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Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

Tennessee Valley Authority

Ash Recovery Project

Kingston, Tennessee

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Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

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Acronyms and Abbreviations

ANOVA	Analysis of Variance
BERA	Baseline Ecological Risk Assessment
CRM	Clinch River mile
DOE	Department of Energy
DQO	Data Quality Objective
dw	Dry weight
EDD	Electronic data deliverable
EPA	U.S. Environmental Protection Agency
ERM	Emory River mile
GSI	Gonadosomatic index
KIF	Kingston Fossil Plant
LERM	Little Emory River mile
MDL	Method Detection Limit
mg/kg	Milligram per kilogram
NRWQC	National Recommended Water Quality Criteria
ORNL	Oak Ridge National Laboratory
QAPP	Quality Assurance Project Plan
QC	Quality Control
RFAI	Reservoir Fish Assemblage Index
SAS	Statistical Analysis Software
Site	Tennessee Valley Authority Kingston Fossil Plant Release Site, in Roane County, Tennessee
TRM	Tennessee River mile
TVA	Tennessee Valley Authority
VA Tech	Virginia Polytechnic Institute and State University

1. Introduction

In May 2012, a *Baseline Ecological Risk Assessment* (BERA) (ARCADIS 2012a) was submitted for the U.S. Environmental Protection Agency (EPA) review, evaluating the potential ecological effects on biota of ash residuals in the river system at the Tennessee Valley Authority (TVA) Kingston Fossil Plant (KIF) Release Site, in Roane County, Tennessee (the Site). The BERA focused primarily on data collected post-dredging in order to evaluate the potential ecological effects of the residual ash from the 2008 TVA KIF ash release. The BERA was developed in support of the *Kingston Ash Recovery Project, Non-Time Critical Removal Action, River System Engineering Evaluation/Cost Analysis* (TVA 2012), which evaluates alternatives for restoration of the river system impacted by the December 22, 2008 ash release.

Monitoring for some ecological receptors continued into 2011; however, these data were only partially available for preliminary analysis at the time the BERA was submitted for review. The purpose of this report is to quantitatively assess the most recent dataset, and when possible, to evaluate temporal differences in constituent concentrations from 2009 to 2011. Due to the project time constraints in releasing this update, only data for selenium, arsenic, and mercury are discussed for each receptor. This report includes an assessment for 2009 through 2011 results for the following biota:

- Fish
- Benthic Invertebrates
- Great Blue Heron
- Canada Geese
- Amphibians (frogs and toads)
- Turtles

Much uncertainty exists when drawing conclusions on trends noted across 3 years of post-release data, thus temporal trends are considered preliminary for most receptors. The overall conclusions presented here do not change the risk management recommendations provided in the BERA.

The data included in this report were reported by analytical laboratories and validated via a quality assurance/quality control review as specified in the Quality Assurance Project Plan for the Tennessee Valley Authority Kingston Ash Recovery Project, Revision 1 (QAPP; TVA 2010). A summary of the data validation process and data quality results are presented in the final chapter of this report.

2. Fish

Many species of fish were selected for use in the BERA because they are ubiquitous in the Emory, Clinch, and Tennessee River locations near the Site. Fish may be exposed to ash-related constituents through their gills, ingestion of sediment and water, consumption of aquatic prey, and maternally transferring constituents to eggs. Exposure to ash-related constituents may lead to bioaccumulation over time which may then affect the health of the community.

The main study objectives were to 1) compare community metric results among locations and across 3 years; 2) evaluate fish reproductive condition among locations and years; 3) compare concentrations of metals and metalloids in fish tissues; 4) evaluate fish health condition among locations and years; and 5) relate concentrations measured at the study sites to reference area concentrations and literature-derived effects values, when available.

2.1 Fish Community

Historically, fish communities have been studied in the Tennessee and Clinch Rivers, and the Emory River monitoring began immediately after the ash release in January 2009. A detailed description of the sampling locations and collection methods can be found in *Evaluation of the Fish Community in the Vicinity of the Kingston Fossil Plant, 2001 – 2010* (Baker 2011a) and in *Evaluation of 2011 Fish Community Survey Results for the Kingston Fossil Plant Ash Recovery Project* (Appendix A).

Fish were collected using boat electrofishing and gill netting. Fifteen 300-meter runs were completed along three area ranges, each of which is named for a designated river mile within the sampled area. The location named Emory River mile (ERM) 2.5 consisted of runs from ERM 1.7 to ERM 4.5, location Clinch River mile (CRM) 1.5 consisted of runs from CRM 0.0 to 2.4, and location CRM 4.4 consisted of runs from CRM 3.8 to 5.3. Experimental overnight gill nets were also used at each area in order to catch fish from deeper habitats. Fish were identified to species, tallied, and examined for diseases, deformities, or any other anomalies (Baker 2011a). Total numbers of species were evaluated using TVA's Reservoir Fish Assemblage Index (RFAI) methodology. The RFAI uses metrics from four categories (species richness and composition, abundance, and fish heath). RFAI scores are ranked and assigned ratings (very poor, poor, fair, good, and excellent) (Baker 2011a).

The RFAI results for the Emory River in the immediate area of the ash release (ERM 2.5) rated "good" during the three annual surveys following the release, while the Clinch River (CRM 1.5 and CRM 4.4) rated "good" to "fair". Results for species richness metrics were similar during pre-release and post-release surveys. The number of indigenous species collected at the sites over the years indicated "moderate" to "good" representation of indigenous species. Compared to historical results near KIF, the number of fish collected in electrofishing samples was highest at sites in 2009 and 2010, but still remained in the moderate

to low range. The principal difference in fish abundance among years occurred in 2011 with historically low numbers of fish collected in electrofishing samples at CRM 4.4 and in gill netting samples at all sites. Sampling results in 2011, both for an initial survey and a repeat survey, were adversely affected by storm events that caused high river flows, high turbidities, and low water temperatures. The low abundance observed for both sampling methods was attributable to concurrent low catch rates for several common but ecologically diverse species. Given that electrofishing catch rates at ERM 2.5 and CRM 1.5 in 2011 were within their respective historical ranges (even with the effects of the storm flows), these declines were more likely related to sampling efficiencies rather than an adverse effect of the ash release being manifested concurrently at the population level for numerous species (Appendix A).

Although the percentages of fish with anomalies were elevated in 2009 and 2011, the majority of the increases were attributable to bluegill infected with common parasites. Given year-to-year variability in the incidence of anomalies and the fact that parasite loads in 2011 were highest at sampling locations in the Clinch River upstream of the Emory River, there is no clear evidence that the increases are ash-related (Appendix A).

Overall, the 2011 RFAI results for each location were within the range of expected variation based on historical results and the intrinsic variability in sampling reservoir fish communities. The one notable exception was the low number of fish collected in electrofishing samples at CRM 4.4. However, observed values for other metrics at this site in 2011 were similar to those observed in other sample years, which resulted in an overall RFAI score (44,"Good") that was similar to previous years. Collectively, the RFAI results for the seven sample years indicate fish assemblages near KIF continue to be representative of those observed prior to the release and, likewise, are representative of those expected in transition zones in upper mainstream reservoirs.

2.2 Fish Reproduction

Oak Ridge National Laboratories (ORNL) conducted fish reproductive studies that focused on assessing the health and condition of fish ovaries. A detailed description of the sampling locations and collection methods can be found in *Evaluating the Effects of the Kingston Fly Ash Release on Fish Reproduction: Spring 2009 – 2010 Studies* (Greeley et al. 2012) and *Preliminary Evaluation of 2011 ORNL Results: Fish Reproduction Studies (*Greeley 2012) (Appendix B).

The ovary was chosen because it provides a route for maternal transfer of metals and metalloids to the developing eggs. In 2011, fish were collected by electrofishing from three reference locations (ERM 8.0, CRM 8.0, Little Emory River mile [LERM] 2.0) and three impacted locations (ERM 3.0, ERM 0.9, and CRM 1.5). Largemouth bass, bluegill sunfish, and redear sunfish were collected in the spring of 2011. After collection and transport to ORNL, fish were euthanized, ovaries were measured, and ovary weight, analysis of ovary stage, oocyte (immature developing eggs) condition, and estimates of fecundity were recorded. The

gonadosomatic index (GSI) or size of the gonad compared with the body size was also used as in indicator of fish reproductive status (Greeley et al. 2012).

Similar to previous years, preliminary evaluation of the 2011 results indicate no adverse effects on female fish reproductive competence, and all location differences observed for GSI and percentages of pre-spawning fish were due to fish having spawned prior to collections. Some spatial differences were found for largemouth bass and bluegill, but less variation was observed in redear sunfish. Laboratory analysis of 2011 samples is still in progress with a complete report anticipated in December 2012.

2.3 Fish Bioaccumulation

A detailed description of the sampling locations and collection methods can be found in *Fish Bioaccumulation Studies Associated with the Kingston Fly Ash Spill, Spring 2009 – Fall 2010* (Adams et al. 2012), *Trace Element Concentrations in Fish: 2010* (ARCADIS 2012b), and in *Preliminary Evaluation of 2011 ORNL Results (rev.1): Fish Bioaccumulation* (Mathews 2012) *(*Appendix C*)*.

Bioaccumulation of metals and metalloids was measured in fillets of channel catfish, largemouth bass, bluegill, and redear sunfish and in whole body gizzard shad. Spring sampling was conducted in April and May in conjunction with the fish health and reproductive studies. Fall sampling was conducted from September through November. Fish were collected from three upstream references (ERM 8.0, LERM 2.0, and CRM 8.0), and four impacted locations (ERM 3.0, ERM 0.9, CRM 1.5, and CRM 3.5). Fish fillets and whole body gizzard shad were sent to Pace Analytical Services, Inc. (Pace) for analysis of 26 metals and metalloids (Adams et al. 2012). A preliminary analysis of the spatial and temporal trends for selenium and arsenic concentrations in fish fillets is presented in this report. In addition, a spatial evaluation of mercury also is presented.

In 2011, selenium concentrations in all species' fillets indicated higher concentrations at ERM 3.0 as compared to upstream locations. Moving downstream, concentrations of selenium increased, with highest concentrations in fillets from CRM 1.5. Selenium concentrations were highest in redear sunfish fillets. While concentrations of selenium in redear sunfish and largemouth bass exhibited an increase over time, all selenium concentrations for all 3 years of study in all fish remain below the EPA proposed ambient water quality criterion of 7.91 milligrams per kilogram (mg/kg) (whole-body, dry weight [dw]). Statistical analysis indicated mean selenium concentrations in redear sunfish from ERM and CRM impacted locations were significantly higher in 2011 than in 2010. Selenium concentrations in largemouth bass were generally lower or similar when compared across the 3 years for most locations; however, at CRM 1.5 concentrations increased from spring 2010 to 2011. A linear regression analysis indicated that concentrations of selenium in largemouth bass and redear increased significantly from 2009 to 2011 at all ash impacted locations (ERM 4.5, ERM 3.0, ERM 0.9, and CRM 1.5), but not at the upstream reference locations ERM 8.0 and LERM 2.0 (Note: CRM 24 and CRM 8.0 locations were excluded from the evaluation).

In general, arsenic concentrations in 2011 were similar or lower than the concentrations measured in 2009 and 2010. However, arsenic concentrations in bluegill, redear, and largemouth bass still indicated a strong spatial gradient with higher concentrations of arsenic at ash-impacted locations than in upstream Emory River and Little Emory River references. Arsenic concentrations also were higher in fillets from the upstream Clinch River, which are likely related to Department of Energy (DOE) facilities. Similar to selenium, the linear regression analysis of temporal trends indicated that concentrations of arsenic in largemouth bass fillets increased significantly from 2009 to 2011 at all ash impacted locations (ERM 4.5, ERM 3.0, ERM 0.9, and CRM 1.5), but not at the upstream reference locations ERM 8.0 and LERM 2.0 (Note: CRM 24 and CRM 8.0 locations were excluded from the evaluation).

Similar to arsenic, mercury concentrations also have been historically higher in the Clinch River than elsewhere in the study area. In the Emory River, concentrations at ERM 3.0 were above the National Recommended Water Quality Criteria (NRWQC) in largemouth bass and catfish and above the NRWQC for largemouth bass at ERM 8.0; however, due to historical inputs there are no clear trends related solely to the ash release.

2.4 Fish Health

A detailed description of the sampling locations and collection methods can be found in *Fish Health Studies Associated with the Kingston Fly Ash Spill, Spring 2009 – Fall 2010* (Adams and Fortner 2012) and in *Preliminary Evaluation of 2011 ORNL Results: Fish Health* (Bevelhimer 2012) (Appendix D).

The fish health study was conducted by ORNL in conjunction with the reproduction and bioaccumulation studies. The species collected (largemouth bass, bluegill, white crappie, and redear sunfish) were the same as in the reproduction study, at the same locations. Only female fish were collected in the spring (in conjunction with the reproductive study), whereas both males and females were collected in the fall. Blood samples from the fish were collected immediately after electroshocking. Condition indices, bioenergetic and hematological responses, histopathological indicators, indicators of carbohydrate-protein metabolism, organ dysfunction responses, and measures of electrolyte homeostasis, feeding and nutrition were all measured and calculated.

While a rigorous statistical analysis is still in progress, 2011 results were preliminarily evaluated graphically. Some bluegill fish indices for 2011 were more indicative of good health (visceral somatic index, blood protein, sodium, and calcium), while others suggested a decline (stomach fullness lower at ERM 0.9, spring condition factor). Similar results were observed for largemouth bass with some indices indicative of good health (condition factors), while others suggested a decline across years (hematocrit, protein, bladder color, liver somatic index, BUN). The sample sizes were low, however, and more statistical tests are needed to fully interpret these results. A more detailed report is anticipated in December 2012.

3. Benthic Invertebrates

Benthic invertebrates are found living within or on top of sediments found in the Emory, Clinch, and Tennessee Rivers. In these rivers, the invertebrate community consists of mostly oligochaetes (aquatic worms), chironomids (larval midges), burrowing mayfly nymphs, along with crustaceans (crayfish and amphipods), bivalves (mussels and clams), snails, larval flies, leeches, and mites. Because of their close association with the sediments and water, benthic invertebrates have an increased potential for bioaccumulation of metals and metalloids. They may also transfer these constituents to their fish and wildlife consumers. Benthic invertebrates serve as a useful receptor for understanding potential effects of exposure to these constituents on the aquatic ecosystem.

The main study objectives in 2011 were to assess risk to the benthic community by 1) comparing community metric results among sites and across 3 years, 2) comparing tissue concentrations of metals and metalloids in snails, mayfly nymphs, and mayfly adults to evaluate differences among sites and years, and finally 3) relating concentrations measured at the study sites to reference area concentrations and literature-derived effects values, when available. A brief discussion of each objective is presented in the subsections below.

3.1 Benthic Invertebrate Community

Benthic invertebrate community evaluations in December 2011 and January 2012 were conducted on the Emory, Clinch, and Tennessee Rivers, similar to previous years. A detailed description of the sampling locations and collection methods can be found in *Evaluation of Benthic Macroinvertebrate Communities in the Vicinity of TVA's Kingston Fossil Plant, 2009-2010* (Baker 2011b).

In 2011 and January 2012, nine transect locations on the Emory River (ERM 6.0, ERM 5.0, ERM 4.1, ERM 3.5, ERM 3.0, ERM 2.6, ERM 2.2, ERM 1.0, and ERM 0.7), six transect locations on the Clinch River (CRM 8.7, CRM 6.0, CRM 4.0, CRM 3.0, CRM 1.5, and CRM 0.5), and two transect locations on the Tennessee River (TRM 573.9 and TRM 566.3) were selected for monitoring. Ten grab samples were collected across each transect, and benthic invertebrates in each sample were identified to the lowest possible taxon. The total number of taxa were tallied and used to generate benthic invertebrate community metrics for assessing the overall health of the benthic invertebrate community. Population density, taxa richness, number of organisms, number of taxa, percent oligochaetes and chironomids, and other metrics were used to assess spatial and temporal patterns. Details on how these metrics are calculated are presented in Baker (2011b). At each sample location, water depth was recorded along with a visual examination of the sediment in each sample to provide qualitative descriptions of percent ash, grain size, and substrate type. For the January 2012 Emory river samples, in addition to the benthic invertebrate community data, co-located sediment samples were collected and analyzed for percent ash, metals, total organic carbon, and the percent sand,

silt, clay, or gravel. The purpose of the co-located data collections was to better interpret the various factors potentially influencing the benthic invertebrate community.

Variations in 2012 Emory River benthic community composition, diversity, and abundance among sites were consistent with the variation seen in previous years. Observed differences (Appendix E) primarily were related to natural temporal variations that were reflected at all sites (including reference sites) or to spatial heterogeneity of available habitat among sites (and even across a transect). The co-located sediment data further confirm the premise that variations in sediment types and substrates translate to variations in the benthic invertebrate community living within and on the different types of sediments. Similarity analysis on the co-located data indicated that, similar to what was found in 2010, the differences in the January 2012 Emory River benthic community data were correlated more strongly with substrate type and water depth than with percent ash content or metal and metalloid concentrations in submerged sediments. The results of the 2011 and 2012 benthic community analysis from the Clinch and Tennessee Rivers are consistent with previous years and do not suggest any adverse impacts from the ash release.

3.2 Benthic Invertebrate Bioaccumulation

Benthic invertebrate bioaccumulation evaluations conducted on the Emory, Clinch, and Tennessee Rivers, in 2011 were similar to previous years. A detailed description of the collection methods can be found in *Evaluation of Invertebrate Bioaccumulation of Fly Ash Contaminants in the Emory, Clinch, and Tennessee Rivers, 2009-2010* (Smith 2012a) and in *Preliminary Evaluation of 2011 ORNL Results: Invertebrate Bioaccumulation* (Smith 2012b) (Appendix F). Similar to previous years, mayfly adults and nymphs (*Hexagenia bilineata*) and a species of snail (*Pleurocera canaliculatum*) were collected for evaluation.

In 2011, four locations on the Emory River (ERM 6.0, ERM 4.0, ERM 2.5, and ERM 1.0), one location on the Little Emory River (LERM 1.0), three locations on the Clinch River (CRM 6.0, CRM 3.5, and CRM 1.5), and three locations on the Tennessee River (Tennessee River mile [TRM] 566.7, TRM 572.5, and TRM 560.8) were selected for collections of snails and mayfly nymphs. Mayfly adults were collected opportunistically as close as possible to core locations on the Emory, Clinch, and Tennessee Rivers. Snails and mayfly nymphs were separated into depurated and non-depurated samples, and adult mayflies were separated by sex and adult developmental stage (i.e., subimago and imago). All samples were analyzed for the same suite of 26 metals and metalloids. A preliminary analysis of the spatial and temporal trends for selenium arsenic, and mercury concentrations in benthic invertebrate tissues was conducted for this report. A more detailed evaluation will be published by December 2012.

Selenium concentrations in snails collected in 2011 remained somewhat elevated at ERM 1.0 and CRM 1.5 locations downstream of the release. Temporally, selenium concentrations in 2011 snails were similar to or lower than those in 2010, possibly indicating a decline over time. Both groups of nymphs exhibited similar spatial trends in all years, with highest concentrations at ERM 2.5 and 1.0, and progressively decreasing

concentrations with increasing distance downstream from the Emory River. Temporally, concentrations decreased in 2011 non-depurated nymphs while they increased in depurated nymphs at ERM 2.5 and 1.0. The highest concentrations of selenium in mayfly adults were generally found at Emory River locations adjacent to and immediately downstream of the ash release, then progressively decreasing with increasing distance from the Emory River. The temporal trends found in mayfly nymphs and adults indicated a potential increase in bioavailablity of selenium from digestible food particles in 2011, but this trend was not observed for snails.

Arsenic concentrations in snails were highest at CRM 6.0, but moderately high concentrations were also found at ERM 1.0 and ERM 2.5. Arsenic in mayfly nymphs from ERM 1.0 was lower in 2011 when compared with other years, however maximum concentrations of arsenic in depurated and non-depurated mayfly nymphs were found at ERM 1.0 in all years. Concentrations at all other locations were similar between 2011 and previous years. Arsenic concentrations in 2011 mayfly adults were similar to concentrations found in 2010, indicating no definitive temporal trends. The data reflect a spatial trend with some of the highest concentrations of arsenic in mayfly adults occurring at ERM 3.0, ERM 2.0, and ERM 1.0 nearest the location of the release.

Maximum concentrations of mercury were found at CRM 6.0 in all groups of invertebrates for all years of study. The next highest concentrations occurred at downstream locations on the Clinch River (CRM 3.5 and CRM 1.5). These spatial and temporal trends indicated an alternative source upstream of CRM 6.0. However, without historical data from the Emory River, it cannot be definitely concluded that the ash release did not contribute to the mercury concentrations downstream of the release.

No clear temporal trends existed for mercury or arsenic measured in snails and mayflies. Temporal trends for selenium in mayflies did suggest a possible increase in bioavailability at sites downstream from the release. However, it should be noted that three years of data may be insufficient to properly support these observed differences. Additional yearly monitoring will help to elucidate these trends.

4. Heron

Great Blue Heron (*Ardea herodias*, hereafter: heron) were selected as a representative aquatic-feeding bird species for the Site. Heron are commonly found in Tennessee and are present year-round. They primarily consume fish and invertebrates, but also consume small birds, mammals, amphibians, and reptiles. Because of their higher position in the food web, heron have the potential for bioaccumulation of metals or metalloids from their prey or incidental ingestion of water and sediments. Heron serve as a useful receptor in order to understand exposure and potential effects of these constituents on the aquatic-feeding bird community.

The main study objectives were to 1) to evaluate the extent of maternal transfer of metals and metalloids to the eggs between locations and over a 3-year period, and 2) to assess risk to heron by comparing concentrations measured at the study sites with literature-derived effects values, when available.

In 2011, heron eggs were collected from the same two colonies sampled in previous years, at ERM 3.0 and TRM 569.5, following the same sampling procedures as described in *Trace Element Concentrations in Great Blue Heron and Osprey: 2009 – 2010* (ARCADIS 2012c). A total of 10 eggs were collected from each location and were analyzed for 26 metals and metalloids. Summary statistics were calculated for heron (sample size, frequency of detection, minimum and maximum detection and detection limits) for each year and location (Appendix G - Table 1). Spatial trends were evaluated for all metals, metalloids, and egg biometrics using a parametric, one-sided Student's t-test (t-test) for 2011 data. Spatial and temporal trends for 2009, 2010, and 2011 data were evaluated using a two-way analysis of variance (ANOVA) test (Statistical Analysis Software [SAS] version 9.3). Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. A non-parametric one-sided Wilcoxon Rank Sum test was used for data that did not fit a normal or lognormal distribution. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05. The results for selenium, arsenic, and mercury are briefly summarized and interpreted below.

Selenium was found above detection limits in all samples (Appendix G - Table 1). In 2011, concentrations of selenium measured in eggs from the Emory River were all statistically significantly greater than the Tennessee River reference eggs (Appendix G - Table 2). When evaluating selenium across 3 years, selenium concentrations in 2011 eggs from the Emory River were slightly higher than the mean concentration in 2009 eggs, but were less than the concentrations in 2010 eggs. Available literature effects values for selenium indicate that concentrations greater than 7.7 mg/kg dw caused adverse effects to hatchability in a sensitive bird species (mallard, Janz et al. 2010). Selenium concentrations in heron eggs collected from the ash-impacted location were all below this adverse effects level.

Arsenic was not detected in any samples, therefore could not be evaluated statistically.

Mercury was also found above detection limits in all samples (Appendix G - Table 1). In 2011, concentrations of mercury in eggs from the Emory River were statistically significantly greater than the Tennessee River reference eggs (Appendix G - Table 2). Although temporal trends for mercury could not be evaluated at this time due to changes in analytical procedures over the study period, spatial trends for mercury concentrations in 2011 showed statistically significantly higher concentrations at the ERM 3.0 colony eggs than in the upstream Tennessee River (TRM 569.5) reference eggs. Other investigations of biota and sediments provide substantial evidence that higher mercury concentrations in the vicinity of the site are more likely related to past releases of mercury from DOE facilities. Mercury effects have been documented in many literature studies. Ranges of concentrations causing effects have been reviewed and summarized by Thompson et al. (1996), who concluded that mercury concentrations in eggs up to 2.5 mg/kg dw appear to have little detrimental effect on reproduction. All of the mercury concentrations measured in heron eggs for this study were below this literature-derived effects value.

5. Canada Geese

Canada geese (*Branta canadensis)* were not included as part of the BERA; however, they are observed nesting near KIF and feeding on the vegetation cover established on areas with residual ash. Canada geese are year-round residents in Tennessee, and they forage by grazing on vegetation in fields or dabbling in the water. Canada geese have the potential for bioaccumulation of metals and metalloids from their prey items or incidental ingestion of water and sediments.

The main study objectives were 1) to evaluate the extent of maternal transfer of metals and metalloids to the eggs between locations and across years, and 2) to assess risk to geese by comparing concentrations measured at the study sites with literature-derived effects values, when available.

Canada goose eggs were collected from similar locations sampled in previous years, on the Emory, Clinch, and Tennessee Rivers, following the same sampling procedures as described in *Trace Element Concentrations and Dietary Exposure Modeling in Canada Geese: 2009 – 2010* (ARCADIS 2012d). A total of 23 eggs were collected in 2011 and were analyzed for 26 metals and metalloids. Summary statistics were calculated (sample size, frequency of detection, minimum and maximum detection and detection limits) for each year and location (Appendix H - Table 1). Spatial trends were evaluated for all metals, metalloids, and egg biometrics using a parametric, one-sided Dunnett's test for 2011 data. Spatial and temporal trends for 2010 and 2011 data were evaluated using a two-way ANOVA test (SAS version 9.3). Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05. The results for selenium, arsenic, and mercury are briefly summarized and interpreted below.

Selenium was found above detection limits in all samples (Appendix H - Table 1). In 2011, concentrations of selenium were higher in impacted-area eggs than in reference-area eggs. Selenium also had spatial and temporal differences in egg concentrations collected on both the Emory and Clinch Rivers. Selenium concentrations greater than 7.7 mg/kg dw have been reported to cause adverse effects to hatchability in a sensitive bird species (mallard, Janz et al. 2010). The mean selenium concentrations in goose eggs were all below this threshold, and only one sample from the 2011 Emory River collections was greater than 7.7 mg/kg (8.28 mg/kg dw). At an individual basis and relative to reference sites, geese may have accumulated ash-related selenium in 2009 through 2011. However, the available information suggests it is unlikely that individual egg concentrations have translated to adverse effects related to the ash-release at either the individual or population level. Furthermore, the mean selenium concentrations documented in 2011 remain below threshold concentrations, and the majority of other constituents analyzed suggest a decreasing temporal trend may be occurring.

Arsenic was not detected in any samples and mercury was only detected in two out of 50 samples (less than 1 percent detection); as a result, these constituents could not be evaluated statistically.

6. Amphibians

Three amphibian species, including the American toad (*Bufo americanus*), spring peeper (*Pseudacris crucifer*), and the upland chorus frog (*Pseudacris feriarum*), were selected as representative amphibian species for the Site. Because they consume aquatic and riparian insects and are also consumed by a wide range of receptors, frogs and toads may bioaccumulate metals and metalloids from their prey and transfer concentrations to their consumers. Frogs and toads are a useful receptor in order to understand exposures and potential effects of these constituents on the amphibian community.

Study objectives were 1) to quantify concentrations of metals and metalloids in whole body tissue in three different amphibian species among locations and across years, and 2) to assess risk to amphibians by relating concentrations measured at the study sites to reference area concentrations and literature-derived effects values, when available.

Amphibians were collected from similar locations as in previous years, including two ash-impacted locations (West and North Embayments), and two un-impacted farms (Dawson Farm and Rocky Top Farm). The reference location in 2011 was moved to a residential property (Walls' Property) in Roane County, Tennessee approximately 9 miles northeast of the Site. Collections were made following the same sampling procedures as described in *Trace Element Concentrations in Amphibians: 2009 – 2010* (ARCADIS 2012e). A total of 150 amphibians were collected in 2011 and were analyzed for 26 metals and metalloids. Summary statistics were calculated for each species, for each year and location (Appendix I - Table 1). Spatial trends were evaluated for all metals and metalloids using a parametric, one-sided Dunnett's test for 2011 data. Spatial and temporal trends for 2009, 2010, and 2011 data were evaluated using a two-way ANOVA test (SAS version 9.3). Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05. The results for selenium, arsenic, and mercury are briefly summarized and interpreted below.

Selenium was found above detection limits in all samples for all species (Appendix I - Tables 1, 2, and 3). Selenium concentrations were statistically significantly greater in the West Embayment in 2011 compared to the reference locations for all species. However, of the three species only American toads from ash-impacted locations had reported selenium concentrations comparable to literature ranges for impacted sites. While there were some differences in selenium concentrations between years, these differences were identified between ash-impacted and reference locations from different years and are unlikely to provide a true temporal comparison. In addition, as with other field studies of mobile species such as American toads, which can migrate at least 0.5 miles during the spring breeding months, there is significant uncertainty in analysis of spatial trends.

Arsenic was not detected with enough frequency to evaluate statistically in spring peepers or upland chorus frogs (less than 30 percent detection); as a result, arsenic could not be evaluated statistically for these two species. Although some spatial differences were identified in American toad 2011 arsenic concentrations, temporal comparisons suggest that concentrations of these constituents may be decreasing over time.

Mercury was found above detection limits in most samples for all species in 2011 (between 70 and 80 percent detection). Only spatial comparisons could be made for mercury, due to changes in analytical procedures over time. The 2011 results show some statistically significantly higher concentrations in mercury concentrations from American toads and spring peepers in the West Embayment. However, these concentrations were well below threshold values and also below the state and federal advisories for fish and game consumption (0.5 and 1 mg/kg, respectively).

7. Turtles

Aquatic turtles were selected as a representative reptile species for the Site. Turtles can occupy various levels in the food web, and can have the potential for bioaccumulation of metals and metalloids. Turtles serve as a useful receptor in order to understand exposure and potential effects of these constituents on turtle populations and provide insight to the health of the reptile community (ARCADIS 2012f).

In 2011, turtle monitoring was conducted by Virginia Polytechnic Institute and State University (VA Tech). Detailed descriptions of study methods are available in *Effects of the Remediated Coal Ash Spill in Kingston, TN on Aquatic and Terrestrial Consumers* (Hopkins et al. 2012).

The main study objectives were to 1) compare community metric results among locations and across years; 2) compare concentrations of metals and metalloids in different species of turtle tissues among locations and years; 3) evaluate turtle reproductive condition among locations and years; and 4) relate concentrations measured at the study sites to reference area concentrations and literature-derived effects values, when available.

7.1 Turtle Community

Similar to previous years of study, turtles were collected from ash-impacted areas in the Emory and Clinch Rivers, and from a reference location in the upstream portion of the Tennessee River (above TRM 568). Comparative statistics were evaluated for body sizes of target species collected from impacted and reference locations, as well as capture rates and body sizes of non-target species. Body mass, carapace length, carapace width, and plastron length, egg and clutch sizes were compared among rivers using a multivariate ANOVA (Hopkins et al. 2012).

During the 2011 collection period, the morphometric assessment found body sizes of turtles were generally similar in the Emory, Clinch, and Tennessee Rivers; the variability in body sizes that was noted was likely due to habitat differences (i.e., rockier habitat, water depth, suitable prey, etc.) (Hopkins et al. 2012).

Both trap effort and mean trap success significantly differed among rivers. The trap effort was greater in the Emory and Tennessee Rivers; however, the Clinch River had greater trap success. While the Clinch River had higher densities, the capture rates from all three rivers were comparable to other studies suggesting there are no adverse impacts to community density (Hopkins et al. 2012). Overall, the community survey results indicated that there were no observable adverse impacts on the turtle communities in the Emory or Clinch Rivers. Species richness and relative abundance of turtles were similar when comparing ash-impacted locations and reference locations.

7.2 Turtle Tissue Concentrations

Similar to previous years, several types of turtle tissue (blood, claw, and carapace) samples were collected from a subset of target turtle species. Target species similar to previous years included the common musk (*Sternotherus odoratus*), the common snapping turtle (*Chelydra serpentina*), and the Eastern spiny softshell turtle (*Apalone spinifera spinifera*). In addition, the common slider (*Trachemys scripta*) was added as a target species. Blood and claw samples were collected from adult turtles and sent for metals and metalloids analysis. In addition, a subset of eggs and hatchlings also were sent for analysis of metals and metalloids (Hopkins et al. 2012).

