as liver (L), ovaries (O), and spleen (Sp). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means.

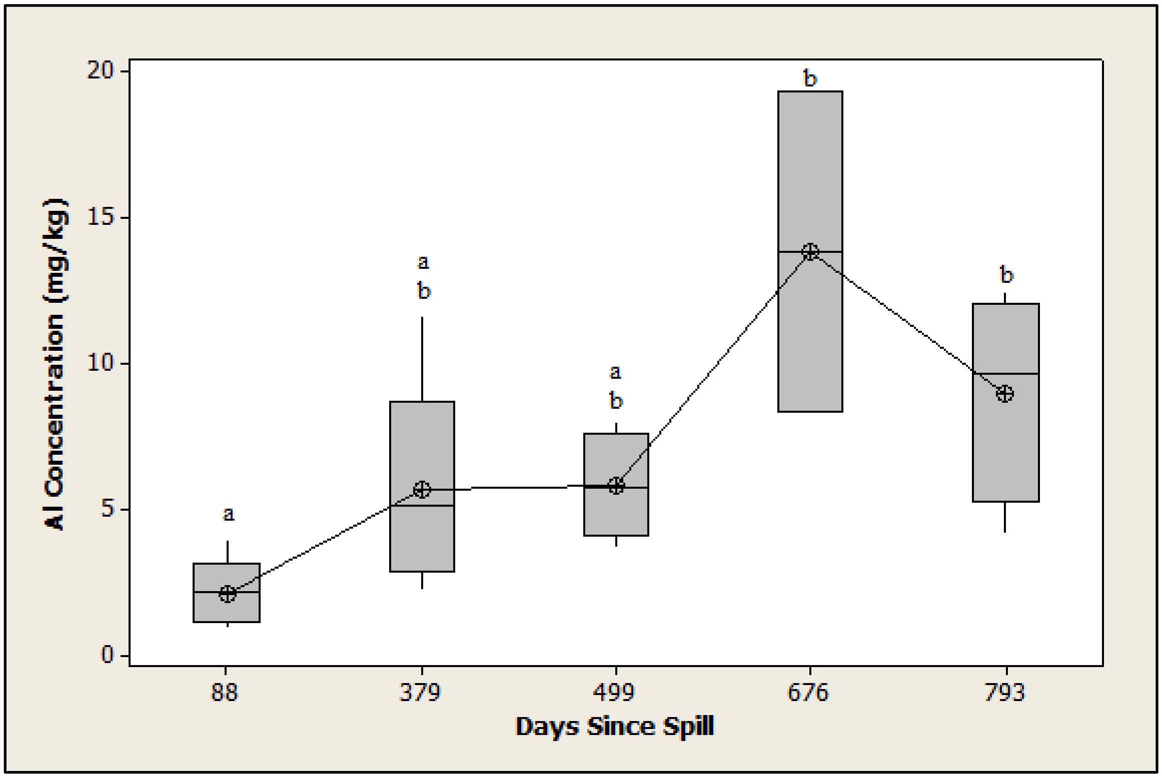

FIGURE S8. *M. salmoides* Muscle Aluminum Concentrations (mg/kg) over the Two Year Period at Clinch River Mile 5.5 (CRM5.5) Associated with the TVA Coal Ash Spill Area in Kingston, TN. Aluminum is indicated as Al and all concentrations are reported in mg/kg dry weight basis. Collections dates are indicated as 88 days since spill (March 2009), 379 days since spill (January 2010), 499 days since spill (May 2010), 676 days since spill (September 2010), and 793 days since spill (January 2011). The boxplots indicate (from top to bottom) the upper ranges, upper quartile ranges, medians, lower quartile ranges, the lower ranges and the circles indicate the means. Days with different letters (a,b) indicate statistical differences among groups at p <0.05.