Data not yet available for claws, carapace, eggs, and hatchlings are currently undergoing QA review and are not yet available for trend analysis; therefore, only blood data have been included for this evaluation. In addition, only data from species collected in previous years were evaluated for spatial and temporal trends in this update. A total of 845 target turtles (of the three species listed above) were collected in 2011, sampled (blood drawn), and analyzed for 26 metals and metalloids. Summary statistics were calculated for each species, for each year and location (Appendix J - Tables 1, 2, and 3). Spatial trends were evaluated using a parametric, one-sided Dunnett's test for 2011 data. Spatial and temporal trends for 2010 and 2011 data were evaluated using a two-way ANOVA test (SAS version 9.3). Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05. A preliminary analysis of the spatial and temporal trends for selenium, arsenic, and mercury (spatial trends only) concentrations in turtle blood tissues was conducted for this report. A more detailed evaluation will be published by VA Tech in March 2013.

Selenium was found above detection limits in all or most samples for each species (Appendix J - Tables 1, 2, and 3). In 2011, selenium concentrations in common musk and softshell turtles collected from the Emory and Clinch Rivers were found to be statistically significantly higher than concentrations of selenium in turtles from the reference location (upstream Tennessee River). No significant differences were identified when comparing just 2010 and 2011 concentrations for any of the three species. There were statistically significant differences among locations with the highest concentrations generally occurring in the Clinch River for snapping and musk turtles and the Emory River for softshell turtles.

Arsenic was found above detection limits in approximately half of the samples for each species (between 50 and 65 percent detection). Arsenic concentrations in common musk and softshell turtles collected from the Emory and Clinch Rivers in 2011 were statistically significantly higher than concentrations of arsenic in turtles from the reference location (upstream Tennessee River). In addition, concentrations of arsenic in snapping turtles collected on the Clinch River were statistically significantly higher than reference concentrations. While there were some exceptions, arsenic concentrations decreased from 2010 to 2011 for most locations and for all three species.

Mercury was found above detection limits in most samples for all species (between 70 and 98 percent detection). No significant differences were identified for 2011 mercury concentrations when compared spatially or temporally (2010 compared with 2011). Different analytical methods were used for mercury in 2010 and 2011. In 2010, the ICP-MS acid digestion method was used by Pace to analyze for mercury, whereas samples from 2011 collections were analyzed using an ICP-MS isotope dilution method in the Environmental Analytical Chemistry Lab at Dartmouth College. There has not been a comparison between methods used to analyze turtle blood for mercury. However, given the observed concentrations and associated uncertainties it is unlikely that differences between these analytical methods would have masked significant temporal trends.

7.3 Turtle Reproduction

Following capture, female turtles were palpated to determine whether they were gravid. Gravid female turtles were injected with oxytocin to induce oviposition and were monitored until eggs had been laid. All eggs were weighed and measured. A subset of eggs was sent for chemical analysis of metals and metalloids, while the remaining eggs were incubated until hatching. Once hatchlings had emerged completely from their eggs, they were weighed and measured. A subset of hatchlings was sent for chemical analysis of metals and metalloids.

Initial findings for turtle reproductive health suggest that the turtle species were similar at both impacted and non-impacted sites. Clutch size, egg hatching success, egg and hatchling size, and other reproductive metrics were similar within each species and did not differ among rivers. In addition, the ranges for the reproductive metrics were all comparable to literature-based ranges from other sites (Hopkins et al. 2012). As a result, preliminary results from this 2011 study do not indicate observable adverse impacts on turtle reproduction.

8. Tree Swallows

Tree swallows were selected as a representative aerial insectivore species for the Site. Tree swallows prey on a variety of insects, primarily emergent aquatic insects such as mayflies (ARCADIS 2012g). They catch their prey while flying over the water or in nearby open areas (U.S. Geological Survey 2003; Blancher and McNiol 1991; Quinney and Ankney 1985). Foraging distances from nesting sites typically range from 100 to 200 meters, thus providing good spatial resolution for results (McCarty and Winkler 1999).

In 2011, tree swallow monitoring was conducted by VA Tech. Detailed descriptions of study methods are available in *Effects of the Remediated Coal Ash Spill in Kingston, TN on Aquatic and Terrestrial Consumers. Annual Report* (Hopkins et al. 2012). Analysis of trace element concentrations, monitored in eggs and nestling blood, has not yet been reported. A more detailed evaluation including these data will be published by VA Tech in March 2013.

The main study objectives were 1) to compare concentrations of metals and metalloids in eggs and nestlings among locations and years; 2) to evaluate tree swallow reproductive success among locations and years; and 3) to relate concentrations measured at the study sites to reference area concentrations and literature-derived effects values, when available.

8.1 Tree Swallow Reproduction and Offspring Health and Survival

Similar to the tree swallow studies in 2009 and 2010, the study of tree swallow reproductive success and trace elements in tissue was continued in 2011 by VA Tech. The 2011 study evaluated egg, nestling, and survival parameters as in the previous year, but also included endocrine responses to stress of fledglings and composition of the nestling diet.

A preliminary evaluation of the reproductive results for tree swallows found no significant differences among impacted and reference colonies in clutch size, egg size, or nestling size in 2011. Hatching success tended to be lower at one impacted site (CRM 2.5) compared to other locations. In addition, one of the impacted colonies on the Emory River experienced twice the reduction in reproductive impacts (hatching success and nestling mortality) compared to other monitored locations during an unusual cold-weather event in May (Hopkins et al. 2012). This difference was similar to other studies that found severe weather events exacerbated the effects of pollutants on reproductive success; however, this difference was also seen at one of the selected reference sites (Fort Loudoun Dam) and a historically contaminated colony upstream on the Clinch River (Melton Hill Dam) (Hopkins et al. 2012). In the absence of trace element data, conclusions on these reproductive differences are premature.

No significant differences were identified among impacted and reference colonies for fledgling success (survival to day17), nestling size, or nestling health. The nestling diet of boluses from the parents was

analyzed and 67 percent of the invertebrates in the diet had an aquatic life stage, providing support to the assumption that tree swallows depend on emergent aquatic insects from the river (Hopkins et al. 2012). Overall, preliminary results from this 2011 study did not indicate observable adverse impacts on the tree swallow reproduction or offspring health and survival in relation to the ash recovery area.

9. Data Quality Assurance/Quality Control Results

This section focuses on the evaluation of data quality and usability.

9.1 Analytical Data Review

TVA's contracted laboratories were required to submit three types of deliverables: a limited (Level 1) data package containing sample results and batch quality control (QC) sample results; a fully-documented (Level 4) data package including raw data for all analyses; and electronic data deliverables (EDDs) for storage in TVA's EarthSoft EQuIS® database.

EDDs were subjected to completeness and correctness testing during loading to TVA's EQuIS database; once loaded to the EQuIS database, the data were subjected to verification. As defined in the TVA-KIF-QAPP, data verification involved comparison of the data loaded in the EQuIS database to the results reported in the Level 1 data package. In addition, data verification included review of the batch QC summary forms for compliance with the applicable methods and for data usability with respect to the project data quality objectives (DQOs) and the TVA-KIF-QAPP.

Following receipt of the Level 4 data package, data were subjected to validation. As defined in the TVA-KIF-QAPP, data validation included review of raw data and associated QC summary forms for compliance with the applicable methods and for data usability with respect to the appropriate guidance documents. As stated in the QAPP: "Initially, 100% of the chemical analysis data will be reported in full documentation data packages for independent data validation. Depending on the nature and frequency of issues identified during data validation, the percentage of data undergoing full data validation may be reduced to a lesser percentage (such as 20%) or data verification may be substituted. The reduction in full data validation may be matrix specific, laboratory specific, or analyte specific. If after the percentage of full data validation has decreased, a trend in frequency of reporting issues, method non-compliances, or data usability issues is identified, data validation will be conducted for specific data points or the percentage of full data validation percentage may be increased until the issues have been minimized to their initial frequency." Data validation expands upon the completeness, correctness, and usability assessment performed during verification to include evaluation of instrumental QC analyses, review of sample preparation information, and recalculation of reported results from raw data. A summary of the data review efforts are presented in Table 9-1.

9.2 Data Quality Summary

Data validation was performed based on the sample results, summary QC data, and raw data provided by the laboratory. Data validation includes a review of the following QC measures (where applicable):

- Sample condition upon laboratory receipt
- Initial calibration linearity
- Blank analysis results greater than the Method Detection Level (MDL)
- Sample preparation and holding times
- Initial calibration verification/continuing calibration verification standard recoveries
- MDLs and linear ranges
- Internal standard recoveries
- Percent moisture
- Matrix spike/matrix spike duplicate
- Laboratory and field duplicate precision
- Quantitation of positive results
- Laboratory control sample/laboratory control sample duplicate recoveries and precision
- Analytical sequence
- Reporting limit standard recoveries (metals only)
- Serial dilutions (metals only)
- Post-digestion spike/post-digestion spike recoveries and precision (metals only)
- Internal standard recoveries
- Inductively coupled plasma interference check standard results (metals only)
- Quantitation of positive results
- MDL verification standards (metals only)
- Standard reference material recoveries (metals only)

The data met the DQOs defined for this task and were acceptable for use for each of the receptors. Table 9-2 summarizes the data quality for each receptor based on the review performed and as compared to the data quality measures identified in the TVA-KIF-QAPP.

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Appendix A

Evaluation of 2011 Fish Community Survey Results for the Kingston Fossil Plant Ash Recovery Project

Evaluation of 2011 Fish Community Survey Results for the Kingston Fossil Plant Ash Recovery Project

June 2012

Introduction

This report provides a summary of the 2011Reservoir Fish Assemblage Index (RFAI) results for fish community surveys conducted near the Kingston Fossil Plant (KIF) with comparisons to results from previous years. This is an interim report for the ongoing monitoring program aimed at evaluating potential effects of the KIF ash release which occurred in December 2008. Sample sites, collection procedures, and data analytical procedures in 2011were the same as those detailed in the previous report, *Evaluation of the Fish Community in the Vicinity of Kingston Fossil Plant, 2001-2010*. The previous report also provides an in-depth discussion of historical results.

Tennessee Valley Authority's Reservoir Fish Assemblage Index (RFAI) methodology has been used to assess fish communities near KIF to satisfy National Pollutant Discharge Elimination System permit renewal requirements since 2001. The RFAI uses 12 fish community metrics from four general categories — species richness and composition, trophic composition, abundance, and fish health — to evaluate the integrity of the fish community.

Fish community samples were collected once every 2 years from 2001 through 2007 at locations upstream (Clinch River mile [CRM] 4.4) and downstream (CRM 1.5) of the KIF heated discharge. Following the ash release in December 2008, fish community sampling has been conducted annually at three locations, including the two sites sampled prior to the release and at an additional site established at Emory River mile (ERM) 2.5 to evaluate immediate near-field effects.

Results

RFAI results including individual metric scores, contributing species, and overall RFAI scores for each sampling site for each sample year are listed in Tables 1 through 4. Metric scoring criteria are shown in Table 5. The total number of each species collected in electrofishing and gill netting samples combined is listed for each sampling site for each sample year in Table 6. The species collected, including trophic level, indigenous, and tolerance classifications, as well as catch per effort during electrofishing and gill netting at each sampling site in 2011, are listed in Tables 7 through 9.

<u>RFAI Scores</u>

- The fish communities at the Emory River site in the immediate area of the release (ERM 2.5) and the site in proximity to the confluence of the Emory and Clinch Rivers (CRM 4.4) attained RFAI scores of 42 and 44, respectively, and rated "Good" (Table 1, Figure 1).
- The fish community at the most downstream site on the Clinch River (CRM 1.5) attained a RFAI score of 37 and rated "Fair".
- The lower rating for CRM 1.5 compared to the two upstream sites was due primarily to lower scores for "number of benthic invertivores", "percent non-indigenous species", and "percent omnivores."

CRM 4.4 also scored higher for “percent top carnivores” than other sites. A summary of individual RFAI metric results is provided below.

Species Richness and Composition

- Twenty-seven to 28 indigenous species were collected at the three sites in 2011 (Figure 2). Thirty species are needed to attain the highest score possible for the metric (metric 1); therefore, all sites received a moderate score. The “number of indigenous species” collected at the sites over the years has ranged from 25 to 35. Moderate species richness also was observed at both Clinch River sites in 2007 and 2009 (25 to 28 species). Year-to-year variations in species richness resulted primarily from species which are not commonly collected each year. For example, of the 50 indigenous species collected since 2001, 15 were collected in only one to four (<25 percent) of the 17 RFAI samples. Thirteen of these “less-common” species were not collected during the 2011 surveys and thereby contributed to the lower representation of indigenous species as compared to some previous years.
- In 2011, all sites received the highest score possible for the metrics “number of centrarchid species” (metric 2), “number of intolerant species” (metric 4), and “number of top carnivore species” (metric 8). Likewise, the number of species that contributed to each of these metrics was within the range observed prior to the release.
- The “number of benthic invertivore species” (metric 3) collected at the sites in 2011 were similar; three species were collected at CRM 1.5 and four were collected at ERM 2.5 and CRM 4.4. Although sites differed by only one species, ERM 2.5 and CRM 4.4 received moderate scores for the metric, while CRM 1.5 received the lowest possible score. As in most previous years (pre- and post-release), fewer benthic invertivore species were collected at CRM 1.5 than at other sites. CRM 1.5 also received the lowest possible score for this metric in 2001, 2007, and 2009 (Table 2). The number of benthic invertivore species collected at the sites in previous years ranged from 2 to 7. The moderate range for this metric is from 4 to 7 species; therefore, no site has received the highest possible score.
- “Percent tolerant individuals” (metric 5) in electrofishing samples has consistently scored the lowest number of possible points, with percentages ranging from approximately 72 to 84 percent during pre-release surveys and from approximately 66 to 90 percent during post-release surveys. Bluegill sunfish typically constituted a majority (~30 to 50 percent) of tolerant individuals in the electrofishing samples, followed by gizzard shad, and then largemouth bass and/or spotfin shiner. In 2011, the catch rate for bluegill sunfish at ERM 2.5 (~292 fish per hour) (Table 9) exceeded those observed at sites in previous years. Bluegill sunfish composed 69.2 percent of the fish collected at the site and contributed to the highest percentage (90 percent) of tolerant individuals observed to date. In gill netting samples at each site in 2011, “percent tolerant individuals” received the highest possible score with the proportions of tolerant individuals ranging from approximately 5 to 10 percent. Historic scores for this metric were variable, with percentages ranging from about 7 to 38 percent, and gizzard shad typically contributing the highest percentages.
- In 2011, “percent dominance by one species” (metric 6) received a moderate score for the electrofishing samples at both Clinch River sites and the lowest possible score at ERM 2.5. Bluegill sunfish composed 30.9 and 35.8 percent of the fish collected at CRM 4.4 and CRM 1.5, respectively, and they composed 69.2 percent at ERM 2.5. Results for this metric have been fairly consistent over time for electrofishing samples, with percentages typically ranging from about 30 to 46 percent, and bluegill sunfish typically being the dominant species. However, scores fluctuated between moderate

and low as the upper bound of the moderate range is at 40 percent. Sauger dominated (30 percent) the gill netting samples at CRM 1.5 and blue catfish dominated at CRM 4.4 and ERM 2.5 (22.9 and 29.5 percent, respectively). Historically, gizzard shad were often the dominant species in gill netting samples, but other species (e.g., skipjack herring, yellow bass, striped bass, and/or blue catfish,) were dominant in any given year. The overall low number of fish collected in gill netting samples in 2011 contributed to the somewhat atypical results, especially at CRM 1.5 due to the higher number of sauger collected at this site.

- The scores for "percent non-indigenous species" (metric 7) in electrofishing and gill netting samples have been the most variable among the 12 metrics from year to year, receiving high, moderate, and low scores. For electrofishing samples in 2011, non-indigenous species composed 4.8 and 5.9 percent (moderate range) of the samples at CRM 4.4 and CRM 1.5, respectively, and composed 1.1 percent ("good") of the sample at ERM 2.5. Scores for electrofishing samples each year were dependent largely upon the collection of Mississippi silverside (taxonomy of the species was revised from inland silverside). Because this is a small, schooling fish, catch rates can be highly variably from year to year. Relatively few were collected in 2011, which resulted in improved scores for this metric compared to recent years. For gill netting samples in 2011, ERM 2.5 and CRM 4.4 received the highest possible rating for "percent non-indigenous species", while CRM 1.5 received a moderate rating. Percentages of striped bass (4.0 percent) and common carp (2.0 percent) accounted for the lower rating at CRM 1.5.
- "Percent top carnivores" (metric 9) for the 2011 electrofishing samples received moderate ratings at CRM 1.5 (8.1 percent) and ERM 2.5 (9.6 percent) and the highest rating at CRM 4.4 (14.6 percent). Consistent with previous years, largemouth bass contributed the highest percentages (6.6 to 10.8 percent) to this metric. Scores have fluctuated between high, moderate, and low over the years, with no discernable difference between pre-release and post-release results. In gill netting samples in 2011, proportions of top carnivores ranged from 32.7 percent at ERM 2.5 to 54.2 percent at CRM 4.4, resulting in moderate and high scores, respectively. The composition of top carnivores at CRM 1.5 was 52.0 percent which is the upper bound of the moderate range (i.e., 26 to 52 percent). The species contributing most to this metric has varied at each site and by year. Notable was the elevated composition of sauger at each site in 2011. This was due in part to the lower overall catch rate at each site in 2011 compared to other years, and the above average number of sauger collected at CRM 1.5 and ERM 2.5.
- "Percent omnivores" (metric 10) in 2011 ranged from 10 to 29.4 percent and scored in the "good" to moderate range in electrofishing samples. As in previous years, gizzard shad was the dominant omnivore at all sites. In gill netting samples in 2011, blue catfish was the dominant omnivore and composed about 27 to 46 percent of the sample, which resulted in moderate scores. "Percent omnivores" scored in the moderate range in gill netting samples most years, but gizzard shad usually accounted for the highest percentages, and blue catfish generally contributed the second highest.

<u>Abundance</u>

- Consistent with previous years, fish abundance (metric 11) was low in electrofishing and gill netting samples at each site in 2011 compared to expectations for transition zones in upper mainstream Tennessee River reservoirs. However, electrofishing catch rates in 2011 were within their respective historic ranges in the immediate area of the release (ERM 2.5) and at CRM 1.5, but fewer fish were collected at CRM 4.4 than in previous years due primarily to low catch rates for several common

species (e.g., bluegill sunfish, gizzard shad, largemouth bass, common carp, and inland silversides). Gill netting catch rates at the sites in 2011 ranged from 4.8 to 6.1 fish per net, which was lower than observed in previous years (~8 to 23 fish per net). These declines were due primarily to low numbers of several common and frequently abundant species (e.g., skipjack herring, gizzard shad, yellow bass, striped bass, etc.).

- As stated, the low fish abundances observed for both sampling methods was attributable to concurrent low catch rates for several common but ecologically diverse species. Given that electrofishing catch rates at ERM 2.5 and CRM 1.5 in 2011 were within their historic ranges, these declines were more likely related to sampling efficiencies rather than an adverse effect of the ash release being manifested concurrently at the population level of numerous species.

Fish Health

- The percentage of fish with anomalies (i.e., visible lesions, bacterial and fungal infections, parasites, muscular and skeletal deformities, and hybridization) in electrofishing samples was higher at all sites in 2011 (3.4 to 8.6 percent) as compared to pre-release surveys (0.1 to 1.3 percent), with the highest incidence of anomalies occurring at CRM 4.4. As in 2009, when percentages ranged from 2.8 to 4.3 percent, a majority of the increases were attributable to bluegill sunfish infected with common parasite(s) (i.e., trematodes in eyes). Because a large proportion (~83 percent) of the infected bluegill sunfish at CRM 4.4 in 2011 were collected at sampling locations in the Clinch River upstream of the confluence of the Emory River, the increased parasite loads observed at each site are unlikely ash-related. Additionally, the percentages of fish with anomalies in gill netting samples ranged from 0 to 2 percent in 2011, which was similar to those observed during pre-release surveys (0 to 2.8 percent).

Summary

- RFAI scores at the Emory River site in the immediate area of the release (ERM 2.5) rated “good” during the three annual surveys following the release.
- Over the seven sample years for the Clinch River sites, RFAI scores rated “good” to “fair”, with the lowest scores occurring in 2007 and 2009, respectively.
- In 2011, the RFAI scores at ERM 2.5and CRM 4.4 were 5 to 7 points higher than at CRM 1.5 due primarily to lower scores for “number of benthic invertivores”, “percent non-indigenous species” and “percent omnivores”.
- Results for species richness metrics (1-4 and 8) were similar during pre-release and post-release surveys. The “number of indigenous species” collected at the sites over the years ranged from 25 to 35, indicating moderate to “good” representation of indigenous species. The highest numbers of indigenous species was observed at ERM 2.5 and CRM 1.5 in 2010. Moderate richness (25 to 28 species) was observed at the Clinch River sites in 2007 and 2009 and at all sites in 2011.
- The metrics “number of centrarchid species”, “number of intolerant species”, and “number of top carnivore species” received the maximum number of points at each site during the three annual surveys following the release, indicating “good” numbers of these species were represented in samples. A moderate to low “number of benthic invertivore species” were collected at the sites each sample year, with no discernable difference between pre-release and post-release surveys.

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- “Percent tolerant individuals” and “percent dominance by one species” usually scored in the moderate to low range at the sites, with no apparent difference between pre-release and post-release samples attributable to ash-related effects. The low scores for “dominance” were largely due to the high percentage of bluegill sunfish and/or gizzard shad in most samples. Likewise, these species are considered tolerant of degraded water quality and contribute to the composition of tolerant individuals.
- “Percent non-indigenous species” have shown a trend of increasing percentages in electrofishing samples due mainly to an increase in the frequency and numbers of Mississippi silversides collected (taxonomy of the species was revised from inland silverside). This species was first collected in Kentucky and Pickwick reservoirs in 1993 and has continued to spread throughout the Tennessee River system. Because this is a schooling fish, catch rates can be highly variable from year to year. Relatively few were collected in 2011 compared to recent years, which resulted in improved scores for this metric.
- Scores for trophic composition (“percent top carnivores” and “percent omnivores”) have fluctuated between high, moderate, and low. The principal difference between pre-release and post-release samples was the improved scores (lower composition) for “percent omnivores” in several electrofishing samples in recent years.
- Fish abundance was low in electrofishing and gill netting samples at the sites each sample year compared to expectations for fish assemblages in transition zones of upper mainstream Tennessee River reservoirs. Respective to historical results at KIF, the number of fish collected in electrofishing samples was highest at sites in 2009 and 2010, but still remained in the moderate to low range. The principle difference in fish abundance among years occurred in 2011 with historically low numbers of fish collected in electrofishing samples at CRM 4.4 and in gill netting samples at all sites. The low abundance observed for both sampling methods was attributable to concurrent low catch rates for several common but ecologically diverse species. Given that electrofishing catch rates at ERM 2.5 and CRM 1.5 in 2011 were within their respective historical ranges, these declines were more likely related to sampling efficiencies rather than an adverse effect of the ash release being manifested concurrently at the population level of numerous species.
- Though the percentages of fish with anomalies were elevated in 2009 and 2011, the majority of the increases were attributable to bluegill sunfish infected with common parasites. Given year-to-year variability in the incidence of anomalies and the fact that parasite loads in 2011 were highest at sampling locations in the Clinch River upstream of the Emory River, there is no clear evidence that the increases are ash related.

Conclusion

- The 2011 RFAI results for each site were within the range of expected variation based on historical results and the intrinsic variability in sampling reservoir fish communities. The one notable exception was the low number of fish collected in electrofishing samples at CRM 4.4. However, observed values for other metrics at this site in 2011 were similar to those observed in other sample years, which resulted in an overall RFAI score (44,”good”) that also was similar to previous years. Collectively, the RFAI results for the seven sample years indicate fish assemblages near KIF continue to be representative of those observed prior to the release and, likewise, are representative of those expected in transition zones within upper mainstream reservoirs.

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Table 1. Reservoir Fish Assemblage Index (RFAI) Scores and Long-Term Average Scores for ERM 2.5, CRM 4.4, and CRM 1.5, 2001-2011.

Site	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	Long-Term Average
ERM 2.5	---	---	---	---	---	---	---	---	44	44	42	43.3
CRM 4.4	45	---	42	---	44	---	36	---	38	42	44	41.6
CRM 1.5	42	---	44	---	41	---	34	---	36	42	37	39.4

RFAI scoring range for five rating categories: 12-21 (“Very Poor”), 22-31 (“Poor”), 32-40 (“Fair”), 41-50 (“Good”), or 51-60 (“Excellent”).

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Table 2. Individual Metric Scores, Contributing Species, and Overall RFAI Scores for CRM 1.5, Autumn 2001-2011.

CRM 1.5																
Metric	Gear	Common Name	2001		2003		2005		2007		2009		2010		2011	
			Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc
1. Number of indigenous species	Combined		**33**	**5**	**33**	**5**	**31**	**5**	**25**	**3**	**28**	**3**	**35**	**5**	**27**	**3**
2. Number of centrarchid species (less Micropterus)	Combined		**8**	**5**	**7**	**5**	**6**	**5**	**6**	**5**	**7**	**5**	**7**	**5**	**8**	**5**
		Black crappie	12		1		--		2		2		4		2	
		Bluegill sunfish	540		365		330		364		970		704		319	
		Green sunfish	11		4		36		16		43		35		30	
		Longear sunfish	12		16		52		106		197		71		38	
		Redbreast sunfish	5		1		6		1		14		5		6	
		Redear sunfish	59		51		40		47		52		107		44	
		Warmouth	2		--		--		--		3		--		6	
		White crappie	8		2		2		--		--		4		1	
3. Number of benthic invertivore species	Combined		**3**	**1**	**4**	**3**	**4**	**3**	**2**	**1**	**3**	**1**	**4**	**3**	**3**	**1**
		Black redhorse	--		--		--		--		--		--		3	
		Freshwater drum	10		30		9		8		11		13		11	
		Golden redhorse	--		1		--		1		1		2		--	
		Logperch	5		6		2		--		--		3		--	
		Northern hog sucker	--		--		4		--		--		--		--	
		Spotted sucker	14		11		18		--		15		11		52	
4. Number of intolerant species	Combined		**5**	**5**	**6**	**5**	**7**	**5**	**3**	**3**	**5**	**5**	**6**	**5**	**5**	**5**
		Black redhorse	--		--		--		--		--		--		3	
		Brook silverside	17		34		5		11		20		21		--	
		Longear sunfish	12		16		52		106		197		71		38	
		Mooneye	--		1		4		--		--		--		--	
		Northern hog sucker	--		--		4		--		--		--		--	
		Rock bass	--		--		--		--		3		3		1	
		Skipjack herring	21		21		22		--		--		10		--	
		Smallmouth bass	6		2		21		1		23		8		7	
		Spotted sucker	14		11		18		--		15		11		52	
5. Percent tolerant individuals	Electrofishing		**83.4%**	**0.5**	**75.7%**	**0.5**	**74.8%**	**0.5**	**76.6%**	**0.5**	**74.1%**	**0.5**	**66.4%**	**0.5**	**75.8%**	**0.5**
		Bluegill sunfish	45.0%		43.8%		34.0%		46.1%		45.5%		40.5%		35.8%	
		Bluntnose minnow	0.2%		0.6%		0.3%		0.1%		1.3%		3.9%		0.1%	
		Common carp	1.6%		1.9%		1.8%		0.6%		0.6%		0.4%		2.0%	
		Gizzard shad	21.6%		21.2%		29.4%		22.4%		6.9%		14.5%		25.8%	
		Golden shiner	0.8%		0.6%		--		--		0.1%		--		--	
		Green sunfish	0.9%		0.5%		3.7%		2.0%		2.0%		2.0%		3.4%	
		Largemouth bass	8.0%		4.1%		2.6%		2.6%		4.6%		3.2%		6.6%	
		Redbreast sunfish	0.4%		0.1%		0.6%		0.1%		0.7%		0.3%		0.7%	
		River carpsucker	0.1%		--		--		--		--		--		--	
		Spotfin shiner	4.6%		2.7%		2.4%		2.7%		12.4%		1.2%		1.4%	
		Striped shiner	--		--		--		--		--		0.2%		--	
		White crappie	0.2%		0.2%		--		--		--		0.2%		--	
	Gill Netting		**27.7%**	**1.5**	**24.5%**	**1.5**	**25.8%**	**1.5**	**37.7%**	**0.5**	**17.7%**	**1.5**	**23.7%**	**1.5**	**10.0%**	**2.5**
		Bluegill sunfish	6.1%		2.8%		--		5.2%		--		0.6%		2.0%	
		Common carp	0.4%		3.5%		3.0%		2.6%		2.5%		4.0%		2.0%	
		Gizzard shad	16.9%		14.7%		19.2%		14.3%		12.7%		17.3%		4.0%	
		Largemouth bass	1.7%		2.1%		1.8%		1.3%		2.5%		0.6%		--	
		Longnose gar	--		1.4%		0.6%		14.3%		--		0.6%		--	
		White crappie	2.6%		--		1.2%		--		--		0.6%		2.0%	
6. Percent dominance by one species	Electrofishing		**45.0%**	**0.5**	**43.8%**	**0.5**	**34.0%**	**1.5**	**46.1%**	**0.5**	**45.5%**	**0.5**	**40.5%**	**0.5**	**35.8%**	**1.5**
		Bluegill sunfish	45.0%		43.8%		34.0%		46.1%		45.5%		40.5%		35.8%	
	Gill Netting		**16.9%**	**1.5**	**14.7%**	**1.5**	**19.2%**	**1.5**	**14.3%**	**1.5**	**21.5%**	**1.5**	**18.5%**	**1.5**	**30.0%**	**0.5**
		Blue catfish	--		--		--		--		21.5%		--		--	
		Gizzard shad	16.9%		14.7%		19.2%		14.3%		--		--		--	
		Sauger	--		--		--		--		--		--		30.0%	
		Yellow bass	--		--		--		--		--		18.5%		--	

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Table 2, continued.

CRM 1.5																		
Metric	Gear	Common Name	2001		2003		2005		2007		2009		2010		2011	
			Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc
7. Percent non-indigenous species	Electrofishing		**1.9%**	**2.5**	**2.3%**	**2.5**	**12.1%**	**0.5**	**0.9%**	**2.5**	**11.0%**	**0.5**	**18.8%**	**0.5**	**5.9%**	**1.5**
		Common carp	1.6%		1.9%		1.8%		0.6%		0.6%		0.4%		2.0%	
		Inland silverside	--		--		10.3%		0.3%		10.3%		18.3%		3.6%	
		Striped bass	--		--		--		--		--		0.1%		0.3%	
		Yellow perch	0.3%		0.4%		--		--		0.1%		--		--	
	Gill Netting		**7.4%**	**1.5**	**9.8%**	**0.5**	**13.8%**	**0.5**	**13.0%**	**0.5**	**6.3%**	**1.5**	**9.8%**	**0.5**	**6.0%**	**1.5**
		Common carp	0.4%		3.5%		3.0%		2.6%		2.5%		4.0%		2.0%	
		Striped x white bass	0.9%		--		--		--		--		--		--	
		Striped bass	6.1%		6.3%		10.8%		10.4%		3.8%		5.8%		4.0%	
8. Number of top carnivore species	Combined		**11**	**5**	**12**	**5**	**11**	**5**	**10**	**5**	**10**	**5**	**12**	**5**	**10**	**5**
		Black crappie	12		1		--		2		2		4		2	
		Flathead catfish	6		15		7		8		1		11		1	
		Largemouth bass	98		37		32		21		125		69		68	
		Longnose gar	--		2		1		11		--		1		--	
		Rock bass	--		--		--		--		3		3		1	
		Sauger	4		4		8		--		14		5		15	
		Skipjack herring	21		21		22		--		--		10		--	
		Smallmouth bass	6		2		21		1		23		8		7	
		Spotted bass	25		8		14		1		8		--		2	
		Spotted gar	2		1		4		1		1		1		--	
		Walleye	--		--		--		1		--		--		--	
		White bass	5		6		6		1		3		17		6	
		White crappie	8		2		2		--		--		4		1	
		Yellow bass	19		22		14		5		5		32		1	
9. Percent top carnivores	Electrofishing		**10.7%**	**1.5**	**6.4%**	**1.5**	**5.3%**	**0.5**	**3.0%**	**0.5**	**5.9%**	**1.5**	**5.0%**	**0.5**	**8.1%**	**1.5**
		Black crappie	0.1%		0.1%		--		--		--		0.2%		--	
		Flathead catfish	0.2%		0.8%		0.3%		0.1%		--		0.2%		--	
		Largemouth bass	8.0%		4.1%		2.6%		2.6%		4.6%		3.2%		6.6%	
		Rock bass	--		--		--		--		0.1%		0.1%		0.1%	
		Skipjack herring	--		--		--		--		--		0.1%		--	
		Smallmouth bass	0.5%		0.2%		0.9%		0.1%		0.8%		0.3%		0.8%	
		Spotted bass	1.5%		0.8%		1.2%		0.1%		0.4%		--		0.1%	
		Spotted gar	0.2%		--		0.3%		0.1%		--		0.1%		--	
		Striped bass	--		--		--		--		--		0.1%		0.3%	
		White bass	--		0.1%		--		--		0.0%		0.5%		0.2%	
		White crappie	0.2%		0.2%		--		--		--		0.2%		--	
		Yellow bass	--		0.1%		--		--		--		--		--	
	Gill Netting		**42.0%**	**1.5**	**52.5%**	**2.5**	**48.6%**	**1.5**	**46.8%**	**1.5**	**37.9%**	**1.5**	**44.6%**	**1.5**	**52.0%**	**1.5**
		Black crappie	4.8%		--		--		2.6%		2.5%		0.6%		4.0%	
		Flathead catfish	1.7%		5.6%		2.4%		9.1%		1.3%		4.6%		2.0%	
		Striped x white bass	0.9%		--		--		--		--		--		--	
		Largemouth bass	1.7%		2.1%		1.8%		1.3%		2.5%		0.6%		--	
		Longnose gar	--		1.4%		0.6%		14.3%		--		0.6%		--	
		Rock bass	--		--		--		--		--		0.6%		--	
		Sauger	1.7%		2.8%		4.8%		--		17.7%		2.9%		30.0%	
		Skipjack herring	9.1%		14.7%		13.2%		--		--		4.6%		--	
		Spotted bass	3.0%		0.7%		1.2%		--		--		--		--	
		Spotted gar	--		0.7%		0.6%		--		1.3%		--		--	
		Striped bass	6.1%		6.3%		10.8%		10.4%		3.8%		5.8%		4.0%	
		Walleye	--		--		--		1.3%		--		--		--	
		White bass	2.2%		3.5%		3.6%		1.3%		2.5%		5.2%		8.0%	
		White crappie	2.6%		--		1.2%		--		--		0.6%		2.0%	
		Yellow bass	8.2%		14.7%		8.4%		6.5%		6.3%		18.5%		2.0%	

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Table 2, continued.

CRM 1.5																
Metric	Gear	Common Name	2001		2003		2005		2007		2009		2010		2011	
			Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc
10. Percent omnivores	Electrofishing		**25.2%**	**1.5**	**27.2%**	**1.5**	**32.2%**	**1.5**	**24.2%**	**1.5**	**9.5%**	**2.5**	**20.1%**	**2.5**	**29.4%**	**1.5**
		Black buffalo	--		--		0.2%		--		--		0.1%		--	
		Bluntnose minnow	0.2%		0.6%		0.3%		0.1%		1.3%		3.9%		0.1%	
		Channel catfish	0.2%		1.6%		0.5%		0.5%		0.5%		0.8%		1.0%	
		Common carp	1.6%		1.9%		1.8%		0.6%		0.6%		0.4%		2.0%	
		Gizzard shad	21.6%		21.2%		29.4%		22.4%		6.9%		14.5%		25.8%	
		Golden shiner	0.8%		0.6%		--		--		0.1%		--		--	
		River carpsucker	0.1%		--		--		--		--		--		--	
		Smallmouth buffalo	0.7%		1.3%		--		0.6%		0.1%		0.2%		0.5%	
		Striped shiner	--		--		--		--		--		0.2%		--	
	Gill Netting		**45.1%**	**1.5**	**37.1%**	**1.5**	**35.4%**	**1.5**	**39.0%**	**1.5**	**48.1%**	**0.5**	**37.5%**	**1.5**	**34.0%**	**1.5**
		Black buffalo	1.3%		--		--		--		--		0.6%		--	
		Blue catfish	13.9%		10.5%		8.4%		9.1%		21.5%		9.8%		26.0%	
		Channel catfish	7.4%		4.9%		3.0%		5.2%		8.9%		4.6%		--	
		Common carp	0.4%		3.5%		3.0%		2.6%		2.5%		4.0%		2.0%	
		Gizzard shad	16.9%		14.7%		19.2%		14.3%		12.7%		17.3%		4.0%	
		Smallmouth buffalo	5.2%		3.5%		1.8%		7.8%		2.5%		1.2%		2.0%	
11. Average number per run	Electrofishing		**77.9**	**0.5**	**54.9**	**0.5**	**64.7**	**0.5**	**52.1**	**0.5**	**142.1**	**1.5**	**115.8**	**1.5**	**59.2**	**0.5**
	Gill Netting		**23.1**	**1.5**	**14.3**	**1.5**	**16.7**	**1.5**	**7.7**	**0.5**	**7.9**	**0.5**	**17.3**	**1.5**	**5**	**0.5**
12. Percent anomalies	Electrofishing		**0.9**	**2.5**	**0.1**	**2.5**	**0.3**	**2.5**	**0.1**	**2.5**	**4.1**	**1.5**	**1.2**	**2.5**	**3.4**	**1.5**
	Gill Netting		**0.4**	**2.5**	**0**	**2.5**	**1.8**	**2.5**	**0**	**2.5**	**2.5**	**1.5**	**0.6**	**2.5**	**2**	**1.5**
RFAI Score				**42**		**44**		**41**		**34**		**36**		**42**		**37**

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Table 3. Individual Metric Scores, Contributing Species, and Overall RFAI Scores for CRM 4.4, Autumn 2001-2011.

CRM 4.4																		
Metric	Gear	Common Name	2001		2003		2005		2007		2009		2010		2011			
			Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc		
1. Number of indigenous species	Combined		**33**	**5**	**34**	**5**	**30**	**5**	**28**	**3**	**28**	**3**	**34**	**5**	**28**	**3**		
2. Number of centrarchid species (less Micropterus)	Combined		**8**	**5**	**7**	**5**	**6**	**5**	**4**	**3**	**8**	**5**	**8**	**5**	**6**	**5**		
		Black crappie	5		2		3		--		3		3		--			
		Bluegill sunfish	483		298		288		305		539		764		157			
		Green sunfish	4		3		14		1		25		49		42			
		Longear sunfish	10		25		13		40		112		31		7			
		Redbreast sunfish	3		--		--		--		2		6		2			
		Redear sunfish	74		50		67		35		73		89		51			
		Warmouth	1		3		--		--		3		3		2			
		White crappie	1		1		3		--		2		3		--			
3. Number of benthic invertivore species	Combined		**5**	**3**	**5**	**3**	**7**	**3**	**5**	**3**	**3**	**1**	**5**	**3**	**4**	**3**		
		Black redhorse	1		1		2		1		2		3		4			
		Freshwater drum	6		17		12		10		8		18		3			
		Golden redhorse	1		2		5		2		--		1		--			
		Logperch	1		2		4		1		--		7		--			
		Northern hog sucker	--		--		--		--		--		--		1			
		River redhorse	--		--		1		--		--		--		--			
		Silver redhorse	--		--		1		--		--		--		--			
		Spotted sucker	17		64		80		15		15		16		25			
4. Number of intolerant species	Combined		**6**	**5**	**7**	**5**	**7**	**5**	**5**	**5**	**6**	**5**	**6**	**5**	**5**	**5**		
		Black redhorse	1		1		2		1		2		3		4			
		Brook silverside	14		44		18		14		40		13		--			
		Longear sunfish	10		25		13		40		112		31		7			
		Mooneye	--		1		--		--		--		--		--			
		Northern hog sucker	--		--		--		--		--		--		1			
		River redhorse	--		--		1		--		--		--		--			
		Rock bass	--		--		--		--		3		--		--			
		Skipjack herring	20		18		12		--		--		4		--			
		Smallmouth bass	6		4		29		5		14		16		10			
		Spotted sucker	17		64		80		15		15		16		25			
5. Percent tolerant individuals	Electrofishing		**81.7%**	**0.5**	**72.2%**	**0.5**	**77.0%**	**0.5**	**77.7%**	**0.5**	**74.4%**	**0.5**	**67.2%**	**0.5**	**71.5%**	**0.5**		
		Bluegill sunfish	52.2%		32.8%		27.0%		44.5%		36.4%		45.4%		30.9%			
		Bluntnose minnow	0.2%		0.2%				0.3%		7.0%		0.6%					
		Common carp	3.8%		1.6%		1.9%		1.2%		1.0%		1.0%		1.8%			
		Gizzard shad	9.5%		24.8%		36.1%		24.6%		6.4%		9.6%		18.2%			
		Golden shiner	0.1%		1.0%				0.1%									
		Green sunfish	0.4%		0.3%		1.3%		0.1%		1.7%		2.9%		8.4%			
		Largemouth bass	9.2%		6.9%		7.4%		4.0%		6.4%		4.7%		10.8%			
		Redbreast sunfish	0.3%								0.1%		0.4%		0.4%			
		Spotfin shiner	6.0%		4.6%		3.1%		2.9%		15.3%		2.4%		1.0%			
		White crappie	--				0.2%				0.1%		0.2%					
	Gill Netting		**22.6%**	**1.5**	**33.4%**	**0.5**	**22.9%**	**1.5**	**29.3%**	**1.5**	**10.4%**	**2.5**	**17.8%**	**1.5**	**8.4%**	**2.5**		
		Bluegill sunfish	1.4%		1.4%		--		1.1%		1.9%		--		6.3%			
		Common carp	3.8%		3.7%		6.4%		5.4%		3.8%		2.0%		--			
		Gizzard shad	16.4%		26.4%		11.0%		21.7%		1.9%		15.8%		--			
		Largemouth bass	0.5%		0.9%		3.7%		--		1.9%		--		--			
		Longnose gar	--		0.5%		--		1.1%		--		--		2.1%			
		River carpsucker	--		--		0.9%		--		--		--		--			
		White crappie	0.5%		0.5%		0.9%		--		0.9%		--		--			
6. Percent dominance by one species	Electrofishing		-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		Bluegill sunfish	**52.2%**	**0.5**	**32.8%**	**1.5**	--		**44.5%**	**0.5**	**36.4%**	**1.5**	**45.4%**	**0.5**	**30.9%**	**1.5**		
		Gizzard shad	--		--		**36.1%**	**1.5**	--		--		--		--			
	Gill Netting		-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		Blue catfish	--		--		--		--		--		--		**22.9%**	**1.5**		
		Gizzard shad	--		**26.4%**	**1.5**	**11.0%**	**2.5**	**21.7%**	**1.5**	--		--		--			
		Striped bass	--		--		--		--		--		**21.8%**	**1.5**	--			

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		Yellow bass	**16.9%**	**1.5**	--		--		--		**25.5%**	**1.5**	--		--	

Table 3, continued.

CRM 4.4																
Metric	Gear	Common Name	2001		2003		2005		2007		2009		2010		2011	
			Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc
7. Percent non-indigenous species	Electrofishing		**4.3%**	**1.5**	**1.8%**	**2.5**	**2.9%**	**2.5**	**4.8%**	**1.5**	**7.3%**	**0.5**	**22.3%**	**0.5**	**4.8%**	**1.5**
		Common carp	3.8%		1.6%		1.9%		1.2%		1.0%		1.0%		1.8%	
		Inland silverside	--		--		0.9%		3.5%		6.2%		21.1%		0.2%	
		Yellow perch	0.5%		0.2%		0.1%		0.1%		0.1%		0.2%		2.6%	
		Muskellunge	--		--		--		--		--		--		0.2%	
	Gill Netting		**4.7%**	**2.5**	**12.0%**	**0.5**	**16.5%**	**0.5**	**15.2%**	**0.5**	**7.6%**	**1.5**	**23.8%**	**0.5**	**0.0%**	**2.5**
		Common carp	3.8%		3.7%		6.4%		5.4%		3.8%		2.0%		--	
		Striped x white bass	--		--		0.9%		--		--		--		--	
		Striped bass	0.9%		8.3%		9.2%		9.8%		3.8%		21.8%		--	
8. Number of top carnivore species	Combined		**10**	**5**	**12**	**5**	**10**	**5**	**9**	**5**	**10**	**5**	**12**	**5**	**9**	**5**
		Black crappie	5		2		3		--		3		3		--	
		Flathead catfish	4		2		2		3		2		3		1	
		Largemouth bass	86		64		97		27		106		90		60	
		Longnose gar	--		1		--		1		--		--		1	
		Rock bass	--		--		--		--		3		--		--	
		Sauger	9		4		6		5		12		5		6	
		Skipjack herring	20		18		12		--		--		4		--	
		Smallmouth bass	6		4		29		5		14		16		10	
		Spotted bass	19		18		12		4		7		2		3	
		Spotted gar	--		5		--		--		--		1		--	
		Walleye	--		--		--		2		--		3		2	
		White bass	17		6		2		3		2		12		5	
		White crappie	1		1		3		--		2		3		--	
		Yellow bass	38		32		9		8		28		5		20	
9. Percent top carnivores	Electrofishing		**12.2%**	**2.5**	**9.9%**	**1.5**	**11.1%**	**2.5**	**5.3%**	**0.5**	**8.1%**	**1.5**	**6.4%**	**1.5**	**14.6%**	**2.5**
		Black crappie	0.2%		0.2%		0.3%		--		0.1%		0.2%		--	
		Flathead catfish	0.1%		0.1%		0.1%		0.1%		--		--		--	
		Hybrid bass	--		--		--		--		--		0.1%		--	
		Largemouth bass	9.2%		6.9%		7.4%		4.0%		6.4%		4.7%		10.8%	
		Rock bass	--		--		--		--		0.2%		--		--	
		Skipjack herring	--		0.2%		--		--		--		--		--	
		Smallmouth bass	0.7%		0.4%		2.0%		0.7%		0.9%		0.5%		1.2%	
		Spotted bass	1.6%		1.3%		1.0%		0.4%		0.3%		0.1%		0.2%	
		Spotted gar	--		0.6%		--		--		--		0.1%		--	
		White bass	0.2%		--		--		--		--		0.5%		0.2%	
		White crappie	--		--		0.2%		--		0.1%		0.2%		--	
		Yellow bass	0.2%		0.2%		0.1%		0.1%		0.1%		--		2.0%	
		Muskellunge	--		--		--		--		--		--		0.2%	
	Gill Netting		**44.1%**	**1.5**	**39.5%**	**1.5**	**42.1%**	**1.5**	**32.7%**	**1.5**	**49.1%**	**1.5**	**45.8%**	**1.5**	**54.2%**	**2.5**
		Black crappie	1.4%		--		--		--		1.9%		--		--	
		Flathead catfish	1.4%		0.5%		0.9%		2.2%		1.9%		3.0%		2.1%	
		Striped x white bass	--		--		0.9%		--		--		--		--	
		Largemouth bass	0.5%		0.9%		3.7%		--		1.9%		--		--	
		Longnose gar	--		0.5%		--		1.1%		--		--		2.1%	
		Sauger	4.2%		1.9%		5.5%		5.4%		11.3%		5.0%		12.5%	
		Skipjack herring	9.4%		7.4%		11.0%		--		--		4.0%		--	
		Smallmouth bass	--		--		--		--		--		--		4.2%	
		Spotted bass	1.9%		2.8%		0.9%		1.1%		--		--		--	
		Striped bass	0.9%		8.3%		9.2%		9.8%		3.8%		21.8%		--	
		Walleye	--		--		--		2.2%		--		3.0%		4.2%	
		White bass	7.0%		2.8%		1.8%		3.3%		1.9%		4.0%		8.3%	
		White crappie	0.5%		0.5%		0.9%		--		0.9%		--		--	
		Yellow bass	16.9%		13.9%		7.3%		7.6%		25.5%		5.0%		20.8%	

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Table 3, continued.

CRM 4.4																		
Metric	Gear	Common Name	2001		2003		2005		2007		2009		2010		2011	
			Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc	Obs	Sc
10. Percent omnivores	Electrofishing		**15.3%**	**2.5**	**30.5%**	**1.5**	**39.1%**	**1.5**	**28.0%**	**1.5**	**15.6%**	**2.5**	**11.6%**	**2.5**	**21.8%**	**2.5**
		Black buffalo	0.1%		0.3%		0.3%		--		--		0.1%		0.4%	
		Blue catfish	--		--		--		--		--		--		0.2%	
		Bluntnose minnow	0.2%		0.2%		--		0.3%		7.0%		0.6%		--	
		Channel catfish	0.8%		0.6%		0.5%		1.5%		1.0%		0.2%		0.8%	
		Common carp	3.8%		1.6%		1.9%		1.2%		1.0%		1.0%		1.8%	
		Gizzard shad	9.5%		24.8%		36.1%		24.6%		6.4%		9.6%		18.2%	
		Golden shiner	0.1%		1.0%		--		0.1%		--		--		--	
		Smallmouth buffalo	0.8%		2.0%		0.3%		0.3%		0.2%		0.1%		0.4%	
	Gill Netting		**44.5%**	**1.5**	**51.9%**	**0.5**	**33.9%**	**1.5**	**45.6%**	**1.5**	**36.8%**	**1.5**	**35.7%**	**1.5**	**27.1%**	**1.5**
		Black buffalo	0.9%		--		--		--		--		--		--	
		Blue catfish	16.4%		12.0%		5.5%		14.1%		19.8%		10.9%		22.9%	
		Channel catfish	7.0%		4.2%		4.6%		3.3%		10.4%		3.0%		4.2%	
		Common carp	3.8%		3.7%		6.4%		5.4%		3.8%		2.0%		--	
		Gizzard shad	16.4%		26.4%		11.0%		21.7%		1.9%		15.8%		--	
		Quillback	--		0.5%		--		--		--		--		--	
		River carpsucker	--		--		0.9%		--		--		--		--	
		Smallmouth buffalo	--		5.1%		5.5%		1.1%		0.9%		4.0%		--	
11. Average number per run	Electrofishing		**61.3**	**0.5**	**60**	**0.5**	**71**	**0.5**	**45.5**	**0.5**	**98.3**	**0.5**	**112.2**	**1.5**	**33.3**	**0.5**
	Gill Netting		**21.3**	**1.5**	**21.6**	**1.5**	**10.9**	**0.5**	**9.2**	**0.5**	**10.6**	**0.5**	**10.1**	**0.5**	**4.8**	**0.5**
12. Percent anomalies	Electrofishing		**1.3**	**2.5**	**0.2**	**2.5**	**1.2**	**2.5**	**0.4**	**2.5**	**2.8**	**1.5**	**1.1**	**2.5**	**8.6**	**0.5**
	Gill Netting		**2.3**	**1.5**	**0**	**2.5**	**2.8**	**1.5**	**0**	**2.5**	**2.8**	**1.5**	**0**	**2.5**	**0**	**2.5**
RFAI Score				**45**		**42**		**44**		**36**		**38**		**42**		**44**

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Table 4. Individual Metric Scores, Contributing Species, and Overall RFAI Scores for ERM 2.5, Autumn 2001-2011.

ERM 2.5								
Metric	Gear	Common Name	2009		2010		2011	
			Obs	Sc	Obs	Sc	Obs	Sc
1. Number of indigenous species	Combined		**32**	**5**	**35**	**5**	**27**	**3**
2. Number of centrarchid species (less Micropterus)	Combined		**8**	**5**	**8**	**5**	**7**	**5**
		Black crappie	3		13		5	
		Bluegill sunfish	515		330		1012	
		Green sunfish	9		43		24	
		Longear sunfish	38		6		6	
		Redbreast sunfish	3		2		--	
		Redear sunfish	132		56		64	
		Warmouth	7		7		8	
		White crappie	2		4		7	
3. Number of benthic invertivore species	Combined		**5**	**3**	**5**	**3**	**4**	**3**
		Black redhorse	1		1		--	
		Freshwater drum	6		6		1	
		Golden redhorse	2		2		4	
		Logperch	--		12		--	
		Northern hog sucker	1		--		1	
		Spotted sucker	19		28		17	
4. Number of intolerant species	Combined		**6**	**5**	**6**	**5**	**5**	**5**
		Black redhorse	1		1		--	
		Brook silverside	56		29		8	
		Longear sunfish	38		6		6	
		Northern hog sucker	1		--		1	
		Skipjack herring	--		25		--	
		Smallmouth bass	2		1		1	
		Spotted sucker	19		28		17	
5. Percent tolerant individuals	Electrofishing		**79.1%**	**0.5**	**78.5%**	**0.5**	**90.0%**	**0.5**
		Bluegill sunfish	39.5%		21.4%		69.2%	
		Bluntnose minnow	3.2%		1.2%		1.8%	
		Common carp	1.0%		1.0%		0.4%	
		Gizzard shad	9.0%		41.1%		7.6%	
		Green sunfish	0.7%		2.8%		1.6%	
		Largemouth bass	11.7%		7.7%		8.6%	
		Redbreast sunfish	0.2%		0.1%		--	
		Spotfin shiner	13.8%		3.1%		0.3%	
		White crappie	--		0.1%		0.5%	
	Gill Netting		**7.4%**	**2.5**	**15.4%**	**2.5**	**4.9%**	**2.5**
		Bluegill sunfish	0.9%		0.7%		--	
		Common carp	0.9%		1.5%		3.3%	
		Gizzard shad	2.8%		11.0%		--	
		Largemouth bass	0.9%		0.7%		1.6%	
		White crappie	1.9%		1.5%		--	
6. Percent dominance by one species	Electrofishing		-	-	-	-	-	-
		Bluegill sunfish l	**39.5%**	**1.5**	--		**69.2%**	**0.5**
		Gizzard shad	--		**41.1%**	**0.5**	--	
	Gill Netting		-	-	-	-	-	-
		Blue catfish	--		--		**29.5%**	**0.5**
		Channel catfish	**47.7%**	**0.5**	--		--	
		Skipjack herring	--		**18.4%**	**1.5**	--	
7. Percent non-indigenous species	Electrofishing		**1.4%**	**2.5**	**9.2%**	**0.5**	**1.1%**	**2.5**
		Common carp	1.0%		1.0%		0.4%	
		Grass carp	--		0.1%		--	
		Inland silverside	--		7.7%		0.6%	
		Striped bass	--		0.1%		--	
		Yellow perch	0.4%		0.3%		0.1%	
	Gill Netting		**1.8%**	**2.5**	**4.4%**	**2.5**	**3.3%**	**2.5**
		Common carp	0.9%		1.5%		3.3%	

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		Striped bass	0.9%	2.9%	--	

Table 4, continued.

ERM 2.5								
Metric	Gear	Common Name	2009		2010		2011	
			Obs	Sc	Obs	Sc	Obs	Sc
8. Number of top carnivore species	Combined		**10**	**5**	**12**	**5**	**9**	**5**
		Black crappie	3		13		5	
		Flathead catfish	3		6		3	
		Largemouth bass	180		125		144	
		Sauger	1		4		10	
		Skipjack herring	--		25		--	
		Smallmouth bass	2		1		1	
		Spotted bass	8		11		--	
		Spotted gar	11		2		--	
		Walleye	--		1		4	
		White bass	1		8		1	
		White crappie	2		4		7	
		Yellow bass	1		22		1	
9. Percent top carnivores	Electrofishing		**12.4%**	**2.5**	**9.7%**	**1.5**	**9.6%**	**1.5**
		Black crappie	--		0.8%		0.3%	
		Flathead catfish	--		0.2%		--	
		Largemouth bass	11.7%		7.7%		8.6%	
		Smallmouth bass	0.2%		0.1%		0.1%	
		Spotted bass	0.5%		0.4%		--	
		Spotted gar	--		0.1%		--	
		Striped bass	--		0.1%		--	
		White bass	--		0.1%		--	
		White crappie	--		0.1%		0.5%	
		Yellow bass	--		0.1%		0.1%	
	Gill Netting		**22.3%**	**0.5**	**51.9%**	**2.5**	**32.7%**	**1.5**
		Black crappie	2.8%		0.7%		1.6%	
		Flathead catfish	2.8%		2.2%		4.9%	
		Largemouth bass	0.9%		0.7%		1.6%	
		Sauger	0.9%		2.9%		16.4%	
		Skipjack herring	--		18.4%		--	
		Spotted bass	--		0.7%		--	
		Spotted gar	10.3%		0.7%		--	
		Striped bass	0.9%		2.9%		--	
		Walleye	--		0.7%		6.6%	
		White bass	0.9%		5.1%		1.6%	
		White crappie	1.9%		1.5%		--	
		Yellow bass	0.9%		15.4%		--	
10. Percent omnivores	Electrofishing		**13.6%**	**2.5**	**44.4%**	**0.5**	**10.0%**	**2.5**
		Black buffalo	0.1%		0.2%		0.1%	
		Bluntnose minnow	3.2%		1.2%		1.8%	
		Channel catfish	0.2%		0.7%		0.1%	
		Common carp	1.0%		1.0%		0.4%	
		Gizzard shad	9.0%		41.1%		7.6%	
		Smallmouth buffalo	0.1%		0.2%		--	
	Gill Netting		**65.3%**	**0.5**	**42.7%**	**1.5**	**45.9%**	**1.5**
		Black buffalo	0.9%		--		--	
		Blue catfish	12.1%		11.8%		29.5%	
		Channel catfish	47.7%		13.2%		13.1%	
		Common carp	0.9%		1.5%		3.3%	
		Gizzard shad	2.8%		11.0%		--	
		Quillback	0.9%		1.5%		--	
		Smallmouth buffalo	--		3.7%		--	
11. Average number per run	Electrofishing		**86.5**	**0.5**	**102.4**	**0.5**	**97.5**	**0.5**
	Gill Netting		**10.7**	**0.5**	**13.6**	**1.5**	**6.1**	**0.5**
12. Percent anomalies	Electrofishing		**4.3**	**1.5**	**1.6**	**2.5**	**4.5**	**1.5**
	Gill Netting		**0.9**	**2.5**	**0**	**2.5**	**0**	**2.5**
RFAI Score				**44**	-	**44**	-	**42**

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Table 5. Scoring Criteria for Forebay, Transition, and Inflow Sections Of Upper Mainstream Reservoirs in The Tennessee River Valley. Upper Mainstream Reservoirs Include Chickamauga, Fort Loudoun, Melton Hill, Nickajack, Tellico, and Watts Bar.

		Scoring Criteria								
		Forebay			Transition			Inflow		
Metric	Gear	1	3	5	1	3	5	1	3	5
1. Number of indigenous species	Combined	<14	14-27	>27	<15	15-29	>29	<14	14-27	>27
2. Number of Centrarchid species	Combined	<2	2-4	>4	<2	2-4	>4	<3	3-4	>4
3. Number of benthic invertivores species	Combined	<4	4-7	>7	<4	4-7	>7	<3	3-6	>6
4. Number of intolerant species	Combined	<2	2-4	>4	<2	2-4	>4	<2	2-4	>4
5. Percent tolerant individuals	Electrofishing	>62%	31-62%	<31%	>62%	31-62%	<31%	>58%	29-58%	<29%
	Gill netting	>28%	14-28%	<14%	>32%	16-32%	<16%			
6. Percent dominance by one species	Electrofishing	>50%	25-50%	<25%	>40%	20-40%	<20%	>46%	23-46%	<23%
	Gill netting	>29%	15-29%	<15%	>28%	14-28%	<14%			
7. Percent non-indigenous species	Electrofishing	>4%	2-4%	<2%	>6%	3-6%	<3%	>17%	8-17%	<8%
	Gill netting	>16%	8-16%	<8%	>9%	5-9%	<5%			
8. Number of top carnivore species	Combined	<4	4-7	>7	<4	4-7	>7	<3	3-6	>6
9. Percent top carnivores	Electrofishing	<5%	5-10%	>10%	<6%	6-11%	>11%	<11%	11-22%	>22%
	Gill netting	<25%	25-50%	>50%	<26%	26-52%	>52%			
10. Percent omnivores	Electrofishing	>49%	24-49%	<24%	>44%	22-44%	<22%	>55%	27-55%	<27%
	Gill netting	>34%	17-34%	<17%	>46%	23-46%	<23%			
11. Average number per run	Electrofishing	<121	121-241	>241	<105	105-210	>210	<51	51-102	>102
	Gill netting	<12	12-24	>24	<12	12-24	>24			
12. Percent anomalies	Electrofishing	>5%	2-5%	<2%	>5%	2-5%	<2%	>5%	2-5%	<2%
	Gill netting	>5%	2-5%	<2%	>5%	2-5%	<2%			

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Table 6. Total Number of Individuals Collected of Each Species in RFAI Electrofishing and Gill Netting Samples Combined and Indigenous Classification (IND).

Common Name	IND	CRM 1.5							CRM 4.4							ERM 2.5		
		2001	2003	2005	2007	2009	2010	2011	2001	2003	2005	2007	2009	2010	2011	2009	2010	2011
Paddlefish	X	1	1	.	1*	.	1	.	.	.	.	.	.	1	.	.	.	.
Lake sturgeon	X	.	.	.	.	.	1	1	.	.	.	.	.	1	1	.	.	1
Longnose gar	X	.	2	1	11	.	1	.	.	1	.	1	.	.	1	.	.	.
Spotted gar	X	2	1	4	1	1	1	.	.	5	.	.	.	1	.	11	2	.
Skipjack herring	X	21	21	22	.	.	10	.	20	18	12	.	.	4	.	.	25	.
Gizzard shad	X	292	196	317	186	157	282	231	122	280	396	188	96	178	91	120	646	111
Threadfin shad	X	3	1	3	.	1	16	4	2	2	.	5	4	.	11	2	12	27
Mooneye	X	.	1	4	.	.	.	.	.	1	.	.	.	.	.	.	.	.
Common carp		20	21	22	7	15	14	19	43	22	27	13	19	19	9	14	18	8
Largescale stoneroller	X	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	3	.
Golden shiner	X	9	5	.	.	3	.	.	1	9	.	1	.	.	.	.	.	.
Emerald shiner	X	44	13	.	.	.	.	.	11	.	9	.	.	.	.	.	.	.
Spotfin shiner	X	54	22	23	21	264	20	12	55	41	33	20	225	40	5	179	48	4
Striped shiner	X	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.
Steelcolor shiner	X	.	.	1	.	.	.	.	.	.	.	.	1	.	.	.	.	.
Bluntnose minnow	X	2	5	3	1	28	68	1	2	2	.	2	103	10	.	41	19	27
Bullhead minnow	X	.	.	.	.	.	.	.	.	.	.	1	.	.	1	1	5	.
Northern hog sucker	X	.	.	4	.	.	.	.	.	.	.	.	.	.	1	1	.	1
River carpsucker	X	1	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
Quillback	X	.	.	.	.	.	.	.	.	1	.	.	.	.	.	1	2	.
Smallmouth buffalo	X	20	16	3	11	4	6	5	7	29	9	3	4	6	2	1	8	.
Black buffalo	X	3	.	2	.	.	2	.	3	3	3	.	.	2	2	2	3	2
Spotted sucker	X	14	11	18	.	15	11	52	17	64	73	15	15	16	25	19	28	17
Silver redhorse	X	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
River redhorse	X	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
Black redhorse	X	.	.	.	.	.	.	3	1	1	2	1	2	3	4	1	1	.
Golden redhorse	X	.	1	.	1	1	2	.	1	2	5	2	.	1	.	2	2	4
Blue catfish	X	32	15	14	7	17	17	13	35	26	6	13	21	11	12	13	16	18
Channel catfish	X	19	20	10	8	18	22	9	22	14	10	13	26	6	6	53	28	10
Flathead catfish	X	6	15	7	8	1	11	1	4	2	2	3	2	3	1	3	6	3
White bass	X	5	6	6	1	3	17	6	17	6	2	3	2	12	5	1	8	1
Yellow bass	X	19	22	14	5	5	32	1	38	32	9	8	28	5	20	1	22	1
Striped bass		14	9	18	8	3	11	5	2	18	10	9	4	22	.	1	5	.
Striped bass x white bass[1]		2	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
Redbreast sunfish	X	5	1	6	1	14	5	6	3	.	.	.	2	6	2	3	2	.
Green sunfish	X	11	4	36	16	43	35	30	4	3	14	1	25	49	42	9	43	24
Warmouth	X	2	.	.	.	3	.	6	1	3	.	.	3	3	2	7	7	8
Bluegill sunfish	X	540	365	330	364	970	704	319	483	298	288	305	539	764	157	514	330	1012
Longear sunfish	X	12	16	52	106	197	71	38	10	25	13	40	112	31	7	38	6	6
Redear sunfish	X	59	51	40	47	52	107	44	74	50	67	35	73	89	51	132	56	64
Hybrid sunfish[1]		.	.	.	.	.	.	3	1	.	.	.	.	.	.	.	.	.
Rock bass	X	.	.	.	.	3	3	1	.	.	.	.	3	.	.	.	.	.
Smallmouth bass	X	6	2	9	1	16	6	7	6	4	21	5	14	8	8	2	1	1
Spotted bass	X	25	8	14	1	8	.	1	19	18	12	4	4	1	1	7	7	.
Largemouth bass	X	98	37	28	21	101	57	59	86	64	83	27	96	79	54	153	120	127
Hybrid bass[1]		.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.