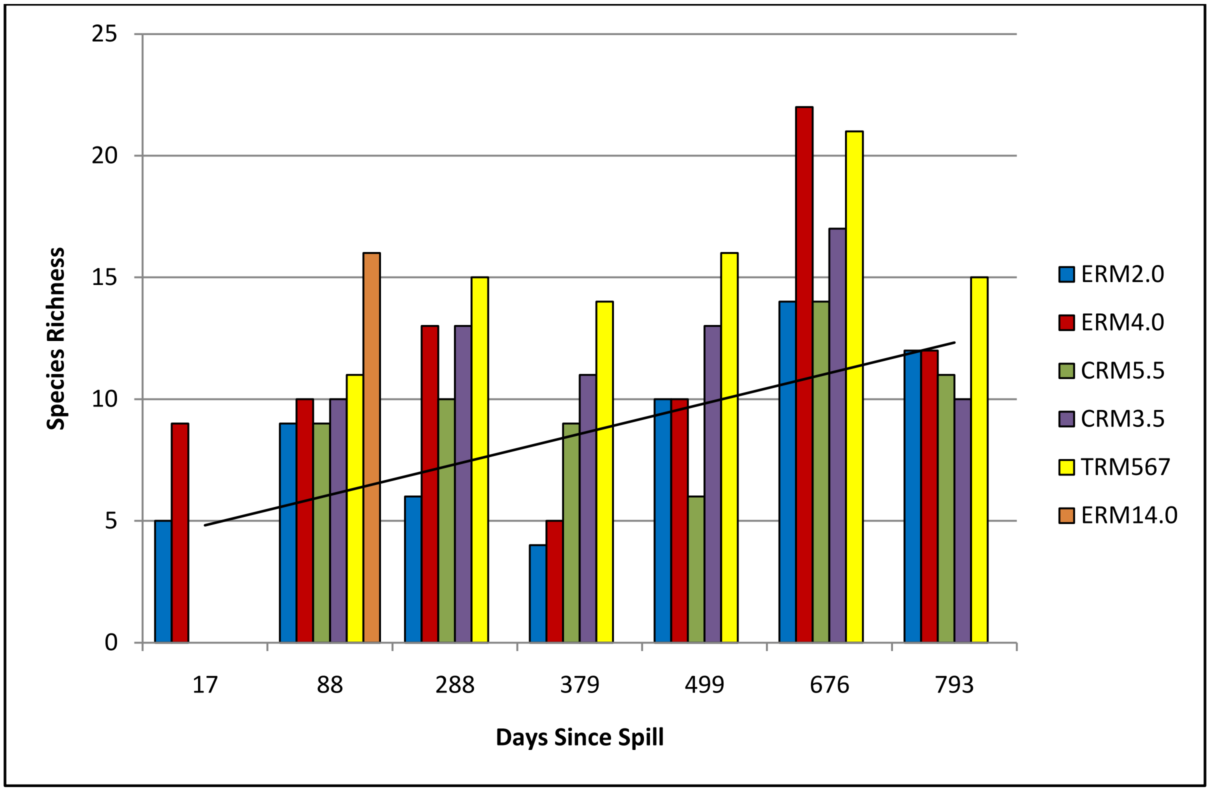

FIGURE S9. Species Richness over the Two Year Period at all Collection Sites Associated with the TVA Coal Ash Spill Area in Kingston, TN. Species richness is reported in numbers of species caught during a 500 second collection period at each site. Collections dates are indicated as 88 days since spill (March 2009), 379 days since spill (January 2010), 499 days since spill (May 2010), 676 days since spill (September 2010), and 793 days since spill (January 2011).