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Table 6, continued.

Common Name	IND	CRM 1.5							CRM 4.4							ERM 2.5		
		2001	2003	2005	2007	2009	2010	2011	2001	2003	2005	2007	2009	2010	2011	2009	2010	2011
White crappie	X	8	2	2	.	.	4	1	1	1	3	.	2	3	.	2	4	7
Black crappie	X	12	1	.	2	2	4	2	5	2	3	.	3	3	.	3	13	5
Snubnose darter	X	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
Yellow perch		3	3	.	.	2	.	.	5	2	1	1	1	4	13	5	4	2
Logperch	X	5	6	2	.	.	3	.	1	2	4	1	.	7	.	.	12	.
Sauger	X	4	4	8	.	14	5	15	9	4	6	5	12	5	6	1	4	10
Walleye	X	.	.	.	1	.	.	.	.	.	.	2	.	3	2	.	1	4
Muskellunge		.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Freshwater drum	X	10	30	9	8	11	13	11	6	17	12	10	8	18	3	6	6	1
Brook silverside	X	17	34	5	11	20	21	.	14	44	18	14	40	13	.	56	29	8
Mississippi silverside		.	.	100	2	219	318	32	.	.	10	24	92	355	1	.	119	9
Chestnut lamprey	X	.	.	.	.	.	1	.	.	.	.	.	.	1	.	.	.	.
Grass carp		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
Total Fish Collected	---	1,400	967	1,137	858	2,211	1,910	938	1,132	1,116	1,167	775	1,581	1,784	547	1,405	1,672	1,523
Total Species Richness	56	36	36	34	28	32	38	30	36	37	34	32	32	38	32	35	40	30
Indigenous Species	50	33	33	31	25	28	35	27	33	34	30	28	28	34	28	32	35	27

**=Only young-of-year collected*

1=Hybrid fish do not contribute to total species richness or number of indigenous species

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Table 7. Species Collected, Ecological Designations, and Electrofishing (EF) and Gill Net (GN) Catch Per Unit Effort at CRM 1.5, Autumn 2011.

Trophic level: benthic invertivore (BI), herbivore (HB), insectivore (IN), omnivore (OM), parasitic (PS), planktivore (PK), top carnivore (TC); Tolerance: tolerant (TOL), intolerant (INT).

Common Name	Scientific Name	Trophic Level	Sunfish Species	Indigenous Species	Pollution Tolerance	Electrofishing Catch Rate Per Run	Electrofishing Catch Rate Per Hour	Total Fish EF	Gill Netting Catch Rate Per Net Night	Total Fish Gill Net	Total Fish Combined	Percent of Total Fish
Lake sturgeon	Acipenser fulvescens	IN	.	X	.	.	.	.	0.1	1	1	0.1%
Gizzard shad	Dorosoma cepedianum	OM	.	X	TOL	15.27	61.56	229	0.2	2	231	24.6%
Threadfin shad	Dorosoma petenense	PK	.	X	.	0.27	1.08	4	.	.	4	0.4%
Common carp	Cyprinus carpio	OM	.	.	TOL	1.2	4.84	18	0.1	1	19	2.0%
Spotfin shiner	Cyprinella spiloptera	IN	.	X	TOL	0.8	3.23	12	.	.	12	1.3%
Bluntnose minnow	Pimephales notatus	OM	.	X	TOL	0.07	0.27	1	.	.	1	0.1%
Smallmouth buffalo	Ictiobus bubalus	OM	.	X	.	0.27	1.08	4	0.1	1	5	0.5%
Spotted sucker	Minytrema melanops	BI	.	X	INT	3.33	13.44	50	0.2	2	52	5.5%
Black redhorse	Moxostoma duquesnei	BI	.	X	INT	0.07	0.27	1	0.2	2	3	0.3%
Blue catfish	Ictalurus furcatus	OM	.	X	.	.	.	.	1.3	13	13	1.4%
Channel catfish	Ictalurus punctatus	OM	.	X	.	0.6	2.42	9	.	.	9	1.0%
Flathead catfish	Pylodictis olivaris	TC	.	X	.	.	.	.	0.1	1	1	0.1%
White bass	Morone chrysops	TC	.	X	.	0.13	0.54	2	0.4	4	6	0.6%
Yellow bass	Morone mississippiensis	TC	.	X	.	.	.	.	0.1	1	1	0.1%
Striped bass	Morone saxatilis	TC	.	.	.	0.2	0.81	3	0.2	2	5	0.5%
Redbreast sunfish	Lepomis auritus	IN	X	X	TOL	0.4	1.61	6	.	.	6	0.6%
Green sunfish	Lepomis cyanellus	IN	X	X	TOL	2	8.06	30	.	.	30	3.2%
Warmouth	Lepomis gulosus	IN	X	X	.	0.4	1.61	6	.	.	6	0.6%
Bluegill sunfish	Lepomis macrochirus	IN	X	X	TOL	21.2	85.48	318	0.1	1	319	34.0%
Longear sunfish	Lepomis megalotis	IN	X	X	INT	2.53	10.22	38	.	.	38	4.1%
Redear sunfish	Lepomis microlophus	IN	X	X	.	2.93	11.83	44	.	.	44	4.7%
Hybrid sunfish[1]	Hybrid lepomis spp.	IN	X	.	.	0.2	0.81	3	.	.	3	0.3%
Rock bass	Ambloplites rupestris	TC	.	X	INT	0.07	0.27	1	.	.	1	0.1%
Smallmouth bass	Micropterus dolomieu	TC	.	X	INT	0.47	1.88	7	.	.	7	0.7%
Spotted bass	Micropterus punctulatus	TC	.	X	.	0.07	0.27	1	.	.	1	0.1%
Largemouth bass	Micropterus salmoides	TC	.	X	TOL	3.93	15.86	59	.	.	59	6.3%
White crappie	Pomoxis annularis	TC	X	X	TOL	.	.	.	0.1	1	1	0.1%
Black crappie	Pomoxis nigromaculatus	TC	X	X	.	.	.	.	0.2	2	2	0.2%
Sauger	Stizostedion canadense	TC	.	X	.	.	.	.	1.5	15	15	1.6%
Freshwater drum	Aplodinotus grunniens	BI	.	X	.	0.67	2.69	10	0.1	1	11	1.2%
Mississippi silverside	Menidia audens	IN	.	.	.	2.13	8.6	32	.	.	32	3.4%
Total						59.21	238.73	888	5	50	938	100.0%
Number Samples						15			10			
Species Collected			9	27		23			16		30	

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Table 8. Species Collected, Ecological Designations, and Electrofishing (EF) and Gill Net (GN) Catch Per Unit Effort at CRM 4.4, Autumn 2011.

Trophic level: benthic invertivore (BI), herbivore (HB), insectivore (IN), omnivore (OM), parasitic (PS), planktivore (PK), top carnivore (TC); Tolerance: tolerant (TOL), intolerant (INT).

Common Name	Scientific Name	Trophic Level	Sunfish Species	Indigenous Species	Pollution Tolerance	Electrofishing Catch Rate Per Run	Electrofishing Catch Rate Per Hour	Total Fish EF	Gill Netting Catch Rate Per Net Night	Total Fish Gill Net	Total Fish Combined	Percent of Total Fish
Lake sturgeon	Acipenser fulvescens	IN	.	X	.	.	.	.	0.1	1	1	0.2%
Longnose gar	Lepisosteus osseus	TC	.	X	TOL	.	.	.	0.1	1	1	0.2%
Gizzard shad	Dorosoma cepedianum	OM	.	X	TOL	6.07	28.71	91	.	.	91	16.6%
Threadfin shad	Dorosoma petenense	PK	.	X	.	0.73	3.47	11	.	.	11	2.0%
Common carp	Cyprinus carpio	OM	.	.	TOL	0.6	2.84	9	.	.	9	1.6%
Spotfin shiner	Cyprinella spiloptera	IN	.	X	TOL	0.33	1.58	5	.	.	5	0.9%
Bullhead minnow	Pimephales vigilax	IN	.	X	.	0.07	0.32	1	.	.	1	0.2%
Northern hog sucker	Hypentelium nigricans	BI	.	X	INT	0.07	0.32	1	.	.	1	0.2%
Smallmouth buffalo	Ictiobus bubalus	OM	.	X	.	0.13	0.63	2	.	.	2	0.4%
Black buffalo	Ictiobus niger	OM	.	X	.	0.13	0.63	2	.	.	2	0.4%
Spotted sucker	Minytrema melanops	BI	.	X	INT	1.53	7.26	23	0.2	2	25	4.6%
Black redhorse	Moxostoma duquesnei	BI	.	X	INT	0.27	1.26	4	.	.	4	0.7%
Blue catfish	Ictalurus furcatus	OM	.	X	.	0.07	0.32	1	1.1	11	12	2.2%
Channel catfish	Ictalurus punctatus	OM	.	X	.	0.27	1.26	4	0.2	2	6	1.1%
Flathead catfish	Pylodictis olivaris	TC	.	X	.	.	.	.	0.1	1	1	0.2%
White bass	Morone chrysops	TC	.	X	.	0.07	0.32	1	0.4	4	5	0.9%
Yellow bass	Morone mississippiensis	TC	.	X	.	0.67	3.15	10	1	10	20	3.7%
Redbreast sunfish	Lepomis auritus	IN	X	X	TOL	0.13	0.63	2	.	.	2	0.4%
Green sunfish	Lepomis cyanellus	IN	X	X	TOL	2.8	13.25	42	.	.	42	7.7%
Warmouth	Lepomis gulosus	IN	X	X	.	0.13	0.63	2	.	.	2	0.4%
Bluegill	Lepomis macrochirus	IN	X	X	TOL	10.27	48.58	154	0.3	3	157	28.7%
Longear sunfish	Lepomis megalotis	IN	X	X	INT	0.47	2.21	7	.	.	7	1.3%
Redear sunfish	Lepomis microlophus	IN	X	X	.	3.27	15.46	49	0.2	2	51	9.3%
Smallmouth bass	Micropterus dolomieu	TC	.	X	INT	0.4	1.89	6	0.2	2	8	1.5%
Spotted bass	Micropterus punctulatus	TC	.	X	.	0.07	0.32	1	.	.	1	0.2%
Largemouth bass	Micropterus salmoides	TC	.	X	TOL	3.6	17.03	54	.	.	54	9.9%
Yellow perch	Perca flavescens	IN	.	.	.	0.87	4.1	13	.	.	13	2.4%
Sauger	Stizostedion canadense	TC	.	X	.	.	.	.	0.6	6	6	1.1%
Walleye	Stizostedion vitreum	TC	.	X	.	.	.	.	0.2	2	2	0.4%
Muskellunge	Esox masquinongy	TC	.	.	INT	0.07	0.32	1	.	.	1	0.2%
Freshwater drum	Aplodinotus grunniens	BI	.	X	.	0.13	0.63	2	0.1	1	3	0.5%
Mississippi silverside	Menidia audens	IN	.	.	.	0.07	0.32	1	.	.	1	0.2%
Total						33.29	157.44	499	4.8	48	547	99.8%
Number of Samples						15			10			
Species Collected			6	28		27			14			

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Table 9. Species Collected, Ecological Designations, and Electrofishing (EF) and Gill Net (GN) Catch Per Unit Effort at ERM 2.5, Autumn 2011.

Trophic: benthic invertivore (BI), herbivore (HB), insectivore (IN), omnivore (OM), parasitic (PS), planktivore (PK), top carnivore (TC); Tolerance: tolerant (TOL), intolerant (INT).

Common Name	Scientific Name	Trophic Level	Sunfish Species	Indigenous Species	Pollution Tolerance	Electrofishing Catch Rate Per Run	Electrofishing Catch Rate Per Hour	Total Fish EF	Gill Netting Catch Rate Per Net Night	Total Fish Gill Net	Total Fish Combined	Percent of Total Fish
Lake sturgeon	Acipenser fulvescens	IN	.	X	.	.	.	.	0.1	1	1	0.1%
Gizzard shad	Dorosoma cepedianum	OM	.	X	TOL	7.4	31.99	111	.	.	111	7.3%
Threadfin shad	Dorosoma petenense	PK	.	X	.	1.8	7.78	27	.	.	27	1.8%
Common carp	Cyprinus carpio	OM	.	.	TOL	0.4	1.73	6	0.2	2	8	0.5%
Spotfin shiner	Cyprinella spiloptera	IN	.	X	TOL	0.27	1.15	4	.	.	4	0.3%
Bluntnose minnow	Pimephales notatus	OM	.	X	TOL	1.8	7.78	27	.	.	27	1.8%
Northern hog sucker	Hypentelium nigricans	BI	.	X	INT	0.07	0.29	1	.	.	1	0.1%
Black buffalo	Ictiobus niger	OM	.	X	.	0.13	0.58	2	.	.	2	0.1%
Spotted sucker	Minytrema melanops	BI	.	X	INT	0.73	3.17	11	0.6	6	17	1.1%
Golden redhorse	Moxostoma erythrurum	BI	.	X	.	.	.	.	0.4	4	4	0.3%
Blue catfish	Ictalurus furcatus	OM	.	X	.	.	.	.	1.8	18	18	1.2%
Channel catfish	Ictalurus punctatus	OM	.	X	.	0.13	0.58	2	0.8	8	10	0.7%
Flathead catfish	Pylodictis olivaris	TC	.	X	.	.	.	.	0.3	3	3	0.2%
White bass	Morone chrysops	TC	.	X	.	.	.	.	0.1	1	1	0.1%
Yellow bass	Morone mississippiensis	TC	.	X	.	0.07	0.29	1	.	.	1	0.1%
Green sunfish	Lepomis cyanellus	IN	X	X	TOL	1.6	6.92	24	.	.	24	1.6%
Warmouth	Lepomis gulosus	IN	X	X	.	0.53	2.31	8	.	.	8	0.5%
Bluegill sunfish	Lepomis macrochirus	IN	X	X	TOL	67.47	291.64	1012	.	.	1012	66.4%
Longear sunfish	Lepomis megalotis	IN	X	X	INT	0.4	1.73	6	.	.	6	0.4%
Redear sunfish	Lepomis microlophus	IN	X	X	.	4.2	18.16	63	0.1	1	64	4.2%
Smallmouth bass	Micropterus dolomieu	TC	.	X	INT	0.07	0.29	1	.	.	1	0.1%
Largemouth bass	Micropterus salmoides	TC	.	X	TOL	8.4	36.31	126	0.1	1	127	8.3%
White crappie	Pomoxis annularis	TC	X	X	TOL	0.47	2.02	7	.	.	7	0.5%
Black crappie	Pomoxis nigromaculatus	TC	X	X	.	0.27	1.15	4	0.1	1	5	0.3%
Yellow perch	Perca flavescens	IN	.	.	.	0.13	0.58	2	.	.	2	0.1%
Sauger	Stizostedion canadense	TC	.	X	.	.	.	.	1	10	10	0.7%
Walleye	Stizostedion vitreum	TC	.	X	.	.	.	.	0.4	4	4	0.3%
Freshwater drum	Aplodinotus grunniens	BI	.	X	.	.	.	.	0.1	1	1	0.1%
Brook silverside	Labidesthes sicculus	IN	.	X	INT	0.53	2.31	8	.	.	8	0.5%
Mississippi silverside	Menidia audens	IN	.	.	.	0.6	2.59	9	.	.	9	0.6%
Total						97.47	421.35	1462	6.1	61	1523	100.0%
Number of Samples						15			10			
Species Collected			7	27		22			14		30	

DRAFT

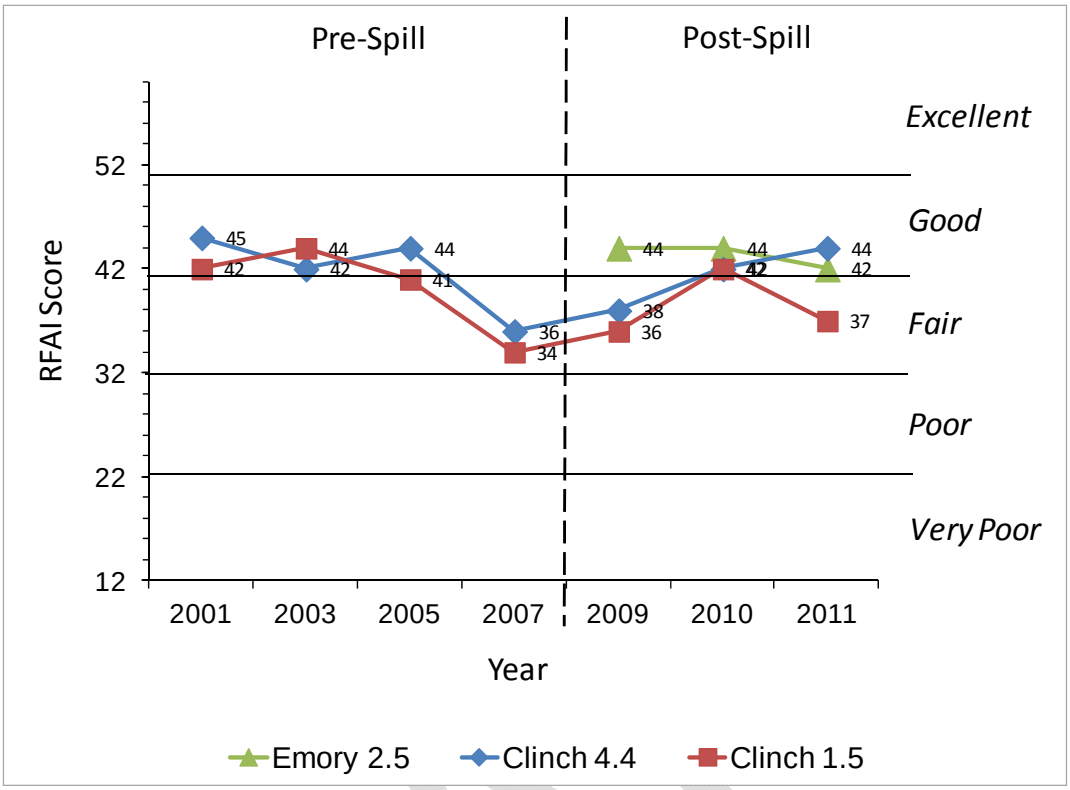

Figure 1. Reservoir Fish Assemblage Index (RFAI) Ratings for Fish Community Sampling Results from 2001 through 2011.

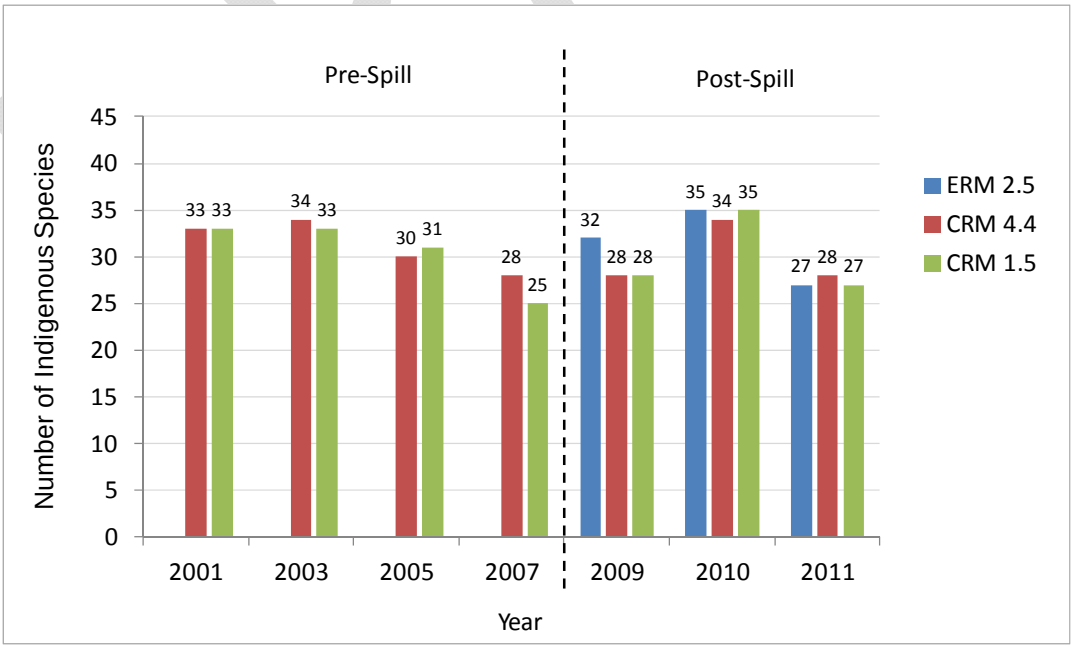

Figure 2. Number of Indigenous Species Collected at ERM 2.5, CRM 4.4, and CRM 1.5 during Fish Community Sampling from 2001 through 2011.

Appendix B

Preliminary Evaluation of 2011 ORNL Results: Fish Reproduction

OAK RIDGE NATIONAL LABORATORY

MANAGED BY UT-BATTELLE FOR THE DEPARTMENT OF ENERGY

P.O. Box 2008
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May 4, 2012

Dr. Neil Carriker
Program Manager, Special Projects
Tennessee Valley Authority Kingston Ash Recovery Project
Kingston, Tennessee

Subject: Preliminary Evaluation of 2011 ORNL Results: *Fish Reproduction*

Dr. Carriker,

I am pleased to submit to you a preliminary evaluation of 2011 *Fish Reproduction* Task results. The attached memorandum covers the spring 2011 fish collections in support of the TVA Kingston Ash Recovery Project from six locations in the Emory and Clinch Rivers. This personal communication is meant to provide a provisional assessment of the reproductive condition of fish collected at the beginning of the spring 2011 breeding season based on ongoing analyses of these samples. Data and tentative conclusions based on these data should be considered preliminary prior to the issuance of a draft report concerning these samples.

We hope this preliminary update is useful to you. If you have any questions or require additional information, please call me at (865) 574-9387.

Sincerely,

Mark S. Greeley, Jr.
Principal Investigator, Fish Reproduction/Fish Early Life Stage Studies
Manager, ORNL Environmental Toxicology Laboratory
Environmental Sciences Division
Oak Ridge National Laboratory

cc: M. J. Peterson, ORNL
D. Jones, ARCADIS
.....

Preliminary Evaluation of 2011 ORNL Results:
Fish Reproduction Studies

Mark S. Greeley, Jr., ORNL

May 4, 2012

Introduction

On December 22, 2008, a dike containing fly ash and bottom ash at the Tennessee Valley Authority's (TVA) Kingston Fossil Plant in East Tennessee failed and released a large quantity of ash into the adjacent Emory River. A byproduct of coal combustion, coal ash contains a variety of metals and other elements, particularly selenium, with known effects on the reproduction and early development of exposed fish populations (Lemly 1993; Besser and others 1996). For instance, female fish in a coal ash-contaminated lake have been shown to have increased frequencies of atretic oocytes (dead or damaged immature eggs) and reductions in the overall numbers of developing oocytes (Sorensen 1988) associated with elevated body burdens of selenium. Similarly, a number of studies have reported increased incidences of developmental abnormalities in larval fish exposed to selenium through maternal transfer to the developing eggs either in the laboratory or in contaminated bodies of water (Woock and others 1987, Lemly 1999, Jezierska and others 2009), including sites where coal ash from power plants is the primary source of selenium contamination.

In order to address the potential risks of the fly ash spill to the reproduction of downstream fish populations in the Emory and Clinch Rivers, females from select sentinel fish species have been sampled from multiple locations in these river systems at the beginning of each spring breeding season from 2009 through 2012. Results of the 2009 and 2010 studies are reported in Greeley and others (2012). Preliminary results of the spring 2011 fish collections are presented in the current memorandum, while laboratory analyses of these samples are ongoing. Spring 2012 fish collections for fish reproduction, fish health, and fish bioaccumulation studies were recently completed and samples currently await analysis.

A significant finding of the 2009 – 2010 reproductive studies reported in Greeley and others (2012) was that the relative sizes of ovaries and developing oocytes in bluegill sunfish were significantly reduced at the Emory River fly ash spill site in the spring of 2010 as compared with other study sites, particularly reference sites located upstream of the spill. Since oocyte atresia was not increased in fish at this location in 2009, this apparent delay in ovarian development at the spill site was probably not the result of direct chemical toxicity of fly ash constituents to the developing oocytes but instead due to the substantial habitat alterations and food chain disruptions that significantly impacted this river reach during this first breeding season following the spill. Other than this apparent developmental delay at the spill site, there were no other significant reproductive abnormalities noted in female bluegill sunfish from any of the study sites sampled in 2009 or from sites sampled in the spring of 2010. Similarly, there was also no conclusive evidence of adverse reproductive effects in female largemouth bass sampled from sites downstream of the fly ash spill in either 2009 or 2010, or in redear sunfish sampled from these same sites in 2010.

Preliminary Results

Largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), and redear sunfish (*Lepomis microlophus*) were sampled in the spring of 2011 from six locations in the Emory and Clinch Rivers, including both upstream reference sites (ERM 8.0, LERM 2.0, and ERM 8.0) as well as downstream sites directly influenced by the spill (ERM 3.0, ERM 0.9, and CRM 1.5) (Fig. 1). All three fish species are multiple spawners, capable of producing several clutches of eggs over a period of a few days through a few weeks. To maximize comparability of reproductive condition across the study sites, fish were collected in April and May immediately prior to or during the first spawn of the season. For each fish sampled, the gonadsomatic index (GSI) − or relative size of the gonad to the fish body − was calculated, and weighed portions of the ovary were saved for later morphometric analysis of oocyte stages and condition and to estimate fecundity.

As the laboratory analyses of 2011 ovary samples are still ongoing, this preliminary evaluation of the relative reproductive condition of female fish at the various study sites in the spring of 2011 is based primarily on the gross morphological appearance of fish and ovary samples and on the GSI as an indicator of reproductive condition. It should be noted that all female fish collected from the Emory and Clinch Rivers in the spring of 2011, regardless of species or location, were reproductively mature based on ovarian size and appearance and overall degree of oocyte development. However, the specific reproductive status of sampled fish within the breeding population – whether pre-spawning, in the midst of a spawn, or immediately post-spawn − did vary substantially from site to site (Table 1). Fish collected prior to the first spawn of the season were characterized by relatively large ovaries and lacked obvious signs of recent spawning activity such as reddened and enlarged gonadal vents, while fish collected during or just after this first spawn still had relatively large but somewhat flaccid ovaries and roughened and red gonadal vents.

Percentages of fish considered to be pre-spawning in the spring of 2011 differed considerably from site to site and from species to species. In the case of bluegill sunfish, percentages of pre-spawning females ranged from 0% in samples from CRM 1.5 to 100% at ERM 8.0 (Table 1). Similar variation in specific reproductive status was observed in the largemouth bass samples, with no pre-spawning fish seen in samples from two study sites (LERM 2.0 and ERM 8.0) as compared with 75-88% pre-spawning fish in samples from the other four study sites. The reproductive status of redear sunfish was by contrast similar across all study sites (Table 1).

Average GSIs of fish sampled in the spring of 2011 differed significantly between some study sites. For instance, the GSIs of bluegill sunfish at CRM 1.5 were significantly lower than at the other study sites (Table 2), apparently due to all fish sampled from this site having spawned prior to sample collection. Similarly, the average GSIs of largemouth bass at two study locations (LERM 2.0 and ERM 8.0) were also significantly lower than at the other four sampling locations, again due to fish spawning at these sites prior to sample collection. In contrast, the GSIs of redear sunfish demonstrated little site to site variation in these spring 2011 samples.

Although the GSI is a widely-used and easily measured indicator of fish reproductive status and environmental stress (Nikolsky 1963), it is evident from these results and elsewhere (Greeley 2002) that it is best suited for use as part of a larger suite of reproductive indicators since it provides only limited data on the specific reproductive condition of the gonads.

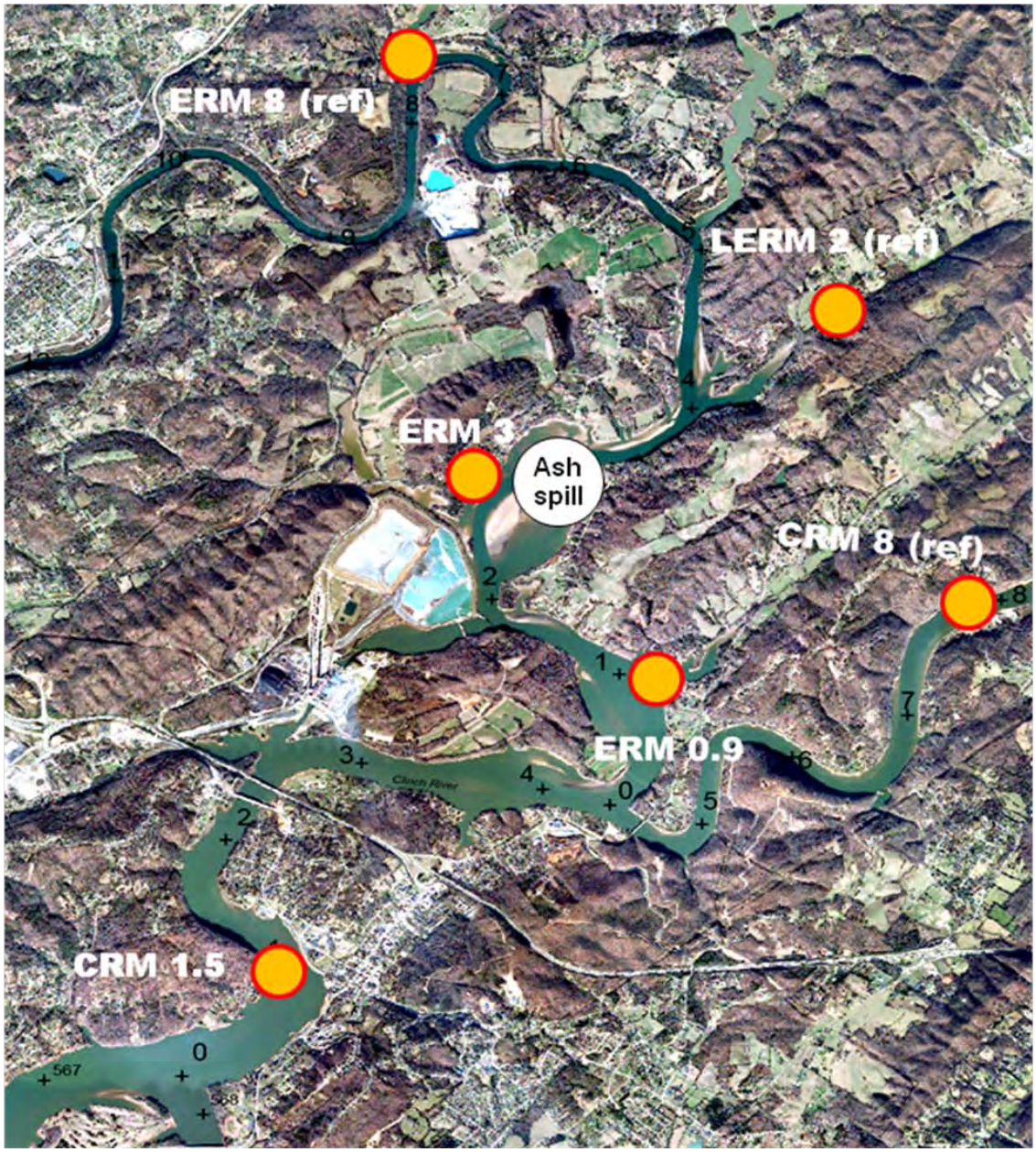

Figure 1. Map showing fish sampling locations in the Emory and Clinch Rivers for the Fish Reproduction Task. ERM = Emory River Mile; CRM = Clinch River Mile; LERM = Little Emory River Mile (sampled only in 2010).

Table 1. Spawning status[a] of fish collected at the beginning of the spring 2011 breeding season from study locations in the Emory and Clinch Rivers. Includes fish from river reaches affected by fly ash (ERM 3.0, ERM 0.9, and CRM 1.5) as well as upstream reference sites (ERM 8.0 and CRM 8.0)[b]

	Spawning Status (% of sampled fish considered to be pre-spawn)		
Location	*Bluegill sunfish*	*Largemouth bass*	*Redear sunfish*
LERM 2.0	67	0	80
ERM 8.0	100	0	75
ERM 3.0	60	88	63
ERM 0.9	55	75	38
CRM 8.0	80	75	89
CRM 1.5	0	88	50

[a] Pre-spawning fish do not have the enlarged and reddened papillae of fish that have begun spawning nor ovaries with shed follicles
[b] ERM = Emory River Mile; CRM = Clinch River Mile; LERM = Little Emory River Mile

Table 2. Gonadosomatic indices (GSIs) of fish collected at the beginning of the spring 2011 breeding season from study locations in the Emory and Clinch Rivers. Includes fish from fly ash-affected river reaches (ERM 3.0, ERM 0.9, and CRM 1.5) as well as upstream reference sites (ERM 8.0 and CRM 8.0)[a]

Location	2011 GSIs (means ± SEM)		
	Bluegill sunfish (n =7-11)	*Largemouth bass (n = 8)*	*Redear sunfish (n=8-10)*
LERM 2.0	7.8 ± 0.5	5.2 ± 0.3[c]	9.2 ± 0.7
ERM 8.0	9.2 ± 0.7	4.3 ± 0.3[c]	8.7 ± 1.0
ERM 3.0	7.5 ± 0.6	9.1 ± 0.9	8.6 ± 1.2
ERM 0.9	7.1 ± 0.6	8.7 ± 0.7	6.0 ± 0.8
CRM 8.0	8.3 ± 0.6	8.8 ± 0.9	7.7 ± 0.5
CRM 1.5	4.5± 0.3[b]	9.3 ± 0.7	8.2 ± 1.4

[a] ERM = Emory River Mile; CRM = Clinch River Mile; LERM = Little Emory River Mile

[b] Significantly lower at $\alpha = 0.05$ from GSIs at all other study sites

[c] Significantly lower at $\alpha = 0.05$ from GSIs at ERM 3.0, ERM 0.9, CRM 8.0, and CRM 1.5

Preliminary Conclusions

- All female fish collected during the spring 2011 fish reproduction, health, and bioaccumulation sampling event were reproductively mature based on the size and appearance of their ovaries.
- However, the reproductive condition of the sampled fish did vary from immediately pre-spawning through post-spawning, although all fish appeared capable of producing additional clutches of eggs during the remainder of the breeding season.
- The few statistically significant differences noted in the GSIs of sampled fish between study sites were apparently due primarily to differences in the pre- versus post-spawning status of bluegill sunfish and largemouth bass at these locations.
- There is no evidence from this preliminary evaluation of adverse reproductive effects or abnormalities in fish at sites influenced by the fly ash spill during the spring of 2011.

Additional Considerations and/or Uncertainties

The GSI provides only limited data as to the actual reproductive condition of the gonads. Completion of the morphometric analyses currently being conducted on ovarian samples from these fish, including the enumeration and staging of the developing oocytes and/or follicles and subsequent estimation of fecundity, is needed to accurately evaluate the reproductive condition of these spring 2011 fish samples.

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Appendix C

Preliminary Evaluation of 2011 ORNL Results (rev. 1): Fish Bioaccumulation

OAK RIDGE NATIONAL LABORATORY
MANAGED BY UT-BATTELLE FOR THE DEPARTMENT OF ENERGY

June 14, 2012

Dr. Neil Carriker
Program Manager, Special Projects
Tennessee Valley Authority Kingston Ash Recovery Project
Kingston, Tennessee

Dr. Carriker,

Subject: Preliminary Evaluation of 2011 ORNL Results (rev.1): *Fish Bioaccumulation*

We are pleased to submit to you a revision of our preliminary evaluation of 2011 *Fish Bioaccumulation* Task results.

This revision includes more basic summary statistics for arsenic, mercury, and selenium concentrations in fish collected across all sampling seasons.

We hope this report is useful to you in evaluating spatial and temporal trends. If you have any questions or require additional information, please call me at (865) 241-9405.

Sincerely,

Dr. Teresa Mathews
Principal Investigator, Fish Bioaccumulation
Environmental Sciences Division
Oak Ridge National Laboratory

c: M. J. Peterson
D. Jones
…..

Preliminary Evaluation of 2011 ORNL Results: *Fish Bioaccumulation*
May 4, 2012

Introduction

In December 2008, an ash dike at the Tennessee Valley Authority (TVA) Kingston Fossil Plant (KIF) ruptured, releasing over 1 billion gallons of coal fly ash into the Emory and Clinch Rivers. Coal fly ash may contain several contaminants of concern, but of these selenium (Se), arsenic (As), and mercury (Hg) have been highlighted because of their toxicity and tendency to bioaccumulate in aquatic food chains. To assess the potential impact of the spilled fly ash on humans and the environment, a comprehensive biological monitoring program was established, for which resident aquatic organisms are collected to determine contaminant exposure and evaluate the risk to humans and wildlife.

Results for fish bioaccumulation for the 2009-2010 sampling seasons have been previously reported (Adams et al. 2012), and these results were used to guide the Baseline Environmental Risk Assessment (BERA) for the TVA-KIF project (Young et al. 2012). Because Se and Hg, two of the primary fly ash contaminants of concern, are accumulated in fish predominantly through food chain rather than aqueous exposure (Pickhardt et al. 2006, Chapman et al. 2010), there may be a lag between the time these contaminants were released into the affected watersheds and the time body burdens in fish are affected. For this reason, although the BERA considers only 2009-2010 data, it was considered critical to evaluate the bioaccumulation data for the 2011 sampling seasons at the time of the BERA release. This letter report presents a preliminary spatial and temporal evaluation of Se and As concentrations in fish. Details of sampling locations, methods, and sampling strategy were presented in Adams et al. (2012). The current letter report also focuses on a spatial analysis of Hg results for samples collected in fall 2011. At the present time, results for mercury for samples collected prior to fall 2011 have not been validated due to low recoveries during analysis (Mathews et al. 2012) . Temporal trends for Hg data, as well as a more detailed statistical analysis of data for Se, As, and other metals will be presented in a future report.

Preliminary Results

Selenium

Selenium concentrations in fillets of bluegill, channel catfish, largemouth bass, and redear sunfish show a strong spatial gradient, with the lowest concentrations observed at reference sites (Little Emory River Mile 2 (LERM 2), Emory River Mile 8 (ERM 8), and Clinch River Mile 8 (CRM 8)) and elevated concentrations at the sites affected by the ash spill in the Clinch and Emory Rivers (ERM 3, CRM 3.5, ERM 0.9, CRM 1.5) (Tables 1-4). Selenium concentrations in fillets of all fish species were elevated in the vicinity of the spill site (ERM 3), and increased with increasing distance downstream such that Se concentrations in fish fillets from all species examined were generally highest at CRM 1.5 across all sampling seasons. Of all fish species, selenium concentrations were highest in fillets of redear sunfish across all seasons sampled. The elevated Se concentrations in redear with respect to other species are likely due to dietary preferences, which will be the focus of a future study (Otter et al. 2012 SUBMITTED). In general, Se concentrations in fish fillets from 2011 were similar to those in 2010, with decreases observed at some sites and increases at others (Tables 1-4).

Summary of 2011 findings for Se:

- Mean selenium concentrations in redear fillets from ash affected sites (ERM 4.5, ERM 3, ERM 0.9, CRM 1.5) were significantly higher in 2011 than in 2010 (one way ANOVA, p= 0.0061). (Fig. 1)

- At all fly ash-affected sites (ERM 3, CRM 3.5, ERM 0.9, CRM 1.5), Se concentrations in catfish fillets were higher in fall 2011 than all previous seasons sampled, but these differences were not statistically significant (one way ANOVA, p = 0.06729). (Fig. 2)

- For largemouth bass, Se concentrations in fillets were significantly higher at fly-ash affected sites with respect to reference sites (one way ANOVA, p = 6.31 E-11). Concentrations in 2011 were generally lower than or similar to concentrations in previous years at most sites, with the exception of at CRM 1.5. A significant increase in Se concentrations was observed between the spring and fall of 2010 (one way ANOVA, p = 0.0006), and concentrations remained elevated in 2011 at this site (Fig. 3).

- In bluegill, Se fillet concentrations at ash affected sites in 2011 were generally similar or slightly higher than in previous years, with the exception of CRM 1.5, where concentrations decreased in 2011. There are strong seasonal fluctuations observed in Se concentrations of these fish, but this is likely due to sex-related differences, as spring collections are predominantly comprised of females, and fall samples are either a mix of both sexes, or biased towards males. These trends will be examined in future studies.

- The draft ambient water quality criterion (AWQC) for Se currently proposed by the U.S. Environmental Protection Agency (EPA) is based on a fish tissue rather than an aqueous Se concentration (7.91 mg/kg whole body, dry wt.), as the tissue concentration integrates the route, duration, and magnitude of exposure and is therefore considered to be a more consistent indicator of exposure and risk (U.S. EPA 2004). All results presented in this report are for fish fillets, on a wet weight basis, while the criterion is for dry weight, whole body fish. A future study will focus on relating fish fillet to whole body concentrations (Mathews et al. 2012 (in prep)), but for a preliminary analysis, a 1:1 relationship can be assumed between fillet and whole body Se concentrations. Under these assumptions and using the % moisture to convert between wet and dry weight concentrations, the highest fillet concentrations, found in redear from CRM 1.5 in 2011 would translate to a whole body dry weight concentration of approximately 6 mg/kg—elevated, but below the draft criterion. Continued monitoring is needed to evaluate whether concentrations in these fish continue to increase.

Arsenic

Arsenic concentrations in fillets of bluegill, redear, and largemouth bass show a strong spatial gradient, but results are considerably more variable across seasons and species than for selenium (Figs. 5-7; Tables 5-8). Most results for channel catfish were below analytical detection limits, and so are not graphically represented here. In order to more clearly visualize the spatial gradients in arsenic concentrations without the noise of temporal variation, arsenic concentrations in largemouth bass fillets from all sampling seasons were averaged together, and results are shown in Figure 8. Figure 8 shows that arsenic concentrations in largemouth bass were highest in fish from the upper Clinch River sites (CRM 24, CRM 8) upstream of the KIF

spill, likely because of inputs from DOE facilities (Mathews et al. 2011). However, while DOE facilities are a significant contributor of arsenic to the watersheds of interest in this study, concentrations in largemouth bass downstream of the KIF were significantly higher than at reference sites not affected by DOE facilities (ERM 8, LERM 2), suggesting that the KIF spill is another significant contributor of arsenic to the Emory and Clinch Rivers (one way ANOVA, $p = 7.14 E-20$). Arsenic concentrations in all fish species in 2011 were either similar to, or lower than concentrations in previous sampling seasons.

Spatial trends for Mercury results in 2011

Mercury concentrations in fillets of bluegill, channel catfish, and largemouth bass were lower than the U.S. EPA's National Recommended Water Quality Criterion (NRWQC) of 0.3 mg/kg (wet wt.) Hg in fish fillets, except at ERM 8 for largemouth bass and ERM 3 for channel catfish and largemouth bass (Table 3). Concentrations in bluegill fillets were low, and no spatial gradient was apparent. Elevated Hg concentrations have historically been found in fish in the Clinch River upstream of the KIF, due to Hg releases from the U.S. Department of Energy (DOE) facilities at Y-12 and ORNL, located approximately at CRM 12 (Brooks & Southworth 2011). The elevated Hg concentrations observed in catfish and largemouth bass fillets at CRM 8 are therefore likely due to inputs from DOE facilities. Elevated Hg concentrations in the Emory River were also observed in the Emory River, upstream of the KIF. Concentrations in the vicinity of the ash spill (ERM 3) were above the NRWQC in bass and catfish, but because of the elevated Hg concentrations throughout the Clinch and Emory River watersheds in the vicinity of the KIF, it is not possible to discern the role of the ash spill on Hg bioaccumulation in fish at this time.

Temporal trends

Linear regression analysis shows that concentrations of As and Se increased significantly between 2009-2011 at ash affected sites (ERM 4.5, ERM 3, ERM 0.9, CRM 1.5), but not at reference sites (ERM 8, LERM 2) (Table 10). The upstream Clinch River sites (CRM 24, CRM 8) were excluded as reference sites from this analysis. Selenium concentrations in redear sunfish from ash affected sites also show a significant increase between 2010 and 2011. While results for other species may not show statistical significance at this time, we emphasize that the data span only a 3 year period. To show robust temporal trends, longer term monitoring is necessary.

Table 1. Basic summary statistics for selenium concentrations in channel catfish fillets(mg/kg wet weight) for the TVA KIF project (2009-2011). N = number of fish collected, FOD = frequency of detects, S.D. = 1 standard deviation of the mean. Where reported results were below the method detection limit (MDL), the MDL was substituted as the result when calculating summary statistics.

Site	Season	N	FOD	Mean	S.D.	Median	Min	Max
LERM 2.0	Fall 2010	6	6	0.29	0.02	0.29	0.26	0.32
	Fall 2011	6	6	0.29	0.08	0.28	0.22	0.44
CRM 24	Fall 2009	6	6	0.28	0.05	0.28	0.19	0.34
CRM 8.0	Spring 2009	12	10	0.23	0.11	0.24	0.05	0.38
	Fall 2009	6	6	0.22	0.05	0.21	0.17	0.30
	Spring 2010	7	7	0.25	0.07	0.23	0.16	0.38
	Fall 2010	6	6	0.27	0.09	0.27	0.15	0.37
	Fall 2011	5	5	0.23	0.05	0.22	0.20	0.32
ERM 8.0	Spring 2009	18	16	0.25	0.09	0.27	0.05	0.38
	Fall 2009	6	6	0.21	0.05	0.20	0.15	0.30
	Spring 2010	8	8	0.24	0.08	0.24	0.16	0.40
	Fall 2010	6	6	0.21	0.02	0.20	0.19	0.23
	Fall 2011	6	5	0.20	0.05	0.19	0.15	0.29
ERM 4.5	Spring 2010	6	6	0.26	0.06	0.25	0.17	0.33
	Fall 2010	6	6	0.24	0.04	0.25	0.19	0.29
ERM 3.0	Fall 2009	6	6	0.34	0.02	0.35	0.32	0.36
	Fall 2010	6	6	0.37	0.07	0.39	0.25	0.44
	Fall 2011	6	6	0.39	0.11	0.41	0.19	0.52
ERM 0.9	Spring 2009	8	8	0.34	0.11	0.30	0.24	0.54
	Fall 2009	7	7	0.35	0.08	0.36	0.26	0.44
	Fall 2010	6	6	0.38	0.08	0.36	0.29	0.53
	Fall 2011	6	6	0.46	0.12	0.51	0.30	0.58
CRM 3.5	Spring 2010	6	6	0.32	0.04	0.32	0.26	0.38
	Fall 2010	5	5	0.25	0.09	0.22	0.17	0.40
CRM 1.5	Spring 2009	15	14	0.37	0.14	0.38	0.10	0.66
	Fall 2009	5	5	0.34	0.11	0.34	0.23	0.51
	Spring 2010	6	6	0.38	0.08	0.40	0.25	0.48
	Fall 2010	6	6	0.38	0.06	0.37	0.30	0.48
	Fall 2011	6	6	0.45	0.10	0.42	0.34	0.61

Table 2. Basic summary statistics for selenium concentrations in largemouth bass fillets(mg/kg wet weight) for the TVA KIF project (2009-2011). N = number of fish collected, FOD = frequency of detects, S.D. = 1 standard deviation of the mean. Where reported results were below the method detection limit (MDL), the MDL was substituted as the result when calculating summary statistics.

Site	Season	N	FOD	Mean	S.D.	Median	Min	Max
LERM2.0	Fall 2009	6	6	0.50	0.05	0.50	0.43	0.58
	Spring 2010	6	6	0.46	0.06	0.44	0.40	0.55
	Fall 2010	6	6	0.52	0.05	0.54	0.45	0.56
	Spring 2011	7	7	0.47	0.08	0.45	0.38	0.58
	Fall 2011	6	6	0.49	0.08	0.49	0.41	0.62
CRM 24	Fall 2009	5	5	0.79	0.06	0.79	0.74	0.90
	Spring 2010	6	6	0.69	0.16	0.65	0.58	1.00
CRM8.0	Spring 2009	6	6	0.60	0.18	0.70	0.34	0.76
	Fall 2009	6	6	0.48	0.04	0.48	0.42	0.53
	Spring 2010	6	6	0.50	0.06	0.49	0.42	0.58
	Fall 2010	6	6	0.55	0.15	0.51	0.40	0.77
	Spring 2011	6	6	0.62	0.14	0.66	0.36	0.73
	Fall 2011	6	6	0.51	0.11	0.52	0.31	0.62
ERM 8.0	Spring 2009	24	24	0.47	0.09	0.45	0.33	0.74
	Fall 2009	6	6	0.44	0.06	0.42	0.38	0.55
	Spring 2010	6	6	0.45	0.05	0.45	0.36	0.49
	Fall 2010	6	6	0.45	0.13	0.40	0.35	0.66
	Spring 2011	7	7	0.40	0.04	0.40	0.35	0.44
	Fall 2011	6	6	0.43	0.06	0.43	0.37	0.52
ERM4.5	Spring 2010	6	6	0.53	0.12	0.53	0.37	0.70
	Fall 2010	6	6	0.62	0.13	0.67	0.46	0.76
ERM3.0	Spring 2009	5	5	0.59	0.11	0.55	0.47	0.74
	Fall 2009	6	6	0.60	0.09	0.59	0.46	0.72
	Spring 2010	6	6	0.66	0.14	0.64	0.46	0.88
	Fall 2010	7	7	0.76	0.05	0.77	0.70	0.85
	Spring 2011	6	6	0.70	0.18	0.75	0.37	0.90
	Fall 2011	6	6	0.58	0.15	0.65	0.36	0.72
ERM0.9	Spring 2009	6	6	0.61	0.09	0.63	0.46	0.73
	Fall 2009	6	6	0.68	0.12	0.69	0.54	0.80
	Spring 2010	6	6	0.62	0.07	0.62	0.50	0.72
	Fall 2010	6	6	0.75	0.05	0.76	0.66	0.81
	Spring 2011	6	6	0.65	0.25	0.73	0.24	0.89
	Fall 2011	6	6	0.66	0.11	0.63	0.53	0.86
CRM3.5	Spring 2010	6	6	0.56	0.08	0.55	0.47	0.67
	Fall 2010	6	6	0.73	0.11	0.70	0.62	0.91
CRM 1.5	Spring 2009	18	18	0.47	0.08	0.45	0.36	0.64
	Fall 2009	6	6	0.42	0.08	0.39	0.36	0.58
	Spring 2010	6	6	0.44	0.05	0.43	0.39	0.54
	Fall 2010	6	6	0.77	0.19	0.78	0.51	1.00
	Spring 2011	6	6	0.77	0.12	0.72	0.66	0.98
	Fall 2011	6	6	0.65	0.20	0.72	0.31	0.85

Table 3. Basic summary statistics for selenium concentrations in bluegill fillets (mg/kg wet weight) for the TVA KIF project (2009-2011). N = number of fish collected, FOD = frequency of detects, S.D. = 1 standard deviation of the mean. Where reported results were below the method detection limit (MDL), the MDL was substituted as the result when calculating summary statistics.

Site	Season	N	FOD	Mean	S.D.	Median	Min	Max
LERM 2.0	Fall 2009	6	6	0.49	0.04	0.50	0.44	0.53
	Spring 2010	6	6	0.50	0.06	0.47	0.44	0.60
	Fall 2010	6	6	0.53	0.10	0.52	0.38	0.68
	Spring 2011	7	7	0.55	0.06	0.53	0.50	0.65
	Fall 2011	6	6	0.56	0.26	0.41	0.36	0.93
CRM 24	Fall 2009	6	6	0.51	0.13	0.50	0.32	0.63
	Spring 2010	3	3	0.56	0.16	0.63	0.38	0.67
CRM 8.0	Spring 2009	6	6	0.59	0.07	0.61	0.50	0.69
	Fall 2009	6	6	0.60	0.09	0.58	0.50	0.74
	Spring 2010	11	11	0.54	0.04	0.53	0.50	0.63
	Fall 2010	6	6	0.63	0.10	0.62	0.49	0.76
	Spring 2011	6	6	0.54	0.07	0.52	0.44	0.65
	Fall 2011	6	6	0.47	0.07	0.45	0.39	0.56
ERM 8.0	Spring 2009	6	6	0.43	0.07	0.44	0.32	0.51
	Fall 2009	6	6	0.55	0.22	0.51	0.27	0.89
	Spring 2010	6	4	0.69	0.28	0.70	0.39	1.00
	Fall 2010	6	6	0.43	0.04	0.44	0.38	0.49
	Spring 2011	5	5	0.46	0.03	0.46	0.43	0.50
	Fall 2011	6	6	0.32	0.04	0.32	0.28	0.37
ERM 4.5	Spring 2010	6	6	0.58	0.10	0.61	0.46	0.69
	Fall 2010	5	5	0.53	0.08	0.56	0.43	0.64
ERM 3.0	Spring 2009	6	6	0.80	0.10	0.78	0.70	1.00
	Fall 2009	6	6	0.83	0.11	0.84	0.69	0.96
	Spring 2010	6	6	0.85	0.16	0.85	0.62	1.10
	Fall 2010	6	6	0.69	0.11	0.69	0.56	0.82
	Spring 2011	6	6	0.92	0.10	0.92	0.79	1.10
	Fall 2011	6	6	0.77	0.11	0.75	0.64	0.93
ERM 0.9	Spring 2009	6	6	0.77	0.07	0.78	0.70	0.86
	Fall 2009	6	6	0.97	0.16	0.95	0.76	1.20
	Spring 2010	5	5	0.74	0.14	0.76	0.56	0.88
	Fall 2010	6	6	0.88	0.13	0.85	0.72	1.10
	Spring 2011	6	6	0.95	0.07	0.98	0.84	1.00
	Fall 2011	6	6	0.79	0.13	0.76	0.66	0.97
CRM 3.5	Spring 2010	6	6	0.78	0.11	0.77	0.65	0.90
	Fall 2010	7	7	1.09	0.30	1.10	0.51	1.30
CRM 1.5	Spring 2009	6	6	0.70	0.16	0.76	0.44	0.86
	Fall 2009	6	6	0.99	0.13	0.99	0.86	1.10
	Spring 2010	6	6	0.82	0.15	0.78	0.65	1.10
	Fall 2010	6	6	1.30	0.17	1.25	1.10	1.50
	Spring 2011	6	6	1.08	0.14	1.05	0.93	1.30
	Fall 2011	6	6	0.84	0.12	0.86	0.64	1.00

Table 4. Basic summary statistics for selenium concentrations in redear sunfish fillets (mg/kg wet weight) for the TVA KIF project (2010-2011). N = number of fish collected, FOD = frequency of detects, S.D. = 1 standard deviation of the mean.

Site	Season	N	FOD	Mean	S.D.	Median	Min	Max
LERM 2.0	Spring 2010	6	6	0.62	0.04	0.62	0.58	0.68
	Spring 2011	7	7	0.74	0.12	0.76	0.58	0.90
CRM 24	Spring 2010	3	3	0.84	0.26	0.83	0.58	1.10
CRM 8.0	Spring 2010	6	6	0.79	0.09	0.76	0.70	0.95
	Spring 2011	6	6	0.82	0.17	0.82	0.61	1.00
ERM 8.0	Spring 2010	6	6	0.56	0.06	0.56	0.49	0.65
	Spring 2011	7	7	0.57	0.12	0.57	0.39	0.73
ERM 3.0	Spring 2010	6	6	0.89	0.18	0.87	0.69	1.20
	Spring 2011	6	6	1.13	0.11	1.10	0.98	1.30
ERM 0.9	Spring 2010	5	5	1.05	0.16	1.00	0.88	1.30
	Spring 2011	6	6	1.17	0.08	1.15	1.10	1.30
CRM 1.5	Spring 2010	6	6	1.02	0.29	1.10	0.48	1.30
	Spring 2011	6	6	1.23	0.27	1.15	0.90	1.60

Table 5. Basic summary statistics for arsenic concentrations in channel catfish fillets (mg/kg wet weight) for the TVA KIF project (2009-2011). N = number of fish collected, FOD = frequency of detects, S.D. = 1 standard deviation of the mean. Where reported results were below the method detection limit (MDL), the MDL was substituted as the result when calculating summary statistics.

Site	Season	N	FOD	Mean	S.D.	Median	Min	Max
LERM 2.0	Fall 2010	6	0	0.027	0.001	0.027	0.026	0.029
	Fall 2011	6	0	0.026	0.001	0.026	0.025	0.028
CRM 24	Fall 2009	6	5	0.045	0.024	0.041	0.015	0.079
CRM 8.0	Spring 2009	12	2	0.042	0.018	0.039	0.026	0.082
	Fall 2009	6	4	0.018	0.003	0.018	0.014	0.022
	Spring 2010	7	4	0.036	0.009	0.038	0.027	0.052
	Fall 2010	6	3	0.042	0.022	0.032	0.026	0.082
	Fall 2011	5	0	0.014	0.001	0.014	0.013	0.015
ERM 8.0	Spring 2009	18	5	0.037	0.011	0.034	0.025	0.050
	Fall 2009	6	0	0.014	0.001	0.014	0.014	0.015
	Spring 2010	8	0	0.027	0.001	0.027	0.025	0.029
	Fall 2010	6	0	0.027	0.001	0.027	0.025	0.028
	Fall 2011	6	0	0.027	0.001	0.026	0.025	0.028
ERM 4.5	Spring 2010	6	1	0.036	0.021	0.028	0.025	0.078
	Fall 2010	6	0	0.027	0.001	0.027	0.026	0.028
ERM 3.0	Fall 2009	6	0	0.059	0.018	0.061	0.030	0.081
	Fall 2010	6	1	0.028	0.002	0.027	0.026	0.032
	Fall 2011	6	0	0.035	0.025	0.029	0.013	0.072
ERM 0.9	Spring 2009	8	1	0.040	0.011	0.045	0.026	0.050
	Fall 2009	7	1	0.015	0.001	0.015	0.014	0.081
	Fall 2010	6	1	0.034	0.019	0.027	0.025	0.072
	Fall 2011	6	0	0.036	0.028	0.024	0.013	0.072
CRM 3.5	Spring 2010	6	2	0.030	0.006	0.028	0.025	0.039
	Fall 2010	5	0	0.033	0.014	0.028	0.025	0.057
CRM 1.5	Spring 2009	15	4	0.039	0.011	0.038	0.027	0.053
	Fall 2009	5	1	0.018	0.008	0.015	0.014	0.032
	Spring 2010	6	4	0.045	0.022	0.038	0.028	0.084
	Fall 2010	6	2	0.046	0.037	0.028	0.025	0.120
	Fall 2011	6	0	0.017	0.005	0.015	0.012	0.025

Table 6. Basic summary statistics for arsenic concentrations in largemouth bass fillets (mg/kg wet weight) for the TVA KIF project (2009-2011). N = number of fish collected, FOD = frequency of detects, S.D. = 1 standard deviation of the mean. Where reported results were below the method detection limit (MDL), the MDL was substituted as the result when calculating summary statistics.

Site	Season	N	FOD	Mean	S.D.	Median	Min	Max
LERM2.0	Fall 2009	6	2	0.108	0.057	0.100	0.052	0.190
	Spring 2010	6	6	0.097	0.038	0.098	0.034	0.140
	Fall 2010	6	6	0.124	0.035	0.135	0.069	0.160
	Spring 2011	7	7	0.148	0.048	0.160	0.066	0.200
	Fall 2011	6	5	0.114	0.056	0.120	0.026	0.190
CRM 24	Fall 2009	5	5	0.262	0.132	0.200	0.140	0.410
	Spring 2010	6	6	0.340	0.110	0.340	0.200	0.490
CRM8.0	Spring 2009	6	6	0.273	0.052	0.280	0.190	0.350
	Fall 2009	6	6	0.202	0.025	0.205	0.160	0.230
	Spring 2010	6	6	0.237	0.069	0.235	0.130	0.320
	Fall 2010	6	6	0.267	0.070	0.295	0.170	0.340
	Spring 2011	6	6	0.285	0.062	0.305	0.170	0.350
	Fall 2011	6	6	0.197	0.063	0.205	0.110	0.280
ERM 8.0	Spring 2009	6	4	0.117	0.059	0.130	0.016	0.180
	Fall 2009	6	6	0.147	0.034	0.150	0.094	0.190
	Spring 2010	6	6	0.147	0.058	0.150	0.072	0.210
	Fall 2010	7	7	0.150	0.042	0.140	0.090	0.210
	Spring 2011	6	5	0.156	0.051	0.165	0.063	0.210
	Fall 2011	6	5	0.156	0.051	0.165	0.063	0.210
ERM4.5	Spring 2010	6	6	0.167	0.044	0.165	0.110	0.240
	Fall 2010	6	6	0.298	0.069	0.290	0.210	0.410
ERM3.0	Spring 2009	5	5	0.148	0.053	0.130	0.088	0.230
	Fall 2009	6	1	0.165	0.022	0.160	0.140	0.200
	Spring 2010	6	6	0.168	0.023	0.170	0.130	0.200
	Fall 2010	7	7	0.209	0.064	0.180	0.160	0.300
	Spring 2011	6	6	0.287	0.039	0.295	0.240	0.330
	Fall 2011	6	4	0.156	0.044	0.160	0.098	0.220
ERM0.9	Spring 2009	6	6	0.218	0.063	0.200	0.140	0.300
	Fall 2009	6	6	0.163	0.066	0.180	0.059	0.230
	Spring 2010	6	6	0.274	0.112	0.325	0.074	0.360
	Fall 2010	6	6	0.302	0.086	0.330	0.140	0.380
	Spring 2011	6	6	0.297	0.090	0.310	0.170	0.420
	Fall 2011	6	6	0.195	0.063	0.210	0.089	0.260
CRM3.5	Spring 2010	6	6	0.245	0.047	0.260	0.170	0.300
	Fall 2010	6	6	0.270	0.068	0.290	0.170	0.330
CRM 1.5	Spring 2009	18	18	0.236	0.050	0.230	0.150	0.350
	Fall 2009	6	6	0.230	0.052	0.220	0.170	0.320
	Spring 2010	6	6	0.185	0.089	0.210	0.058	0.280
	Fall 2010	6	6	0.208	0.105	0.250	0.059	0.330
	Spring 2011	6	6	0.190	0.070	0.180	0.100	0.290
	Fall 2011	6	6	0.208	0.053	0.190	0.170	0.310

Table 7. Basic summary statistics for arsenic concentrations in bluegill sunfish fillets (mg/kg wet weight) for the TVA KIF project (2009-2011). N = number of fish collected, FOD = frequency of detects, S.D. = 1 standard deviation of the mean. Where reported results were below the method detection limit (MDL), the MDL was substituted as the result when calculating summary statistics.

Site	Season	N	FOD	Mean	S.D.	Median	Min	Max
LERM 2.0	Fall 2009	6	0	0.024	0.022	0.016	0.014	0.068
	Spring 2010	6	5	0.041	0.014	0.038	0.028	0.067
	Fall 2010	6	2	0.030	0.005	0.028	0.027	0.038
	Spring 2011	7	0	0.028	0.002	0.028	0.025	0.029
	Fall 2011	6	0	0.033	0.014	0.028	0.024	0.062
CRM 24	Fall 2009	6	1	0.067	0.056	0.046	0.016	0.120
	Spring 2010	3	2	0.088	0.089	0.045	0.028	0.190
CRM 8.0	Spring 2009	6	6	0.060	0.009	0.060	0.047	0.069
	Fall 2009	6	6	0.052	0.016	0.053	0.034	0.072
	Spring 2010	11	11	0.085	0.042	0.073	0.034	0.160
	Fall 2010	6	6	0.079	0.026	0.083	0.040	0.110
	Spring 2011	6	5	0.042	0.010	0.042	0.027	0.055
	Fall 2011	6	1	0.062	0.029	0.053	0.036	0.110
ERM 8.0	Spring 2009	6	6	0.022	0.005	0.020	0.019	0.032
	Fall 2009	6	0	0.018	0.008	0.016	0.013	0.034
	Spring 2010	6	2	0.146	0.147	0.086	0.025	0.400
	Fall 2010	6	1	0.030	0.005	0.029	0.025	0.040
	Spring 2011	5	0	0.028	0.002	0.028	0.025	0.029
	Fall 2011	6	0	0.025	0.009	0.028	0.013	0.033
ERM 4.5	Spring 2010	6	6	0.085	0.022	0.081	0.058	0.110
	Fall 2010	5	3	0.030	0.008	0.033	0.026	0.039
ERM 3.0	Spring 2009	6	6	0.039	0.007	0.039	0.030	0.047
	Fall 2009	6	0	0.034	0.019	0.030	0.015	0.068
	Spring 2010	6	6	0.072	0.013	0.071	0.055	0.090
	Fall 2010	6	6	0.091	0.036	0.077	0.050	0.140
	Spring 2011	6	6	0.051	0.022	0.043	0.036	0.094
	Fall 2011	6	0	0.061	0.024	0.063	0.025	0.093
ERM 0.9	Spring 2009	6	1	0.072	0.020	0.077	0.039	0.099
	Fall 2009	6	5	0.053	0.025	0.052	0.015	0.084
	Spring 2010	5	5	0.077	0.040	0.070	0.039	0.150
	Fall 2010	6	6	0.118	0.073	0.101	0.052	0.240
	Spring 2011	6	6	0.055	0.021	0.051	0.032	0.091
	Fall 2011	6	0	0.036	0.019	0.033	0.018	0.072
CRM 3.5	Spring 2010	6	4	0.160	0.038	0.150	0.120	0.230
	Fall 2010	7	7	0.140	0.076	0.130	0.086	0.290
CRM 1.5	Spring 2009	6	0	0.059	0.015	0.059	0.037	0.079
	Fall 2009	6	4	0.037	0.030	0.024	0.015	0.092
	Spring 2010	6	6	0.160	0.100	0.140	0.055	0.320
	Fall 2010	6	6	0.069	0.036	0.069	0.033	0.110
	Spring 2011	6	6	0.063	0.028	0.065	0.027	0.098
	Fall 2011	6	0	0.046	0.018	0.041	0.026	0.078

Table 8. Basic summary statistics for arsenic concentrations in redear sunfish fillets (mg/kg wet weight) for the TVA KIF project (2009-2011). N = number of fish collected, FOD = frequency of detects, S.D. = 1 standard deviation of the mean. Where reported results were below the method detection limit (MDL), the MDL was substituted as the result when calculating summary statistics.