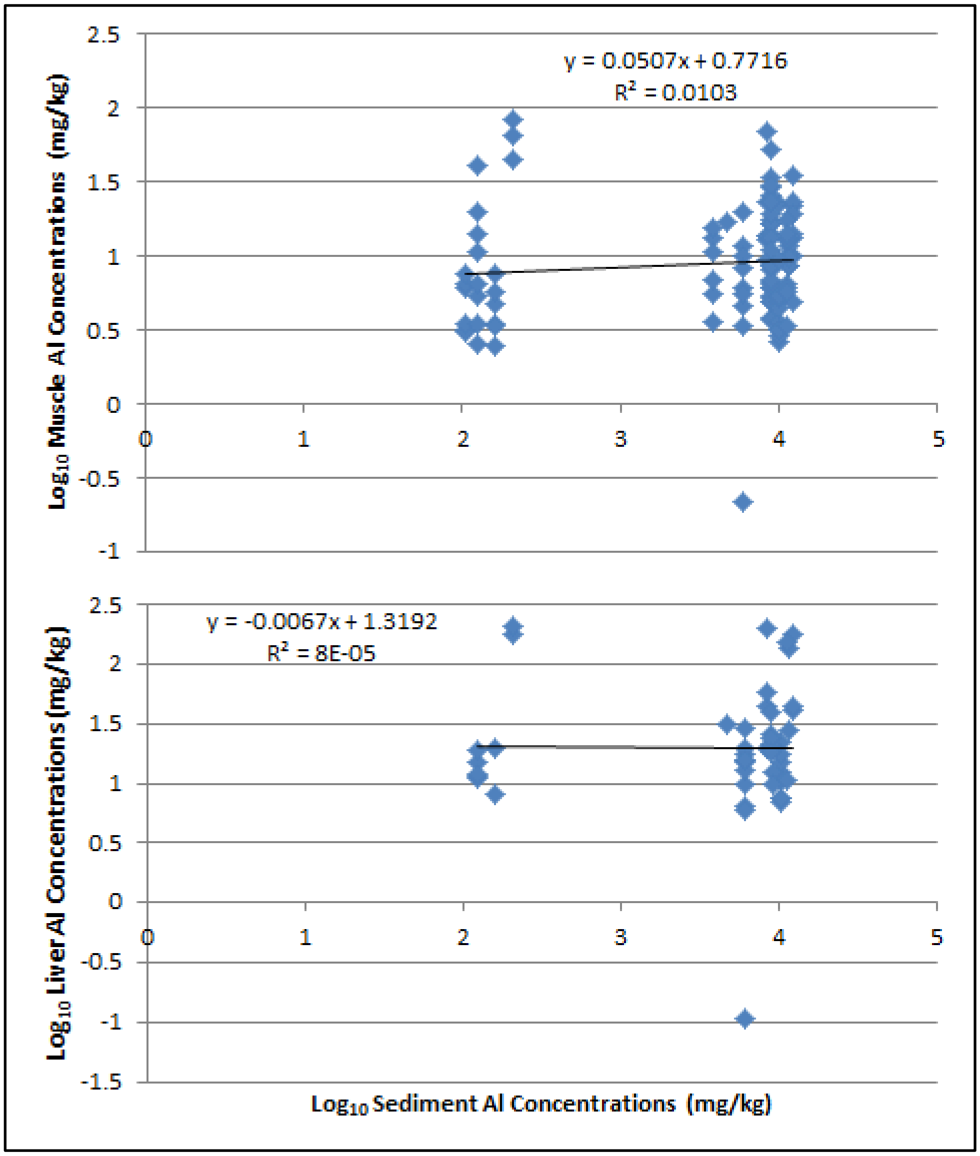

FIGURE S10. Relationship between $\log_{10}$ Liver and Muscle Aluminum Concentrations (mg/kg) from all *L. microlophus* Samples and $\log_{10}$ Sediment Aluminum Concentrations (mg/kg) Over the Two Year Period from all Collection Sites Associated with the TVA Coal Ash Spill Area in Kingston, TN. Aluminum is indicated as Al and all concentrations are reported as $\log_{10}$ mg/kg dry weight basis. R^2 indicates the correlation coefficient between the outcomes and their expected values. The slope of the line is indicated as y=mx+b.

Biographical Sketch

Yosuke Sakamachi was born in Tokyo, Japan, on December 16, 1986. He was the first born to Kenichi and Masako Sakamachi. He then moved to the United State of America in 1990, where he resided in New York and later North Carolina. He attended The Japanese School of Raleigh and East Chapel Hill High School, graduating in the spring of 2004. He then attended Appalachian State University in the following fall and received a Bachelor of Science in ecology, with a minor in chemistry in 2009, and a Master of Science in biology in 2011.