Site	Season	N	FOD	Mean	S.D.	Median	Min	Max
LERM 2.0	Spring 2010	6	5	0.064	0.029	0.058	0.028	0.110
	Spring 2011	7	5	0.077	0.037	0.066	0.042	0.150
CRM 24	Spring 2010	3	3	0.243	0.032	0.230	0.220	0.280
CRM 8.0	Spring 2010	6	6	0.208	0.071	0.195	0.130	0.310
	Spring 2011	6	6	0.248	0.142	0.260	0.067	0.430
ERM 8.0	Spring 2010	6	5	0.061	0.024	0.062	0.028	0.093
	Spring 2011	7	0	0.041	0.031	0.028	0.026	0.110
ERM 3.0	Spring 2010	6	6	0.107	0.031	0.097	0.080	0.150
	Spring 2011	6	6	0.148	0.061	0.150	0.056	0.210
ERM 0.9	Spring 2010	5	5	0.230	0.041	0.240	0.170	0.270
	Spring 2011	6	6	0.225	0.086	0.225	0.130	0.340
CRM 1.5	Spring 2010	6	6	0.227	0.062	0.220	0.150	0.320
	Spring 2011	6	6	0.193	0.048	0.190	0.140	0.270

Table 9. Basic summary statistics for mercury concentrations in fish fillets(mg/kg wet weight) for the TVA KIF project (Fall 2011). Red font indicates mean concentrations above the U.S. Environmental Protection Agency's National Recommended Water Quality Criterion (0.3 mg/kg wet wt).

	Bluegill						Catfish						Bass					
Site	*n*	Mean	Std. dev.	Median	Min	Max	*n*	Mean	Std. dev.	Median	Min	Max	*n*	Mean	Std. dev.	Median	Min	Max
LERM 2	6	0.07	0.02	0.07	0.05	0.09	6	0.15	0.12	0.09	0.07	0.37	6	0.19	0.03	0.20	0.15	0.22
CRM 24		0.05	0.00															
CRM 8	6	0.09	0.02	0.09	0.06	0.11	5	0.24	0.19	0.16	0.05	0.54	6	0.23	0.10	0.22	0.10	0.39
ERM 8	6	0.11	0.04	0.21	0.15	0.56	6	0.26	0.15	0.21	0.15	0.56	6	0.39	0.16	0.34	0.25	0.71
ERM 4.5																		
ERM 3	6	0.08	0.03	0.08	0.04	0.13	6	0.37	0.60	0.12	0.08	1.60	6	0.33	0.23	0.19	0.17	0.63
CRM 3.5																		
ERM 0.9	6	0.09	0.02	0.09	0.06	0.12	6	0.10	0.03	0.10	0.06	0.14	6	0.21	0.09	0.21	0.11	0.37
CRM 1.5	6	0.08	0.02	0.08	0.06	0.11	6	0.15	0.07	0.13	0.10	0.29	6	0.19	0.06	0.20	0.10	0.26

Table 10. Summary statistics for temporal trends in arsenic and selenium concentrations 2009-2011. Values shown are p values for linear regression, with year of samples collected as the independent variable and concentration of Se or As as dependent variables. Sites were separated into ash affected sites (ERM 4.5, ERM 3, ERM 0.9, CRM 1.5) and reference sites (ERM 8, LERM 2). The upstream Clinch River sites (CRM 24, CRM 8) were excluded from this analysis. Asterisks indicate statistically significant values ($p < 0.05$).

	As		Se	
	ash	reference	ash	reference
Largemouth bass	.017*	0.325	0.004*	0.368
Bluegill	0.822	0.22	0.901	0.7
Channel catfish	ND	ND	0.786	0.083
Redear	0.899	0.774	0.004*	0.691

Fig. 1. Mean (± 1 standard deviation) selenium concentrations in redear sunfish fillets (mg/kg wet wt.) 2010-2011.

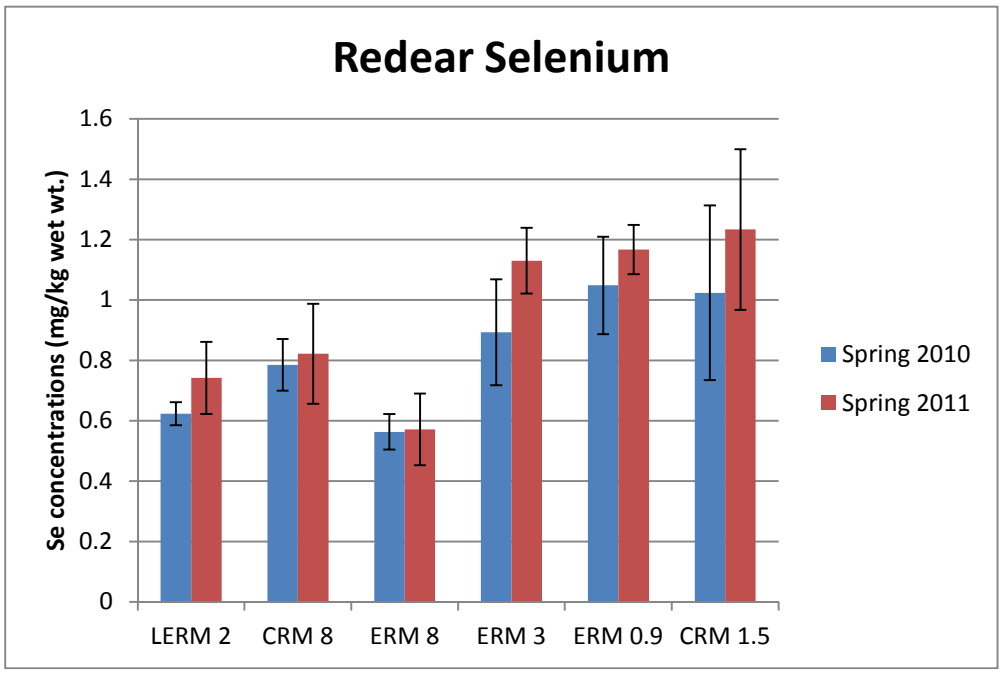


Fig. 2. Mean selenium concentrations in channel catfish fillets (mg/kg wet wt.) 2009-2011.

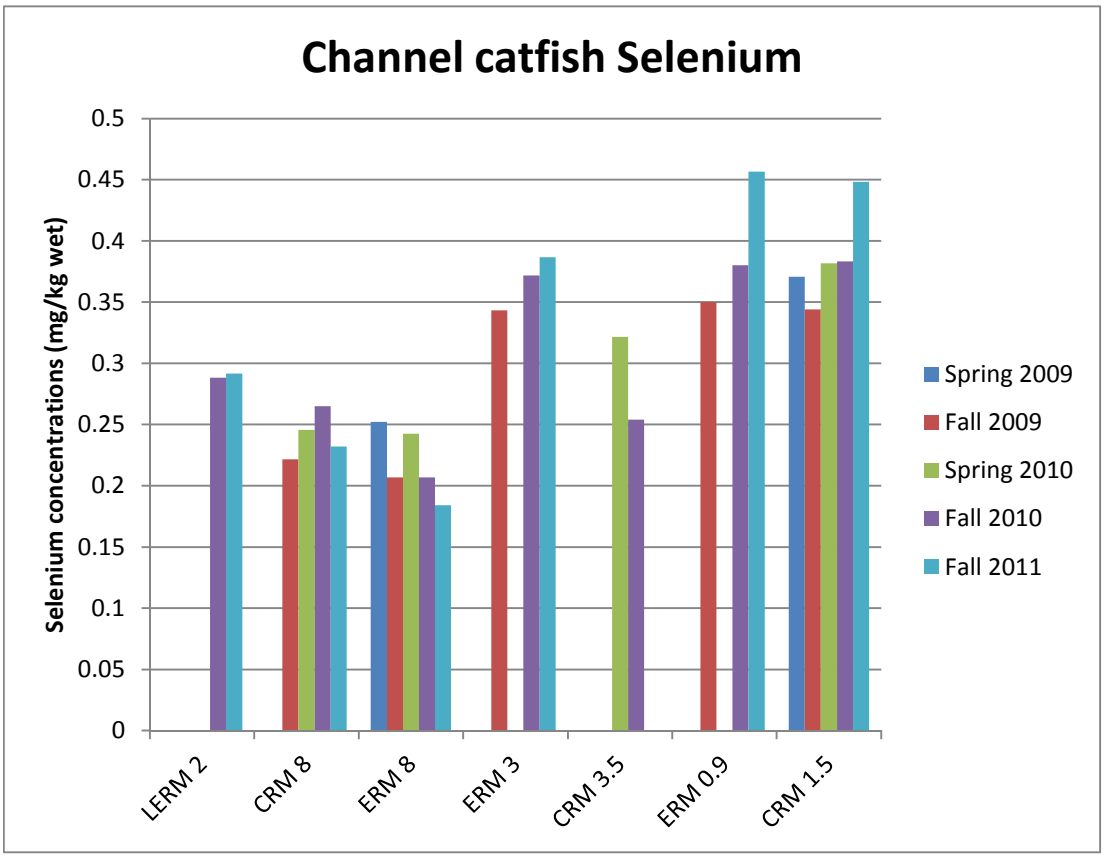


Fig. 3. Mean selenium concentrations in largemouth bass fillets (mg/kg wet wt.) 2009-2011.

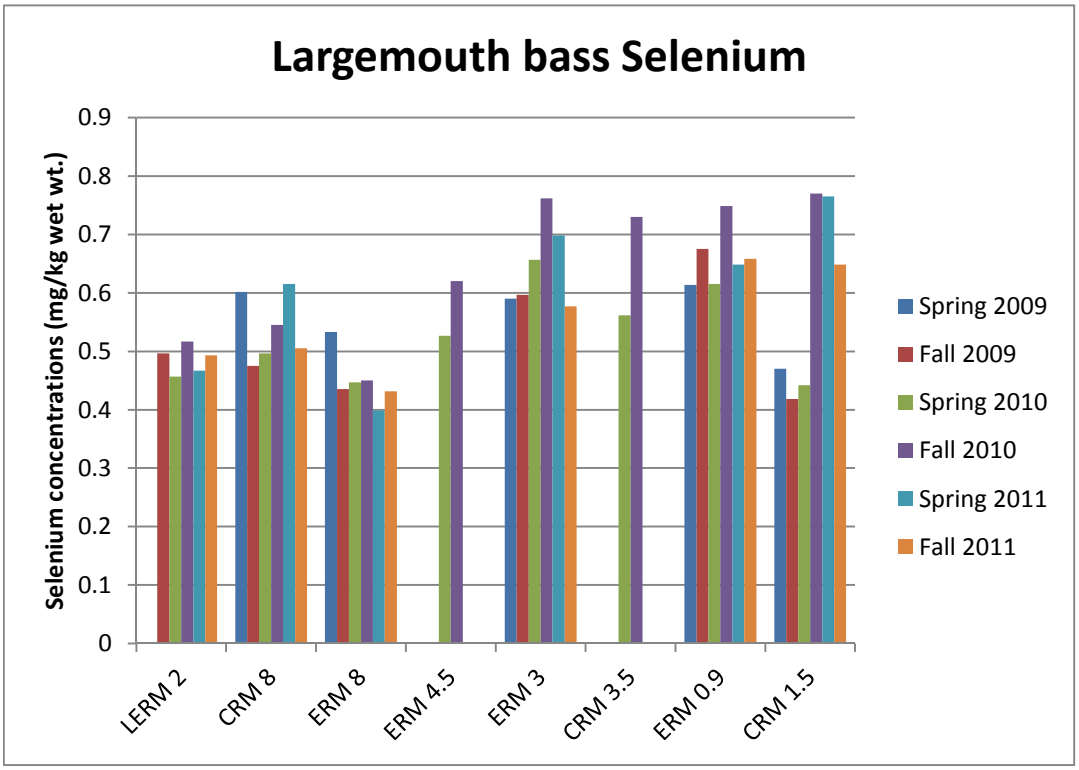


Fig. 4. Mean selenium concentrations in bluegill fillets (mg/kg wet wt.) 2009-2011.

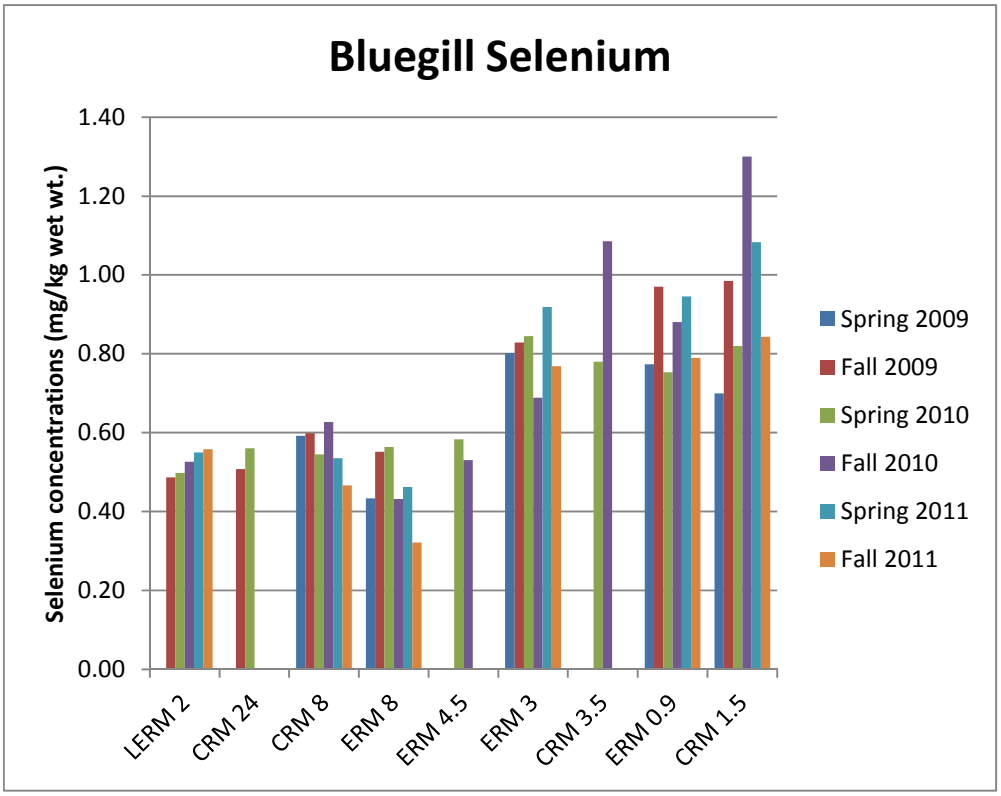


Fig. 5. Mean arsenic concentrations in bluegill fillets (mg/kg wet wt.) 2009-2011.

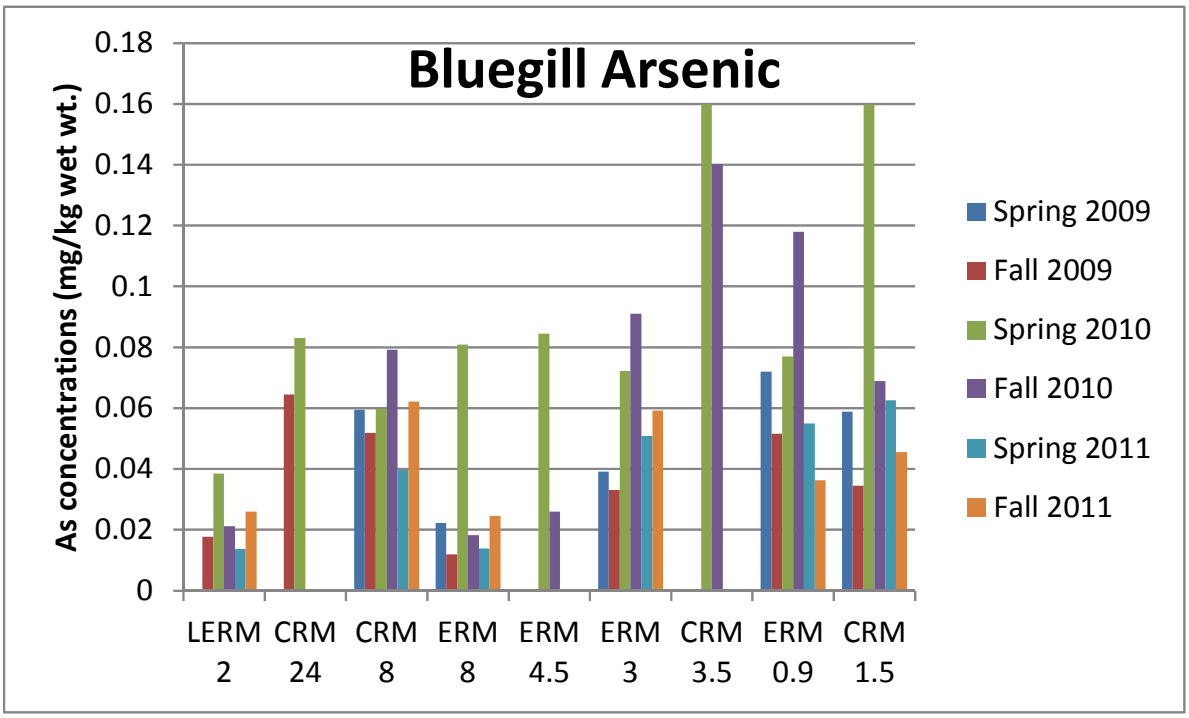


Fig.6. Mean arsenic concentrations in largemouth bass fillets (mg/kg wet wt.) 2009-2011.

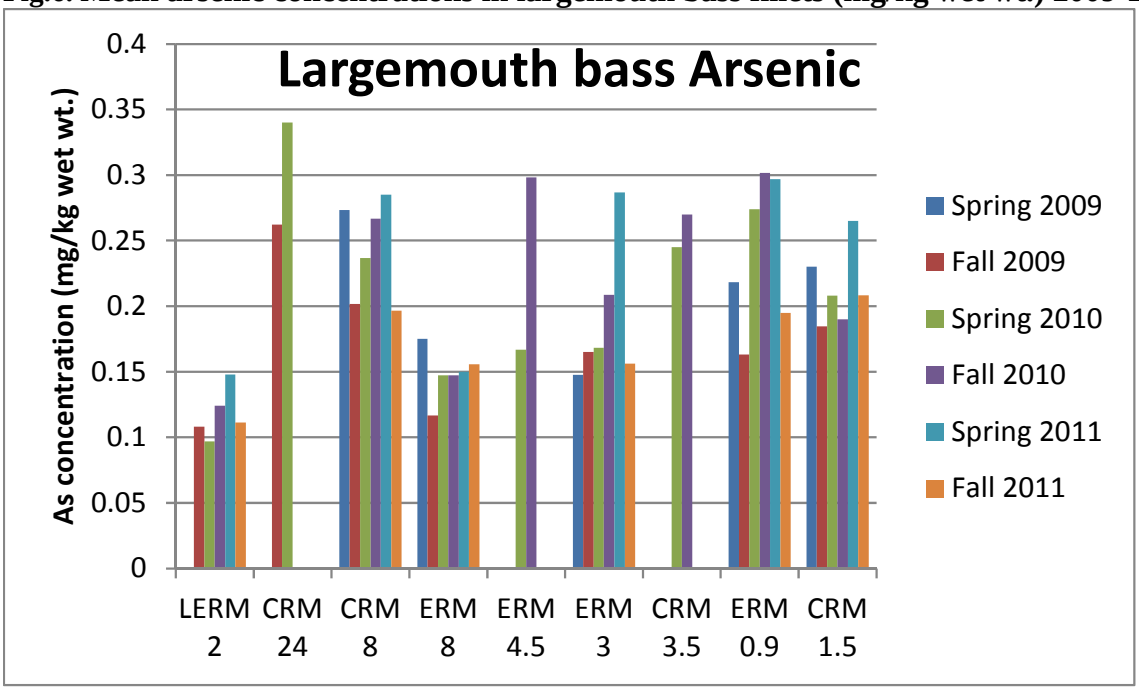


Fig. 7. Mean arsenic concentrations in redear fillets (mg/kg wet wt.) 2010-2011.

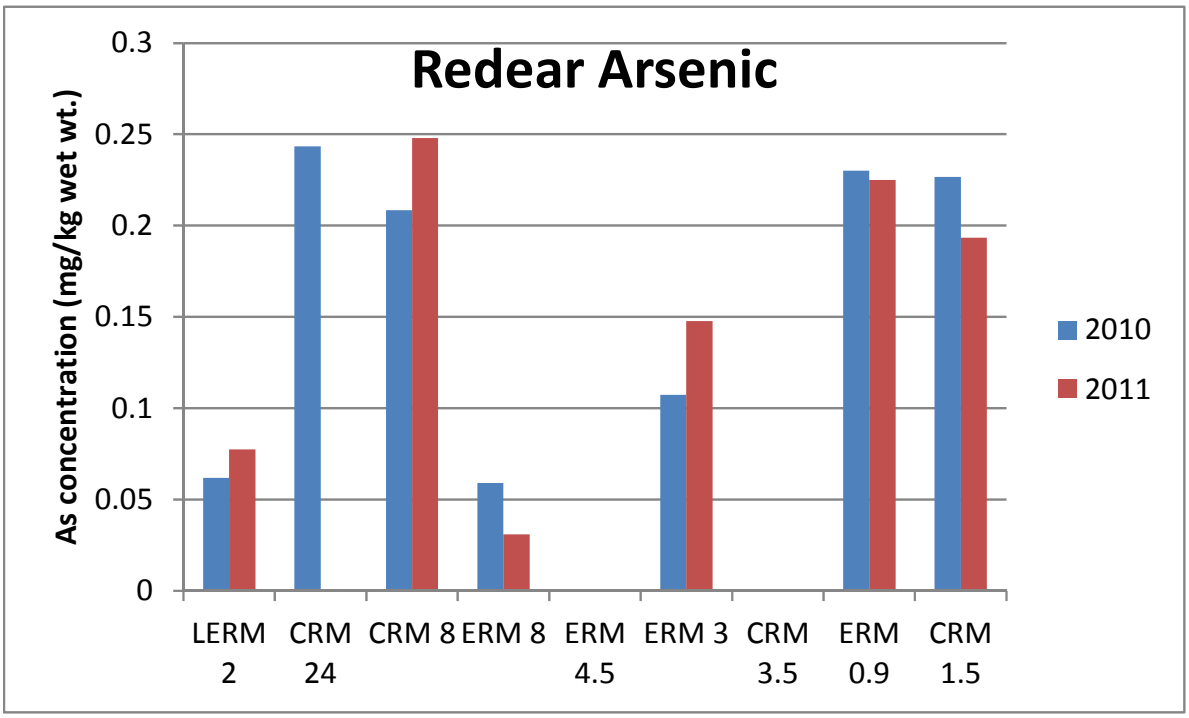
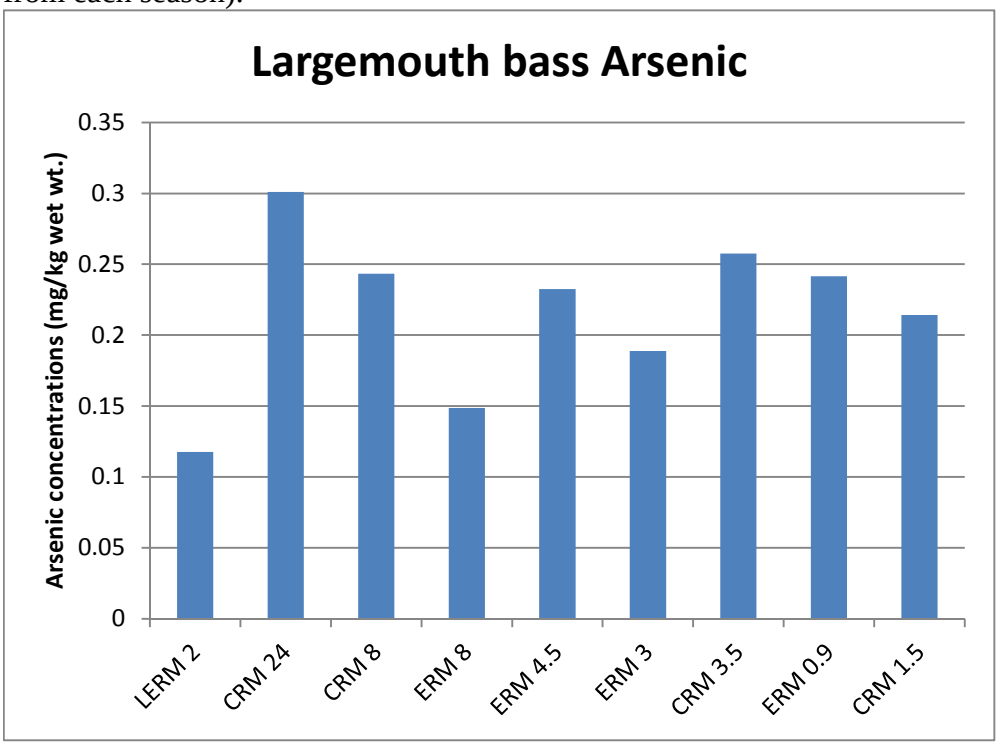


Fig. 8. Mean arsenic concentrations in largemouth bass fillets (mg/kg). Data shown in this figure represent the mean concentrations of all available data, 2009-2011. (See Table 6 for data from each season).



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Appendix D

Preliminary Evaluation of 2011
ORNL Results: Fish Health

OAK RIDGE NATIONAL LABORATORY
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May 4, 2012

Dr. Neil Carriker
Program Manager, Special Projects
Tennessee Valley Authority Kingston Ash Recovery Project
Kingston, Tennessee

Dr. Carriker,

Subject: Preliminary Evaluation of 2011 ORNL Results: *Fish Health*

We are pleased to submit to you our preliminary evaluation of 2011 *Fish Health* Task results.

The enclosed report covers the initial analysis of the fish health metrics collected from fish from the 2011 spring and fall fish collections.

We hope this preliminary update is useful to you. If you have any questions or require additional information, please call me at (865) 576-0266.

Sincerely,

Mark Bevelhimer, PhD
Principal Investigator, Fish Health
Environmental Sciences Division
Oak Ridge National Laboratory

c: M. J. Peterson
D. Jones, ARCADIS

Preliminary Evaluation of 2011 ORNL Results: *Fish Health*
May 4, 2012

Introduction

This report summarizes the results of samples collected in spring (April-May) and fall (October-November) 2011 for the fish health task for the Kingston Fossil Plant Coal Ash Spill Project. As in 2009 and 2010, fish were collected in 2011 from reference sites (ERM 8.0, LERM 2.0, CRM 8.0) and sites at and downstream of the Kingston fly ash spill (ERM 3.0, ERM 1.5, CRM 1.5). A variety of fish health measures were collected from 8-10 fish of each of four species from each site -- bluegill, largemouth bass, and redear sunfish in the spring and bluegill, largemouth bass, and channel catfish in the fall. The specific objective of the fish health component of the overall biological monitoring program is to determine if exposure to fly ash-associated metals causes short, intermediate, or long-term health effects on these sentinel fish species. The fish health study conducted in conjunction with the bioaccumulation and reproductive study is critical for assessing and evaluating possible causal relationships between contaminant exposure (bioaccumulation) and the response of fish to exposure as reflected by the various measurements of fish health.

Preliminary Results

The early analysis of the 2011 fish health data concentrated on comparing 2011 values to the data collected in 2009 and 2010. For a rapid assessment, we created data blocks of the mean values for each species-metric combination arranged by site and collection period (season/year) and then color-coded so that we could make easy visual analysis (Tables 1 and 2). (Note: not all metrics are included in these tables.) Values were color-coded such that the lowest values are the lightest color and highest values the darkest. Specifically, we looked for evidence of:

1. Higher or lower values at any of the three spill sites as compared to the three reference sites
2. A consistent increase or decrease at any of the spill sites from 2009-2011 (that was not also evident at the reference sites).
3. Decreasing or increasing trend within the spill sites in a downstream direction (i.e., ERM 3.0, ERM 0.9, CRM 1.5)

The visual assessment did not reveal any consistent patterns that suggest an obvious health effect of exposure to the fly ash spill; however, we did make the following observations of the 2011 data that will require further evaluation:

Bluegill

- Spring visceral somatic index was higher at all sites than in 2010 (that's a good thing)
- Spring blood protein was high at ERM 0.9 (a good thing)
- Spring blood sodium was highest at spill sites (a good thing)
- Spring stomach fullness was low at ERM 0.9
- Spring condition factor (CF) was lowest at two of the spill sites
- Spring liver-somatic index (LSI) was lowest at the spill sites just like in 2010
- Fall protein and calcium higher at ERM 0.9 than other sites (a good thing)
- Fall stomach fullness low at spill sites especially ERM 0.9

Largemouth Bass

- Spring hematocrit at spill sites lower than in 2010
- Spring glucose is higher at spill sites than reference sites and lower than in 2010
- Spring blood protein is slightly lower than in 2010
- Spring gall bladder color higher than 2010 with spill sites highest
- Spring CF highest at three spill sites (a good thing)
- Spring LSI lowest at ERM 0.9
- Fall BUN elevated at ERM 3.0
- Fall sodium slightly lower at most sites
- Fall CF slightly lower at spill sites

With small sample sizes (10 or less per species per site per period in this study) most statistically significant results should be apparent in a careful visual assessment of the data. We saw little here, but we caution that conclusions should not be made until the appropriate statistical tests are applied to these data.

Future Analysis

Future analyses will include a rigorous statistical analysis to determine if there are significant differences in the health metric values among reference and spill sites and from year to year. This analysis will include the multivariate cononical discriminant analysis that has been performed on past years' data.

We also intend to combine the fish health results and the bioaccumulation results in an analysis to look for any relationships between contaminant body burden and fish health response. Because both sets of data were collected from the same individual fish this should be a very powerful analysis and will overcome possible problems with highly mobile species, such as largemouth bass and catfish, whose spot of capture may not actually reflect the amount of exposure to fly ash that can be inferred by a less mobile species, such as bluegill.

In addition to the data presented here, select 2011 samples of gill, liver, and gonad tissue will soon be analyzed for histopathological anomalies as in previous years, and these results will be included in the multi-parameter analyses.

Table 1. Fish health metrics for 9 blood parameters for bluegill and largemouth bass collected at 6 sites over three years. Parameters include ALBumin, ALkaline Phosphatase, ALanine Transaminase, AMYlase, Blood PROtein, Blood Urea Nitrogen, CAlcium, CREATinine, and GLOBulin. Cells within each species group are color-coded with lowest values in light shades and highest values in dark shades.

		ALB							ALP							ALT					
		2009		2010		2011			2009		2010		2011			2009		2010		2011	
	Row Labels	Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall
	BLUGIL																				
Ref	CRM8.0	16.1	14.3	19.9	19.1	19.9	18.4	####	37.7	24	28.6	21.5	30.5	16.9	####	22.4	13.2	28.5	14.3	31.5	19.1
Ref	ERM8.0	15.6	16.4	22.3	18.9	21.8	21.1	####	30.8	28.4	36.4	22.9	52.4	8.33	####	21.5	17.1	20.1	13.3	25.2	12.2
Ref	LERM2.0		16.7	20	20	24	24.7	####		22.2	34.8	14.4	23	8.67	####		9.1	17.7	14.3	15.6	16.4
Spill	ERM3.0	15.6	13.9	19.9	19.8	23.1	21.4	####	27.5	24.3	36.5	15.5	23.3	3.33	####	20	13.1	20.5	19.5	22.6	13.9
Spill	ERM0.9	15.7	16.6	20.4	19.1	25.8	22.1	####	35.7	29.1	39.8	18.5	32.5	7.11	####	20.2	31.3	21.9	15.1	19.6	10.3
Spill	CRM1.5	12.9	18.3	18.4	20.7	23.6	19.8	####	29.9	30.8	39.4	21.8	21.3	14	####	20.8	13.1	29.1	12.7	21.7	12.3
	LMBASS																				
Ref	CRM8.0	17.8	15	21.6	20.6	18.6	18.9	####	36.2	34.6	34.6	43.3	22.4	30.1	####	42	22.7	25.9	24.6	34.3	15.9
Ref	ERM8.0	18.9	21.1	20.8	19.3	21.3	21.1	####	30.9	36.6	23.8	26	29.9	18.4	####	34.3	17.4	35.4	20.5	47.9	17.9
Ref	LERM2.0		18.8	22.4	19.3	20.8	23.4	####		26.6	24.3	25.1	29.8	28.3	####		25.3	38.5	20.3	26	24.5
Spill	ERM3.0	18.4	18.4	24	20.3	21.7	21.5	####	29.6	36.1	28	32.3	30.6	21.8	####	25.3	18.5	42	17.9	44.4	17
Spill	ERM0.9	18.8	20	20.4	20.8	23.1	21.4	####	31.7	40.6	32.5	37.1	28.9	24	####	35	24.8	30.9	20.4	35.6	27.8
Spill	CRM1.5	18.9	16.4	22.8	20.9	23.4	21.8	####	31.1	25.9	31.3	36.3	34.1	24.1	####	32.1	43.3	35	21	27.1	13.9

		AMY							BPRO							BUN					
		2009		2010		2011			2009		2010		2011			2009		2010		2011	
		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall
	BLUGIL																				
Ref	CRM8.0	43.7	21.6	58.5	31.6	63.2	24.8	####	41.2	34.9	41.6	38.8	41.2	37.9	####	3.58	2.67	2.63	1.88	2.1	1.33
Ref	ERM8.0	36.1	20.1	42.6	35.1	78	31.6	####	37.4	36.3	43	38.6	42	40.3	####	2.44	2.56	2.71	3	3.2	2.67
Ref	LERM2.0		21.4	56.2	30.6	65.6	29.1	####		35.6	41.1	37.6	43	40.6	####		2.2	2.7	2.43	1.78	2.56
Spill	ERM3.0	53.8	19.5	47.9	28.6	52.4	22	####	38.5	32.6	41.3	39.3	42	40.1	####	3.38	1.75	2.09	2.75	2.11	2.44
Spill	ERM0.9	52.7	18.1	65.1	24.3	64.6	28.3	####	40	39.6	41.1	38.6	46	42.6	####	3.56	2.14	2.25	1.38	2.13	3.11
Spill	CRM1.5	25.7	20.9	54.9	29.7	65.7	25.8	####	34.5	37.8	37.1	40.3	43.1	39	####	2	2.11	2.1	1.89	1.78	2
	LMBASS																				
Ref	CRM8.0	159	152	220	224	207	186	####	52.2	44.7	59.4	48.9	43.9	45.2	####	3.2	2.86	3.75	2.25	2.43	2.44
Ref	ERM8.0	214	201	236	194	184	216	####	49	45.8	46.6	46.9	48.1	48.4	####	2.24	1.88	2.25	2.63	2.71	2.13
Ref	LERM2.0		205	185	198	209	262	####		44.5	50.4	45.1	51.3	45.8	####		2	1.88	2.88	3.78	2.63
Spill	ERM3.0	151	173	211	179	242	206	####	53.6	44.5	57.3	49.6	50.2	48.1	####	3.29	2.75	1.86	2.29	4.78	4.13
Spill	ERM0.9	193	227	234	284	298	220	####	51.9	53.5	60.6	47.9	52.6	47.9	####	3.36	3.38	3.38	2	4	3
Spill	CRM1.5	189	189	231	270	266	209	####	52	46.6	55.5	50.3	55	49.1	####	1.94	2.75	2.5	2.63	4.75	2.88

		CA							CREAT							GLOB					
		2009		2010		2011			2009		2010		2011			2009		2010		2011	
		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall
	BLUGIL																				
Ref	CRM8.0	19.2	13	21.8	14.1	19.7	13.2	####	0.05	0.02	0.05	0.06	0.01	0.01	####	25	21.1	21.9	19.6	21.3	19.4
Ref	ERM8.0	15.3	13.1	20.2	14.7	18.7	14.2	####	0.12	0	0.03	0.03	0	0.06	####	22.8	20.4	20.7	19.6	20.2	19.1
Ref	LERM2.0		12.2	19.9	13.8	19.5	13.9	####		0.12	0.11	0.03	0.01	0	####		18.9	21.4	17.9	19	16.1
Spill	ERM3.0	16.9	11.3	19.9	14.5	19.3	14.1	####	0.06	0.04	0.1	0	0	0.07	####	22.9	19.1	21.3	19.8	18.4	18.7
Spill	ERM0.9	18.9	13.8	18.8	14.1	20	15.3	####	0.08	0.07	0.08	0.09	0	0.08	####	24.3	23	20.6	19.8	20.1	20.7
Spill	CRM1.5	15.4	13.6	19.6	14.7	19.2	14	####	0.08	0.09	0.04	0.16	0.01	0.06	####	21.9	19.6	17.1	19.6	19.8	19.2
	LMBASS																				
Ref	CRM8.0	18.4	12.4	19.3	14.4	15.3	13.2	####	0.02	0.11	0.28	0.18	0.1	0.09	####	34.6	30.3	37.9	28.4	25.4	26.2
Ref	ERM8.0	16.2	13.8	19.1	13.1	19.7	14.8	####	0.14	0.03	0.14	0.09	0.1	0.18	####	30.1	24.6	26	27.8	26.9	27.4
Ref	LERM2.0		13.2	20.2	13.1	17.6	13.9	####		0.09	0.16	0.05	0.16	0.03	####		25.8	28	26	30.8	22.4
Spill	ERM3.0	19.7	13.3	20.3	14.2	18.2	14.4	####	0.06	0.05	0.11	0.03	0.09	0.16	####	35.1	26	33.1	29.4	28.3	26.5
Spill	ERM0.9	17.5	14.9	17	15.3	18.4	14.2	####	0.13	0.25	0.29	0.58	0.15	0.05	####	33	33.4	30.9	27.4	29.5	26.3
Spill	CRM1.5	17.3	13	21.9	14.3	19.2	13.5	####	0.13	0.08	0.15	0.18	0.16	0.18	####	33.9	25.9	33	29.1	31.5	27.8

Table 2. Fish health metrics for 7 blood parameters and 2 condition indices for bluegill and largemouth bass collected at 6 sites over three years. Parameters include GLUcose, HematoCriT, Potassium (K), LeuCocriT, Sodium (NA), PHOSphorus, Total BILirubin, Condition Factor, and Liver Somatic Index. Cells within each species group are color-coded with lowest values in light shades and highest values in dark shades.

		GLU							HCT							K					
		2009		2010		2011			2009		2010		2011			2009		2010		2011	
		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall
	BLUGIL																				
Ref	CRM8.0	66.9	56	47.3	41.5	57.4	46.9	####	25.8	23.6	26.8	27.4	30.5	28.8	####	5.78	6.94	3.59	5.66	3.45	5.24
Ref	ERM8.0	63.7	67.4	68.9	53.5	56	46	####	27.9	24.2	38.3	26.8	27.6	31.7	####	5.24	4.83	4.2	5.18	4.08	3.9
Ref	LERM2.0		39.1	48.8	41.9	54	55.3	####		21.6	31.7	25	31.7	30.9	####		4.72	2.5	5.29	2.2	5.58
Spill	ERM3.0	51.4	43	59.9	76.3	63.1	54.2	####	28.1	24.3	29.2	28	28.3	29	####	6.04	5.39	3.95	4.31	3.16	4.09
Spill	ERM0.9	62.3	91.9	54.9	50.9	61.4	46.6	####	25.3	34.9	33.4	27.5	30.8	28.8	####	5.57	6.4	4.81	5.8	3.01	5.19
Spill	CRM1.5	75.7	58.6	93.6	40.7	71.2	48.1	####	25.9	24.9	29.9	26.3	32.7	34.1	####	4.92	5.52	3.78	5.46	3.53	3.93
	LMBASS																				
Ref	CRM8.0	66.2	56.6	100	57	47	43.1	####	30.5	34.9	36.5	31	27.3	32.1	####	4.4	3.14	1.55	2.81	3.33	2.01
Ref	ERM8.0	76.4	42.6	51.6	49.4	61.1	60.9	####	35	34	31	30	32.3	35	####	2.75	2.86	1.99	2.63	2.13	2.61
Ref	LERM2.0		36	65.3	55	76.9	50.4	####		29.9	29.6	30.1	27.9	39.1	####		2.09	1.58	2.66	2.03	1.63
Spill	ERM3.0	98.7	58.1	145	73.1	105	67.3	####	31	31.5	37.4	34	31	32.6	####	2.14	3.09	1.17	2.31	2.8	1.56
Spill	ERM0.9	68.5	140	113	68	86	55	####	31.5	42.3	38.5	33.3	32.6	32.1	####	2.67	2.61	1.49	2.7	2.03	2.05
Spill	CRM1.5	67.4	46.4	174	67.1	105	49	####	29.8	28.6	38.4	35.8	31.6	32.1	####	3.86	4.95	1.43	2.15	2.09	1.64
		LCT							NA							PHOS					
		2009		2010		2011			2009		2010		2011			2009		2010		2011	
		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall
	BLUGIL																				
Ref	CRM8.0	0.81	1.06	1	1.13	0.75	1	####	152	151	148	153	155	151	####	12.6	9.16	13.5	7.89	13.1	8.52
Ref	ERM8.0	0.5	0.61	0.57	1	1.2	0.94	####	151	147	151	156	148	148	####	11	8.73	11.9	10.5	12.3	8.84
Ref	LERM2.0		0.8	0.6	1.07	1.11	1.06	####		149	147	151	150	152	####		6.39	11	8.19	11.1	9.74
Spill	ERM3.0	0.8	1.5	0.91	1	1.15	1.39	####	154	142	148	152	153	150	####	10	7.09	11.2	10.9	13.2	10.1
Spill	ERM0.9	0.91	0.81	0.56	0.75	1.22	0.78	####	156	155	151	153	155	152	####	10.8	13.2	12.6	8.84	12.9	8.96
Spill	CRM1.5	0.93	0.78	0.7	0.94	1.11	0.89	####	148	153	148	155	154	148	####	9.95	8.64	15	8.72	12.7	8.81
	LMBASS																				
Ref	CRM8.0	0.67	0.29	0.06	0.58	1	0.83	####	157	148	155	155	153	150	####	7.6	7.47	10.9	10.3	7.29	7.54
Ref	ERM8.0	0.58	0.44	1.5	0.69	1.13	0.69	####	156	149	152	150	151	150	####	9.62	9.13	9.2	7.6	8.64	8.78
Ref	LERM2.0		0.56	1.38	0.69	0.89	0.25	####		149	152	154	150	153	####		7.13	8.79	8.74	6.86	8.93
Spill	ERM3.0	1.29	0.63	0.75	0.5	0.78	0.63	####	156	152	155	155	154	151	####	9.81	8.54	12.3	9.09	7.03	9.01
Spill	ERM0.9	1.18	0.56	0.56	0.5	0.81	0.81	####	157	160	153	156	152	150	####	9.17	12.7	8.49	8.55	8.79	9.99
Spill	CRM1.5	0.86	0.64	0.29	0.69	0.75	1	####	152	152	155	155	155	147	####	9.05	9.96	16.5	9.69	6.73	7.58
		TBIL							CF							LSI					
		2009		2010		2011			2009		2010		2011			2009		2010		2011	
		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall		Spring	Fall	Spring	Fall	Spring	Fall
	BLUGIL																				
Ref	CRM8.0	0.39	0.51	0.34	0.54	0.67	0.62	####	1.81	1.66	1.85	1.74	1.66	1.70		1.40	0.72	1.93	0.77	1.91	0.80
Ref	ERM8.0	0.53	0.41	0.54	0.89	0.46	0.96	####	1.76	1.63	1.67	1.69	1.73	1.71		1.80	1.10	2.03	1.00	1.85	0.83
Ref	LERM2.0		0.7	0.37	0.73	0.5	0.96	####		1.66	1.7	1.75	1.73	1.55			0.98	1.90	0.87	1.83	0.77
Spill	ERM2.0	0.43	0.39	0.3	0.74	0.38	0.8	####	1.68	1.56	1.84	1.77	1.71	1.74		1.30	0.73	1.89	0.92	1.61	0.73
Spill	ERM0.9	0.34	0.61	0.33	0.43	0.51	0.54	####	1.73	1.66	1.82	1.69	1.66	1.71		1.30	0.82	1.54	0.66	1.50	0.76
Spill	CRM1.5	0.45	0.46	0.45	0.61	0.54	0.46	####	1.64	1.66	1.72	1.78	1.66	1.72		1.20	0.79	1.61	1.62	1.50	0.80
	LMBASS																				
Ref	CRM8.0	0.22	0.39	0.3	0.4	0.21	0.36	####	1.34	1.21	1.57	1.32	1.40	1.34		1.49	0.84	1.76	0.64	1.46	0.60
Ref	ERM8.0	0.31	0.46	0.28	0.44	0.26	0.44	####	1.43	1.38	1.37	1.31	1.40	1.31		1.47	0.73	1.14	0.74	1.47	0.84
Ref	LERM2.0		0.48	0.24	0.46	0.24	0.43	####		1.37	1.41	1.35	1.37	1.22			1.07	1.26	0.70	1.47	0.67
Spill	ERM2.0	0.26	0.36	0.26	0.5	0.21	0.46	####	1.42	1.30	1.54	1.39	1.70	1.28		1.84	0.82	1.32	0.94	1.44	0.71
Spill	ERM0.9	0.22	0.45	0.3	0.51	0.26	0.44	####	1.50	1.29	1.44	1.38	1.57	1.26		1.70	0.60	1.40	0.72	1.33	0.59
Spill	CRM1.5	0.35	0.3	0.25	0.39	0.23	0.46	####	1.42	1.37	1.43	1.41	1.56	1.30		1.75	0.75	1.54	0.76	1.55	0.61

Appendix E

Supplemental Benthic Invertebrate Community

Supplemental Benthic Invertebrate Community

June 2012

Introduction

The Tennessee Valley Authority (TVA) Kingston Fossil Plant (KIF), one of TVA's larger fossil plants, is located at the confluence of the Emory and Clinch Rivers on Watts Bar Reservoir in Roane County, Tennessee. Ash, a by-product of a coal-fired power plant, is stored in unlined containment areas, including a former Dredge Cell. Failure of the Dredge Cell dike released about 5.4 million cubic yards (cy) of coal ash covering approximately 300 acres. Fly ash also entered the channel and overbank areas of the riverine section of the Emory River. While the released fly ash itself is primarily composed of fine silica particles very similar to sand, it also contains trace amounts of arsenic, chromium, copper, lead, mercury, nickel, selenium, thallium, vanadium, zinc, and other metals which occur naturally in the coal.

Evaluations of the spatial extent of ash deposition indicate that ash may have traveled upstream as far as Emory River Mile (ERM) 6.0, and as far downstream as Tennessee River Mile (TRM) 566 (Jacobs 2010). In the upstream direction, the depth of ash appeared to diminish quickly beyond about ERM 3.5. In the downstream direction, ash deposition generally diminishes quickly below about ERM 1.0, with pockets of greater depth occurring in depositional areas in the lower Emory River and Clinch River. Downstream of Clinch River mile (CRM) 2.0, ash deposits generally vary from trace amounts to 2 inches. Ash deposition of 0.5 to 1 inch was observed in the Tennessee River at TRM 566 (south of the Clinch River). Only trace amounts of ash have been observed further downstream (Jacobs 2010).

Dredging efforts in the Emory River began on March 20, 2009 and continued until roughly May 29, 2010. Hydraulic dredging in the river began during an initial dredging pilot program on March 20, 2009. This pilot study continued until July 20, 2009 (during the time-critical removal action). Phase I production dredging began in August 2009 and focused on removing the greatest volume of ash in the quickest time frame to reduce the potential for upstream flooding by clearing the river channel and to minimize downriver migration risk. At the end of the pilot and Phase I dredging, approximately 1.96 million cy of ash had been removed from the river. Phase II dredging began in February 2010 in order to further minimize the potential future ash migration down river. This period of dredging was considered "precision" dredging and was focused on returning the river channel to its original (pre-release) depths while minimizing disturbance of legacy sediment. An estimated 780,000 cy of ash was removed during the Phase II dredging. During dredging operations, turbidity was expected to increase in the immediate area of the dredging. Engineering controls (silt curtains) and operational controls (i.e., reduce cutter head speed, reduce rate of advance, and reverse cutter head rotation) were implemented to minimize suspending solids during the dredging operations.

Benthic invertebrates are found living within or on top of sediments found in the Emory, Clinch, and Tennessee Rivers. These rivers consist of mostly oligochaetes (aquatic worms), chironomids (larval midges), burrowing mayfly nymphs, and also crustaceans (crayfish and amphibods), bivalves (mussels and clams), snails, larval flies, leeches, and mites (Figure 1). Because of their close association with the sediments and water, they have the potential for bioaccumulation of metals and metalloids. They may also transfer these constituents to their fish and wildlife consumers. Benthic invertebrates serve as a useful receptor in order to understand exposure and potential effects of these constituents on the benthic invertebrate community.

The main study objectives were to assess risk to the benthic invertebrate community by comparing community metric results among sites and across 3 years and spatially relating any changes in the community to sediment chemistry or physical properties.

Study Methods

Collections

Benthic invertebrate community evaluations in December 2011 and January 2012 were conducted on the Emory, Clinch, and Tennessee Rivers, similar to previous years. A detailed description of the sampling locations and collection methods can be found in *Evaluation of Benthic Macroinvertebrate Communities in the Vicinity of TVA's Kingston Fossil Plant, 2009-2010* (Baker 2011). Sampling was conducted using a ponar or Peterson grab sampler to collect from the upper 6 inches of sediments along a transect line (Figure 2). The benthic invertebrates were collected once the sample was washed through a screen.

In December 2011 and January 2012, nine transect locations on the Emory River (ERM 6.0, ERM 5.0, ERM 4.1, ERM 3.5, ERM 3.0, ERM 2.6, ERM 2.2, ERM 1.0, and ERM 0.7), six transect locations on the Clinch River (CRM 8.7, CRM 6.0, CRM 4.0, CRM 3.0, CRM 1.5, and CRM 0.5), and two transect locations on the Tennessee River (TRM 573.9 and TRM 566.3) were selected for monitoring. All benthic invertebrate community sampling locations since the ash release are listed below (Exhibit 1).

Exhibit 1. Benthic Invertebrate Community Sampling Sites: 2009-2012

River Mile	January 2009	December 2009	December 2010	December 2011 - January 2012
Sampling Period	**1**	**2**	**3**	**4**
ERM 6.0*	X	X	X	X
ERM 5.0	X	X	X	X
ERM 4.1		X	X	X
ERM 3.5				X
ERM 3.0			X	X
ERM 2.6			X	X
ERM 2.2		X	X	X
ERM 1.0	X	X	X	X
ERM 0.7				X
CRM 8.7*	X	X	X	X
CRM 6.0*	X	X	X	X
CRM 4.0	X	X	X	X
CRM 3.0	X	X	X	X
CRM 1.5	X	X	X	X
CRM 0.5	X	X	X	X
TRM 573.9*	X	X	X	X
TRM 566.3	X	X	X	X

* - Reference Site.

Ten grab samples were collected from each transect, and benthic invertebrates within each sample were identified to the lowest possible taxon. The total number of taxa were tallied and used to generate benthic invertebrate community metrics in order to assess the overall health of the benthic invertebrate community. Population density, taxa richness, number of organisms, number of taxa, percent oligochaetes and chironomids, and many other types of metrics were used to assess the community spatially and temporally. Details on how these metrics are calculated are presented in Baker (2011).

At each sample location, water depth was also recorded along with a physical description of the sediment in the sample in order to estimate: percent ash, grain size, and substrate type. In addition to the benthic invertebrate community data collections, sediment chemistry data (percent ash, metals, total organic carbon, and the percent sand, silt, clay or gravel) was also collected in January 2012 from the Emory River. The purpose of the co-located data collections was to better interpret the various factors potentially influencing the benthic invertebrate community.

Assessment Methods

Benthic invertebrate community data were described using summary statistics and analyzed using standard multi-metric procedures, as described in Baker (2011). In addition to the community assessment, the 2012 Emory River benthic invertebrate community metrics were also compared with co-located sediment data. The physical and chemical data were normalized to adjust for differences in scale, and were compared for similarity between locations using the Euclidian distance resemblance matrix. The benthic invertebrate community data were log transformed and compared for similarity between locations using the Bray Curtis similarity resemblance matrix. The physical/chemical and benthic invertebrate community resemblance matrices were compared to each other using Spearman rank correlation techniques to determine the variable, or set of variables, that were correlated most to the benthic invertebrate community.

Results and Discussion

The fourth period of collections began in December 2011 and finished in January 2012 for the Emory, Clinch, and Tennessee Rivers. Results are consistent with previous years as the benthic invertebrate metrics do not suggest any adverse impacts from the ash release (Tables 1 and 2). Species composition was similar with slightly greater oligochaetes and bivalves in the Emory River fourth period compared to the third. While it was hypothesized that over time, reductions of benthic invertebrate community metrics (e.g., density and richness) may occur due to impacts from the ash release, the results from the fourth period sampling show the opposite. Community density metrics were similar to previous years or much higher (Figures 3 through 13). Average taxa richness only decreased in the reference locations for the Clinch River (Figure 4), and the average chironomid abundance decreased during the fourth sampling period compared with the third for all sites, including reference locations (Figure 5).

Overall, the results of the community analysis from the Clinch and Tennessee River are consistent with previous years and do not suggest any adverse impacts from the ash release. The 2012 Emory River benthic invertebrate community data are generally consistent with the 2010 Emory River benthic invertebrate community data (habitat, composition, diversity, abundance, metrics), as described below.

- Habitat – 2010 and 2012 substrate characterization were similar (i.e., percent sand, silt, and clay).

- Composition, Diversity, Abundance – 2010 and 2012 mean percent benthic invertebrate abundance and diversity metrics were similar (Table 1).
- Metrics – 2010 and 2012 benthic invertebrate community abundance, diversity, tolerance, composition, feeding guild, and organism habit metrics (a total of ten metrics) were similar.

Some temporal differences between 2010 and 2012 community metrics were noted; however, these differences appear to be more related to temporal variation than to potential site-related impacts as differences in 2012 were also generally noted in the upstream reference transect.

A similarity analysis on co-located data indicated that differences in the 2012 Emory River benthic invertebrate community data (taxa and counts) were correlated more to substrate type and water depth, similar to 2010, than percent ash composition or concentrations of metals and metalloids in the sediment. Analytical results, grain size, and water depth for sediment samples can be found in Tables 3 and 4.

Comparison between sediment data (physical and chemical) and benthic invertebrate community resemblance matrices using Spearman rank correlation showed some agreement in multivariate pattern. The highest correlation was rho=0.508 for boron, water depth, gravel, medium sand, and fine sand. The best fit for a single variable was for water depth (rho=0.290), then medium sand (rho=0.263), then silt (rho=0.252), then fine sand (rho=0.250). Boron and percent ash were weekly correlated at rho=0.113 and 0.129, respectively.

Some negative relationships with metal concentrations may exist, although, these were few and appeared to be negligible. The linear relationship between metals concentrations (26) and benthic diversity (number of taxa) and abundance (number of organisms) was generally positive for all metals with the exception of arsenic and boron. Note: three sample locations tend to skew the results – ERM 3.5-03, ERM 2.6-08, ERM 2.2-10. The ponar was only 15 percent and 40 percent full from samples ERM 3.5-03 and ERM 2.6-08, respectively, which provides an explanation for the low diversity and abundance; however, the ponar was 70 percent full from ERM 2.2-10 and was not considered low recovery. Additionally, all three samples have comparatively low benthic diversity and abundance but high metals concentrations and percent ash.

Conclusions

Differences across four periods of benthic invertebrate community surveys were more related to habitat (mostly substrate types and water depth) than to proximity to the ash release. Community composition, richness, density, and abundance of benthic invertebrates were similar across all years of post-release data with some metrics increasing in 2012 relative to previous years.

While one sample out of ten from transects at ERM 3.5, 2.6, and 2.2 in 2012 had lower diversity and abundance when compared spatially, and metal concentrations were higher in those samples than other sites, these results do not change the overall interpretation that the benthic invertebrate community lacks evidence of being adversely impacted by habitat changes and the presence of residual ash. Continuation of monitoring the benthic invertebrate community and co-located sediment data on the Emory River will help determine if impacts from the ash release occur over time.

References

Baker, T.F. 2011. Evaluation of Benthic Macroinvertebrate Communities in the Vicinity of TVA's Kingston Fossil Plant, 2009 – 2010. November. (BERA Appendix M).

Jacobs. 2010. Kingston Ash Recovery Project Non-Time-Critical Removal Action for the River System Sampling and Analysis Plan (SAP). Document No. EPA-AO-021. May 2010.

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Table 1. Benthic Invertebrate Community Results for the Emory River: 2009 – 2012

Tennessee Valley Authority **Kingston, Tennessee**

	River	**Emory River**																							
	Transect	**ERM 6.0**				**ERM 5.0**				**ERM 4.1**			**ERM 3.5**	**ERM 3.0**		**ERM 2.6**		**ERM 2.2**			**ERM 1.0**				**ERM 0.7**
	Sample Period	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**2**	**3**	**4**	**4**	**3**	**4**	**3**	**4**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**4**
	Number of Samples	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**20**	**10**	**20**	**10**	**10**	**20**	**10**	**10**	**10**	**20**	**10**	**10**
Average Abundance (# / m^2)	Population	752	737	452	707	587	733	348	1275	988	2140	1300	1050	2433	2258	2093	2157	1315	2073	1823	967	1108	1577	2832	2648
	Oligochaetes	273	110	48	402	344	43	58	905	255	507	592	333	1108	1407	628	743	260	543	1190	373	465	689	1025	847
	Chironomids	375	415	227	247	84	383	213	245	552	1105	422	587	1056	697	1219	948	378	1026	508	320	318	718	1230	1347
	Hexagenia	9	12	5	3	7	35	8	8	10	35	37	50	20	13	55	55	168	126	23	35	115	39	168	193
	Sphaeriidae	0	0	0	0	30	0	0	3	5	0	22	2	94	40	53	255	360	104	42	163	128	64	242	145
Average Compostion	% Oligochaetes	34.8	14.8	18.8	54.7	46.1	6.7	13.5	50.3	23.1	26.4	41.1	33.9	41.4	60.6	22.7	32.5	12.1	14.1	45.9	33.0	46.7	35.8	36.1	34.9
	% Chironomids	50.2	57.5	47.7	34.9	16.9	57.2	55.9	25.4	56.5	47.0	36.2	47.0	46.7	31.3	56.1	42.5	27.5	50.1	42.6	37.1	27.9	48.9	40.9	47.5
Taxa Richness	Total Richness	26	23	23	28	23	22	27	52	45	41	57	51	47	43	39.5	41	47	40.5	48	23	24	39.5	55	43
	Average Richness	8.6	7.7	5.9	8.0	6.4	7.7	5.4	11.2	11.5	11.3	12.3	11.4	13.5	12.8	13.9	13.9	11.0	12.5	13.4	9.0	9.2	11.2	17.6	15.1
	Total EPT Richness	2	2	3	1	3	2	3	6	6	5	5	5	3	2	3	1	4	2.5	3	1	3	3.5	3	2
	Average EPT Richness	0.5	0.5	0.4	0.2	0.6	0.6	0.5	0.7	1.0	1.1	0.8	0.8	0.7	0.3	1.0	0.7	1.5	1.0	0.6	0.6	0.9	0.9	1.1	0.6

Note: Sample periods are defined as follows: 1- January 2009; 2- December 2009; 3- December 2010; 4- December 2011- January 2012.

#/m^2 - Number per square meter.
% - Percent.
EPT - Ephemeroptera, Plecoptera, and Trichoptera.
ERM - Emory River Mile.

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Table 2. Benthic Invertebrate Community Results for the Clinch and Tennessee Rivers: 2009 – 2012

Tennessee Valley Authority **Kingston, Tennessee**

	River	Clinch River																							
	Transect	**CRM 8.7**				**CRM 6.0**				**CRM 4.0**				**CRM 3.0**				**CRM 1.5**				**CRM 0.5**			
	Sample Period	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
	Number of Samples	**10**	**10**	**10**	**10**	**10**	**10**	**20**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**7**	**9**	**10**	**10**	**10**	**10**
Average Abundance (# / m^2)	Population	3448	582	1857	2542	3013	972	2478	1603	1652	1300	1643	2638	1587	947	1207	1320	1380	1230	981	1826	1153	1328	1297	2118
	Oligochaetes	2738	92	1025	613	1838	345	1005	242	550	57	527	417	190	70	160	303	150	333	217	526	302	362	228	373
	Chironomids	307	85	602	267	790	238	1040	487	393	325	702	695	575	318	573	370	420	380	488	469	388	425	517	542
	Hexagenia	147	0	20	10	145	88	91	445	150	278	188	470	345	140	245	402	292	252	176	404	147	225	280	732
	Sphaeriidae	30	2	7	8	90	30	22	3	477	352	143	195	388	392	150	103	402	188	10	215	187	247	183	357
Average Compostion	% Oligochaetes	74.2	15.7	54.2	30.7	62.4	30.3	37.7	21.6	46.7	7.0	28.7	18.3	9.2	11.7	11.9	26.0	15.5	25.5	14.8	27.8	26.0	26.6	17.3	19.2
	% Chironomids	9.1	19.0	33.2	20.8	24.7	17.8	42.3	28.7	22.7	29.9	46.3	26.4	35.1	32.6	45.8	28.1	30.9	32.6	43.0	25.8	31.9	30.2	36.0	26.1
Taxa Richness*	Total Richness	34	25	43	46	36	34	48	47	35	21	53	56	25	19	27	41	17	20	26	41	34	24	31	36
	Average Richness	12.9	6.6	13.3	11.2	15.1	8.8	16.9	14.8	10.2	8.7	16.4	16.9	9.9	8.6	11.2	14.2	10.0	10.1	9.6	14.8	11.5	10.1	12.5	15.1
	Total EPT Richness	3	4	4	7	2	4	4.5	2	2	2	4	7	2	1	2	4	2	1	2	3	5	1	3	1
	Average EPT Richness	1.1	0.7	0.7	1.0	1.1	0.9	1.2	1.0	0.7	1.0	1.2	1.8	1.1	1.0	1.2	1.4	1.1	1.0	0.7	1.0	1.7	1.0	1.2	1.0

** In December 2010, replicate transects were sampled at selected locations (i.e., 20 samples). For these transects, Total Richness and Total EPT Richness were calculated for each replicate and then averaged.*

	River	Tennessee River											
	Transect	**TRM 573.9**				**TRM 566.3**				**TRM 560.8**			
	Sample Period	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
	Number of Samples	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Average Abundance (# / m^2)	Population	1975	1285	1835	1772	1998	1553	1315	2060	828	897	930	1140
	Oligochaetes	1120	173	95	195	1052	718	207	405	55	50	58	68
	Chironomids	460	263	467	352	550	498	602	672	650	400	435	677
	Hexagenia	68	262	375	383	105	142	223	443	30	98	58	112
	Sphaeriidae	163	427	827	632	82	137	152	408	60	323	322	248
Average Compostion	%Oligochaets	55.6	15.7	8.7	12.2	50.8	37.6	15.7	23.1	6.5	5.9	6.6	6.8
	%Chironomids	24.1	22.8	32.5	19.9	29.2	36.4	48.8	31.2	79.0	53.3	47.7	57.8
Taxa Richness	Total Richness	31	31	24	25	35	20	26	34	15	14	18	21
	Average Richness	13.2	10.0	10.6	10.0	12.1	9.1	11.0	13.2	6.8	6.3	8.2	10.0
	Total EPT Richness	2	3	3	2	4	1	2	1	3	3	3	1
	Average EPT Richness	1.0	1.1	1.4	1.1	1.1	0.8	1.1	1.0	0.9	0.7	1.2	0.9

Note: Sample periods are defined as follows: 1- January 2009; 2- December 2009; 3- December 2010; 4- December 2011- January 2012.

#/m^2 - Number per square meter.

% - Percent.

CRM - Clinch River Mile.

EPT - Ephemeroptera, Plecoptera, and Trichoptera.

TRM - Tennessee River Mile.

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Table 3. 2012 Emory River Sediment Analytical Results (mg/kg)
Tennessee Valley Authority Kingston, Tennessee

Sample ID	Aluminum	Antimony	Arsenic	Barium	Beryllium	Boron	Cadmium	Calcium	Chromium
ERM 0.7-02	13500	2.21	22.9	196	1.81	12.7	0.53	3020	20.6
ERM 0.7-05	4890	1.36	11.2	70.2	0.681	5.72	0.136	1390	7.63
ERM 0.7-06	6200	1.47	16.3	130	1.06	11.9	0.147	2450	9.84
ERM 0.7-09	14500	1.91	23.3	240	2.26	16.1	0.306	3640	21.4
ERM 0.7-10	10800	1.67	16	144	1.37	7.48	0.167	3260	17.5
ERM 1.0-01	10400	1.49	26.3	211	1.94	14.6	0.179	3050	19.6
ERM 1.0-03	12400	2.09	19.9	195	1.71	12	0.209	3290	17.9
ERM 1.0-05	6970	1.89	5.99	75.1	0.872	7.58	0.265	1780	9.55
ERM 1.0-06	3550	1.31	9.67	59.4	0.524	5.24	0.131	1200	5.45
ERM 1.0-08	11300	1.68	19.2	216	1.74	13.5	0.168	3180	18
ERM 1.0-10	17900	2.01	22.7	225	2.33	15.2	0.281	3280	26.5
ERM 2.2-01	7330	1.5	8.27	80.6	0.6	6	0.15	1020	9.29
ERM 2.2-02	10200	1.85	24.1	161	1.55	11.2	0.259	2410	15.5
ERM 2.2-08	8190	2.17	17.1	159	1.17	9.6	0.217	2590	10.7
ERM 2.2-09	2520	1.35	20.4	72.4	0.539	5.39	0.135	1070	4.23
ERM 2.2-10	15700	1.28	94	378	3.89	50	0.384	6020	26.2
ERM 2.6-01	15900	2.36	12.9	143	1.56	9.46	0.236	2020	19.3
ERM 2.6-03	9000	1.89	10.3	97.3	1.13	7.54	0.189	1720	12.1
ERM 2.6-05	5090	1.54	6.28	57.2	0.646	6.16	0.154	900	6.49
ERM 2.6-08	15900	1.51	111	329	2.92	46.9	0.349	3650	32.7
ERM 2.6-09	10500	2.24	17.6	149	1.39	8.96	0.538	2380	14.6
ERM 2.6-10	11100	1.62	56.6	194	1.75	12	0.389	2870	17.7
ERM 3.0-02	3730	1.35	12.3	47	0.542	5.42	0.135	635	6.47
ERM 3.0-04	6920	2.25	11.2	112	1.03	9	0.225	3070	8.41
ERM 3.0-07	7500	2.26	10.8	105	1.27	9.04	0.226	2250	10.5
ERM 3.0-08	10200	2.7	18.2	129	1.46	10.8	0.324	2170	14.5
ERM 3.0-10	6260	1.65	19.3	103	1.02	6.59	0.165	1460	10.7
ERM 3.5-01	4120	1.28	3.27	17.8	0.511	5.11	0.128	313	4.7
ERM 3.5-03	9230	1.49	74	291	2.36	28.8	0.174	4100	22.1
ERM 3.5-05	894	1.31	2.79	10.5	0.522	5.22	0.131	147	1.91
ERM 3.5-07	1010	1.33	1.41	11.9	0.531	5.31	0.133	188	1.73
ERM 3.5-09	1830	1.48	2.38	23.6	0.594	5.94	0.148	468	2.76
ERM 3.5-10	3300	1.37	2.85	35.9	0.549	5.49	0.137	451	5.19
ERM 4.1-02	5860	1.86	6.88	70	0.781	7.44	0.186	1090	7.81
ERM 4.1-05	4370	1.7	4.37	60.1	0.678	6.78	0.17	1260	5.32
ERM 4.1-06	1330	1.23	1.23	15.5	0.491	4.91	0.123	210	2.23
ERM 4.1-08	620	1.31	1.31	7.12	0.524	5.24	0.131	131	1.52
ERM 4.1-10	4040	1.3	3.28	34.8	0.521	5.21	0.13	445	5.52
ERM 5.0-02	6560	2.13	5.14	85.3	0.893	8.5	0.255	1310	7.74
ERM 5.0-03	15700	1.82	78.7	264	2.65	29.7	0.4	3310	30.2
ERM 5.0-05	407	1.3	1.3	5.62	0.521	5.21	0.13	130	0.755
ERM 5.0-07	665	1.29	1.72	7.18	0.514	5.14	0.129	134	1.88
ERM 5.0-09	2990	1.42	1.65	25.7	0.57	5.7	0.142	386	4.61
ERM 5.0-10	2520	1.38	2.02	25.3	0.552	5.52	0.58	274	3.59
ERM 6.0-01	1850	1.48	1.48	21.6	0.592	5.92	0.148	336	2.84
ERM 6.0-02	2370	1.41	2.49	34	0.565	5.65	0.141	572	3.11
ERM 6.0-03	5990	2.05	4.09	77.8	0.9	8.18	0.205	1120	7.41
ERM 6.0-04	2010	1.27	1.45	29	0.508	5.08	0.128	461	3.64
ERM 6.0-05	4810	1.64	3.65	70.5	0.69	6.58	0.164	944	5.42
ERM 6.0-06	721	1.26	1.26	8.55	0.506	5.06	0.126	126	1.67
ERM 6.0-07	1640	1.34	2.23	21.1	0.537	5.37	0.134	398	3.06
ERM 6.0-08	8140	2.49	6.12	105	1.24	9.95	0.249	2050	9.2
ERM 6.0-09	6400	1.91	4.2	67.3	0.917	7.64	0.267	2910	6.92
ERM 6.0-10	7260	1.8	3.57	47.9	0.721	7.21	0.18	1410	14.4

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Table 3. 2012 Emory River Sediment Analytical Results (mg/kg)
Tennessee Valley Authority Kingston, Tennessee

Sample ID	Cobalt	Copper	Iron	Lead	Magnesium	Manganese	Mercury	Molybdenum	Nickel
ERM 0.7-02	16.1	24.5	23000	17.1	1400	577	0.13	8.83	26.9
ERM 0.7-05	6.59	9.29	11400	6.05	604	256	0.045	5.45	11
ERM 0.7-06	7.78	15.4	12600	8.37	747	279	0.052	5.87	13.5
ERM 0.7-09	15.8	31.1	19900	17.3	1480	352	0.14	7.66	26.6
ERM 0.7-10	8.75	19.9	16500	12.1	1020	316	0.11	6.68	14.7
ERM 1.0-01	12.8	27.5	17800	14.9	1290	560	0.12	5.96	20.6
ERM 1.0-03	15.3	22.7	22700	18.5	1350	584	0.086	8.34	25.5
ERM 1.0-05	12.3	17.5	14900	13.8	1030	414	0.49	7.58	17.3
ERM 1.0-06	5.19	6.89	7970	5.27	496	231	0.043	5.24	7.68
ERM 1.0-08	12.5	23.2	21000	11.6	1210	260	0.1	6.71	22.7
ERM 1.0-10	17.8	38.9	24400	21.4	1780	506	0.25	8.04	31.4
ERM 2.2-01	8.45	7.59	16400	9.71	655	429	0.049	6	9.59
ERM 2.2-02	13	21.3	18400	14.3	1040	484	0.063	7.39	21.5
ERM 2.2-08	10.9	19	14800	12.6	916	384	0.071	8.69	17.9
ERM 2.2-09	1.97	5.87	6370	4.01	401	94.3	0.044	5.39	4.04
ERM 2.2-10	13.7	56.3	10400	27.4	1190	66.9	0.12	5.11	29.2
ERM 2.6-01	19	22.7	23800	20.9	1590	429	0.13	9.46	30.4
ERM 2.6-03	12.1	15.5	15700	13.1	898	276	0.07	7.54	19.3
ERM 2.6-05	7.26	7.48	10000	8.25	495	221	0.052	6.16	11.4
ERM 2.6-08	13	48.8	17200	22.8	1260	70	0.11	4.99	28.8
ERM 2.6-09	14.7	15.9	18800	15.4	1110	453	0.072	8.96	22.3
ERM 2.6-10	10.7	25.4	16000	15.1	1110	359	0.092	6.48	17.4
ERM 3.0-02	5.09	5.63	8310	5.31	407	182	0.044	5.42	7.77
ERM 3.0-04	11.7	12.5	11900	10.8	733	457	0.076	9	17.7
ERM 3.0-07	14	13.8	15900	14.6	837	451	0.1	9.04	20.2
ERM 3.0-08	15.3	18.3	19000	17.3	994	412	0.1	10.8	24
ERM 3.0-10	8.14	19	11100	10.5	588	284	0.077	6.59	13.5
ERM 3.5-01	4.65	3.43	10700	5.09	417	239	0.042	5.11	4.65
ERM 3.5-03	10.6	34.3	16600	13.9	836	73.8	0.045	4.97	22.3
ERM 3.5-05	1.91	1.41	2880	1.75	131	31.3	0.043	5.22	3
ERM 3.5-07	2.07	1.33	3000	1.99	134	84.1	0.042	5.31	3.03
ERM 3.5-09	3.53	3.15	4980	4.25	204	113	0.047	5.94	5.52
ERM 3.5-10	4.92	5.16	8370	5.99	307	184	0.046	5.49	7.91
ERM 4.1-02	9.6	9.6	13800	11	623	351	0.063	7.44	14
ERM 4.1-05	9.05	6.78	10700	8.68	531	428	0.053	6.78	12.6
ERM 4.1-06	2.33	1.23	3910	2.63	160	85.2	0.041	4.91	3.32
ERM 4.1-08	1.52	1.31	2070	1.57	131	23.1	0.042	5.24	2.28
ERM 4.1-10	4.45	4.04	12200	5.57	354	344	0.043	5.21	5.65
ERM 5.0-02	14.2	10.2	14600	12.5	724	440	0.072	8.5	19.9
ERM 5.0-03	16.6	45.3	17900	25.4	1260	336	0.12	7.27	30.2
ERM 5.0-05	1.3	1.3	1430	1.33	130	16.7	0.042	5.21	1.74
ERM 5.0-07	1.59	1.29	3070	3.83	129	27.1	0.042	5.14	2.44
ERM 5.0-09	3.28	2.73	4950	3.87	269	164	0.046	5.7	5.15
ERM 5.0-10	3.54	2.76	5870	4.2	320	62.6	0.044	5.52	6.24
ERM 6.0-01	3.88	2.61	4840	3.88	236	111	0.048	5.92	5.77
ERM 6.0-02	5.03	3.14	6720	5	259	255	0.045	5.65	6.7
ERM 6.0-03	12.4	8.84	15000	11.6	639	407	0.067	8.18	17
ERM 6.0-04	4.34	3.88	6120	5.22	254	142	0.041	5.08	6.53
ERM 6.0-05	10.1	6.51	11600	9.57	499	465	0.054	6.58	12.5
ERM 6.0-06	1.77	1.26	2430	1.87	126	25.1	0.042	5.06	2.83
ERM 6.0-07	4.21	2.95	4620	4.05	193	73	0.043	5.37	5.9
ERM 6.0-08	16.4	12.6	18300	16.7	860	535	0.095	9.95	23.7
ERM 6.0-09	11.9	8.64	12900	11.8	704	192	0.063	7.64	18
ERM 6.0-10	9.48	7.46	25100	9.37	413	301	0.059	7.21	8.86

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Table 3. 2012 Emory River Sediment Analytical Results (mg/kg)
Tennessee Valley Authority Kingston, Tennessee

Sample ID	Potassium	Selenium	Silver	Sodium	Strontium	Thallium	Vanadium	Zinc
ERM 0.7-02	1230	2.91	5.08	221	101	2.21	39.7	74
ERM 0.7-05	488	1.39	0.681	136	29.3	1.36	12.8	26.3
ERM 0.7-06	614	2.61	0.734	147	64.2	1.47	20.2	42.2
ERM 0.7-09	1380	3.25	0.957	191	144	1.91	47.2	62.5
ERM 0.7-10	820	2.61	0.835	167	75.4	1.67	31.5	66.6
ERM 1.0-01	1090	3.25	0.745	149	119	1.49	41.6	47.3
ERM 1.0-03	1130	3.63	1.04	209	94.5	2.09	35.3	70
ERM 1.0-05	425	1.89	0.947	189	13.2	1.89	13.2	79.4
ERM 1.0-06	319	1.62	0.655	131	21	1.31	10.2	19.5
ERM 1.0-08	1110	3.42	0.839	168	126	1.68	37.7	42.4
ERM 1.0-10	1810	3.86	1	201	118	2.01	51.6	82.9
ERM 2.2-01	490	1.5	0.75	150	9.89	1.5	16.7	28.9
ERM 2.2-02	908	3.14	0.924	185	70.9	1.85	31.6	54.2
ERM 2.2-08	749	2.87	1.09	217	47.9	2.17	21.6	50.2
ERM 2.2-09	196	2.48	0.673	135	32.5	1.35	14.4	7.14
ERM 2.2-10	1910	6.44	0.639	283	358	1.28	91.5	44.7
ERM 2.6-01	1480	3.26	1.18	236	35.4	2.36	33.4	87.9
ERM 2.6-03	738	1.89	0.943	189	33.3	1.89	22.3	56.8
ERM 2.6-05	435	1.79	0.77	154	14	1.54	11.4	33.8
ERM 2.6-08	2460	5.67	0.706	264	190	1.25	73.5	42.9
ERM 2.6-09	878	3.14	4.21	224	61.3	2.24	28.1	68
ERM 2.6-10	1090	3.14	3.57	162	132	1.62	43.5	47.5
ERM 3.0-02	357	1.35	0.677	135	13.3	1.35	9.43	23.8
ERM 3.0-04	584	2.25	1.12	225	26.1	2.25	15.4	57.4
ERM 3.0-07	558	2.26	1.13	226	26.4	2.26	18.1	65.7
ERM 3.0-08	798	2.86	1.35	270	37	2.7	25.5	77.6
ERM 3.0-10	561	1.91	0.824	165	48.3	1.65	21.9	36.7
ERM 3.5-01	295	1.28	0.639	128	5.11	1.28	8.23	13.7
ERM 3.5-03	985	3.06	0.622	154	212	1.24	62	30.3
ERM 3.5-05	131	1.31	0.653	131	5.22	1.31	2.61	9.66
ERM 3.5-07	133	1.33	0.664	133	5.31	1.33	2.65	9.96
ERM 3.5-09	158	1.48	0.742	148	5.94	1.48	4.28	17.3
ERM 3.5-10	273	1.37	0.686	137	5.49	1.37	7.36	25.3
ERM 4.1-02	513	1.86	0.93	186	9.49	1.86	13.5	47.5
ERM 4.1-05	394	1.7	0.848	170	7.19	1.7	8.48	41.6
ERM 4.1-06	132	1.23	0.614	123	4.91	1.23	2.9	10.7
ERM 4.1-08	131	1.31	0.655	131	5.24	1.31	2.62	7.41
ERM 4.1-10	321	1.3	0.651	130	5.21	1.3	8.8	16
ERM 5.0-02	511	2.13	1.06	213	8.5	2.13	12.8	65.8
ERM 5.0-03	1810	6.11	0.909	215	160	1.82	68.3	64
ERM 5.0-05	130	1.3	0.651	130	5.21	1.3	2.6	6.64
ERM 5.0-07	129	1.29	0.643	129	5.14	1.29	2.57	9.18
ERM 5.0-09	272	1.42	0.712	142	5.7	1.42	4.9	22.2
ERM 5.0-10	240	1.38	0.691	138	5.52	1.38	4.31	21.2
ERM 6.0-01	175	1.48	0.74	148	5.92	1.48	3.44	21.1
ERM 6.0-02	189	1.41	0.707	141	5.65	1.41	4.72	20.7
ERM 6.0-03	440	2.05	1.02	205	8.18	2.05	11.5	56.6
ERM 6.0-04	206	1.27	0.635	127	5.08	1.27	3.73	20.9
ERM 6.0-05	362	1.64	0.822	164	6.58	1.64	8.68	43.3
ERM 6.0-06	126	1.26	0.632	126	5.06	1.26	2.53	9.2
ERM 6.0-07	178	1.34	0.671	134	5.37	1.34	4.4	17.3
ERM 6.0-08	629	2.49	1.24	249	9.95	2.49	16.1	78.7
ERM 6.0-09	471	1.91	0.955	191	7.64	1.91	11.5	65.2
ERM 6.0-10	607	1.8	0.901	180	7.21	1.8	20.9	25.4

ERM - Emory River Mile.
mg/kg - Milligrams per kilogram.

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Table 4. 2012 Emory River Sediment Grain Size and Water Depth Results
Tennessee Valley Authority Kingston, Tennessee

Sample ID	Percent Ash	Clay	Fraction of Organic Carbon	Gravel	Sand	Sand, Coarse
ERM 0.7-02	20	14.1	2.5	0	5.7	0.1
ERM 0.7-05	8	3.1	0.6	0.1	72	0.2
ERM 0.7-06	23	4.5	1.1	0.2	56.5	1
ERM 0.7-09	22	14	2.1	0.2	3.2	0.1
ERM 0.7-10	19	17.9	0.9	0.1	18.8	0.3
ERM 1.0-01	22	12.3	0.8	1.2	22.9	5.3
ERM 1.0-03	12	11.7	2.9	0.2	13.2	0.4
ERM 1.0-05	6	13.9	2.5	0.1	23.3	0.1
ERM 1.0-06	9	2.2	0.7	0.1	85.1	0.4
ERM 1.0-08	26	9	1.7	0.1	19.8	0.1
ERM 1.0-10	24	12.3	1.7	0	2.4	0.2
ERM 2.2-01	3	21.1	0.9	0.3	27.9	0.3
ERM 2.2-02	21	15.2	3.9	0.6	20.5	2.1
ERM 2.2-08	18	7.1	3.8	1.4	44.7	3.2
ERM 2.2-09	9	3.2	2.9	23	64.8	23.2
ERM 2.2-10	62	19.9	0.5	0	4	0
ERM 2.6-01	7	16.6	1.8	0	8.1	0
ERM 2.6-03	10	13.8	2.4	0	19.2	0.1
ERM 2.6-05	3	7.5	1.3	0.1	53.9	0.2
ERM 2.6-08	66	17.7	1.5	0	4.5	0
ERM 2.6-09	9	12.3	3.20	0	9.5	0.1
ERM 2.6-10	37	16.3	0.8	0.3	15	0.1
ERM 3.0-02	1	1.9	0.5	0	96.9	0.1
ERM 3.0-04	3	2.2	23.8	0	91.1	6.3
ERM 3.0-07	7	12.6	4.2	0	21.1	0.2
ERM 3.0-08	25	18.3	3.9	0	4.5	0
ERM 3.0-10	13	10.9	1.7	0	34.6	0.6
ERM 3.5-01	1	10.9	0.7	0	62.2	0
ERM 3.5-03	62	14.5	1	1.2	24.2	0.9
ERM 3.5-05	1	0.2	0.5	0	99	0
ERM 3.5-07	1	0.8	0.5	0	97.4	0
ERM 3.5-09	1	2	0.5	0	94.2	0
ERM 3.5-10	1	4.1	0.9	0	87.6	0
ERM 4.1-02	8	16.7	2.2	0	30	0
ERM 4.1-05	1	7.3	2.5	0	61.3	0
ERM 4.1-06	1	2.6	0.5	0	94.2	0
ERM 4.1-08	1	0.7	0.5	0.1	97.9	0.3
ERM 4.1-10	1	11.9	0.5	0	73.6	0
ERM 5.0-02	1	15.5	3.3	0.2	52	0.3
ERM 5.0-03	34	33.6	2.5	0	5.5	0
ERM 5.0-05	1	0.7	0.5	1.1	97.5	1.2
ERM 5.0-07	1	0.7	0.5	0	98.6	0.2
ERM 5.0-09	1	2.5	0.5	0	94.2	0
ERM 5.0-10	1	2	0.5	0	96.9	0
ERM 6.0-01	1	2.5	0.9	0	92.5	0
ERM 6.0-02	1	5.2	1	0	82.5	0
ERM 6.0-03	1	11.5	2.2	0	53.1	0
ERM 6.0-04	1	0.9	0.5	0	97.3	0
ERM 6.0-05	1	6.2	1.3	0	82.3	0.1
ERM 6.0-06	1	0.8	0.5	0	98.3	0
ERM 6.0-07	1	1.9	1.2	1.9	94.5	2.2
ERM 6.0-08	1	11.2	2	0	75.3	0
ERM 6.0-09	1	10.4	3.5	0.3	62.6	2.4
ERM 6.0-10	1	39.1	0.9	0	36.5	0.2

ERM - Emory River mile.
ft - Feet.
PLM - Polarized Light Microscopy.

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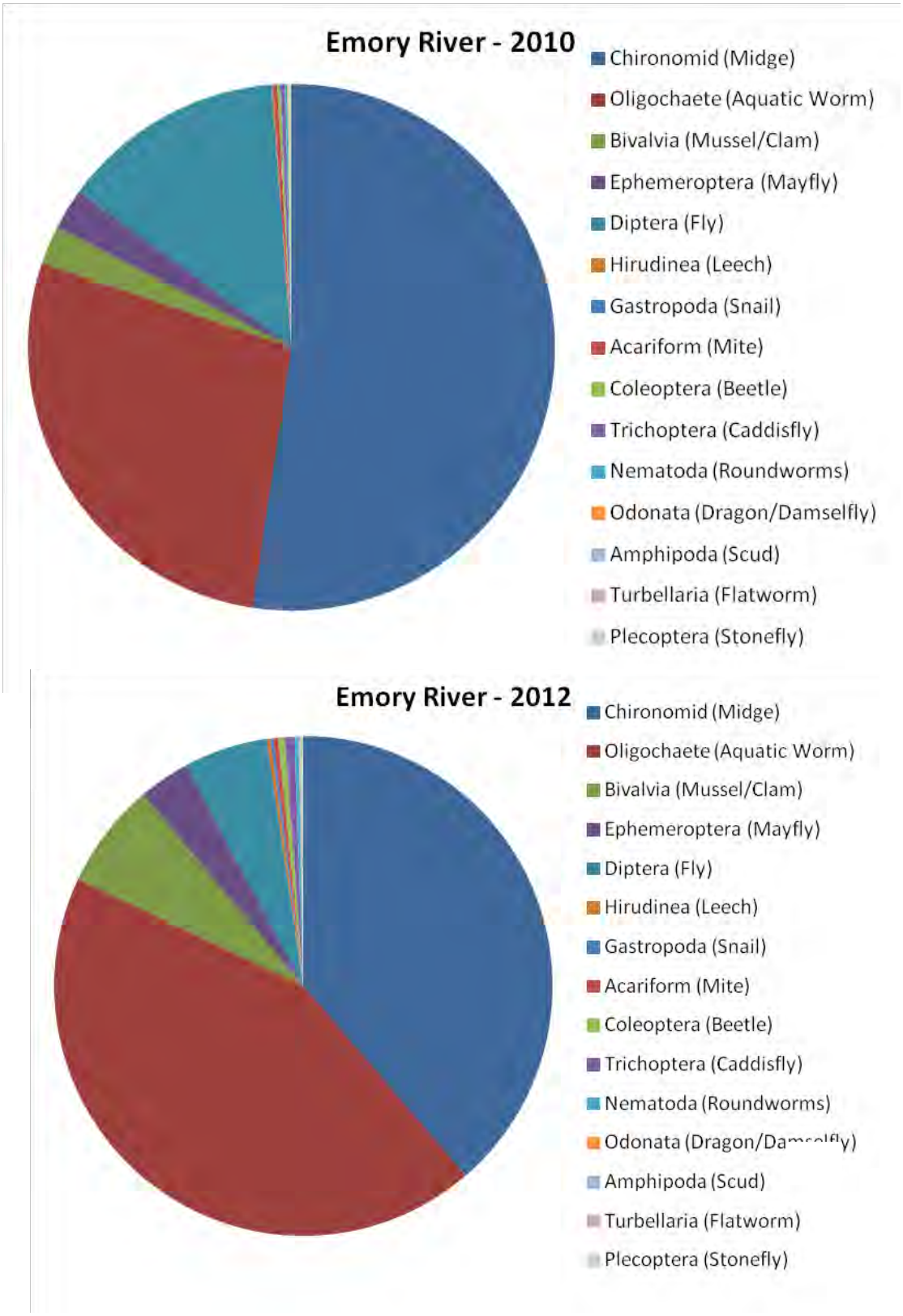
Table 4. 2012 Emory River Sediment Grain Size and Water Depth Results
Tennessee Valley Authority Kingston, Tennessee

Sample ID	Sand, Fine	Sand, Medium	Silt	Total Organic Carbon	Water Depth (ft)
ERM 0.7-02	5.5	0.1	80.2	22000	11.4
ERM 0.7-05	71.2	0.6	24.8	5800	8.8
ERM 0.7-06	53.4	2.1	38.8	10000	4.1
ERM 0.7-09	3	0.1	82.6	20000	2.1
ERM 0.7-10	17.1	1.4	63.2	7400	3
ERM 1.0-01	13.8	3.8	63.6	9600	11
ERM 1.0-03	12.8	0	74.9	27000	14
ERM 1.0-05	23	0.2	62.7	24000	22.5
ERM 1.0-06	83.4	1.3	12.6	2300	17.6
ERM 1.0-08	19.6	0.1	71.2	20000	12.1
ERM 1.0-10	2.2	0	85.3	13000	9.6
ERM 2.2-01	26.7	0.9	50.7	6100	3
ERM 2.2-02	16.7	1.7	63.7	22000	4.7
ERM 2.2-08	40.8	0.7	46.8	28000	10.5
ERM 2.2-09	15.9	25.7	9	1900	15.1
ERM 2.2-10	4	0	76.1	2900	19.4
ERM 2.6-01	8	0.1	75.3	29000	6.7
ERM 2.6-03	18.8	0.3	67	26000	7.1
ERM 2.6-05	53.3	0.4	38.5	12000	4
ERM 2.6-08	4.4	0.1	78.5	2200	29.9
ERM 2.6-09	9.2	0.2	78.2	17000	27.3
ERM 2.6-10	14.6	0.3	68.4	6200	7.7
ERM 3.0-02	90.4	6.4	1.2	2600	3.4
ERM 3.0-04	81.7	3.1	6.7	120000	26
ERM 3.0-07	20.5	0.4	66.3	30000	2.4
ERM 3.0-08	4.2	0.3	77.2	21000	4.8
ERM 3.0-10	33.8	0.2	54.5	12000	4.8
ERM 3.5-01	62	0.2	26.9	1400	5.7
ERM 3.5-03	18.6	4.7	60.1	2800	30.4
ERM 3.5-05	98.9	0.1	0.8	1600	23.5
ERM 3.5-07	97.2	0.2	1.8	1400	10.8
ERM 3.5-09	94	0.2	3.8	5000	2.3
ERM 3.5-10	84.7	2.9	8.7	7300	2.1
ERM 4.1-02	29.6	0.4	53.3	23000	5
ERM 4.1-05	60.3	1	31.4	19000	14
ERM 4.1-06	94	0.2	3.2	3000	16.5
ERM 4.1-08	96.1	1.5	1.2	1400	27.5
ERM 4.1-10	72.4	1.2	14.5	2100	9.1
ERM 5.0-02	50.7	1	32.3	9700	31
ERM 5.0-03	5.3	0.2	60.9	9900	35
ERM 5.0-05	93.1	3.2	0.7	1300	35
ERM 5.0-07	92.1	6.3	0.7	1400	34
ERM 5.0-09	93.5	0.7	3.3	3600	24
ERM 5.0-10	96.9	0	1.2	5900	15
ERM 6.0-01	92.3	0.2	5	11000	8
ERM 6.0-02	81.9	0.6	12.3	9000	7.5
ERM 6.0-03	52.5	0.6	35.4	18000	22.7
ERM 6.0-04	94.9	2.7	1.9	3000	33
ERM 6.0-05	81.7	0.5	11.5	11000	34
ERM 6.0-06	97.7	0.6	0.8	1700	35
ERM 6.0-07	86.4	5.9	1.7	3200	28
ERM 6.0-08	72.4	2.9	13.5	16000	34
ERM 6.0-09	58	2.2	26.7	23000	35
ERM 6.0-10	35.2	1.1	24.4	9600	24

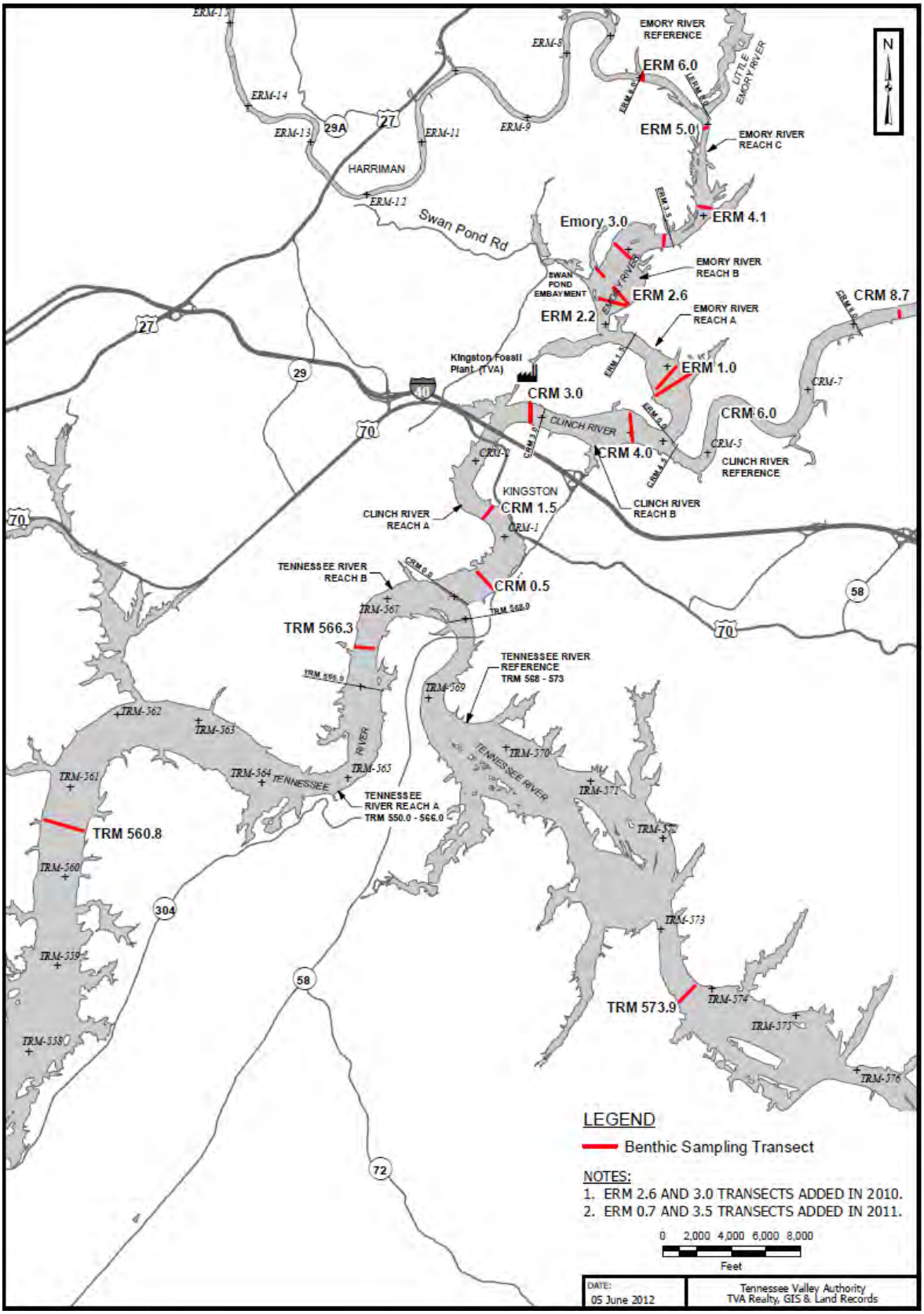
ERM - Emory River mile.
ft - Feet.
PLM - Polarized Light Microscopy.



ARCADIS

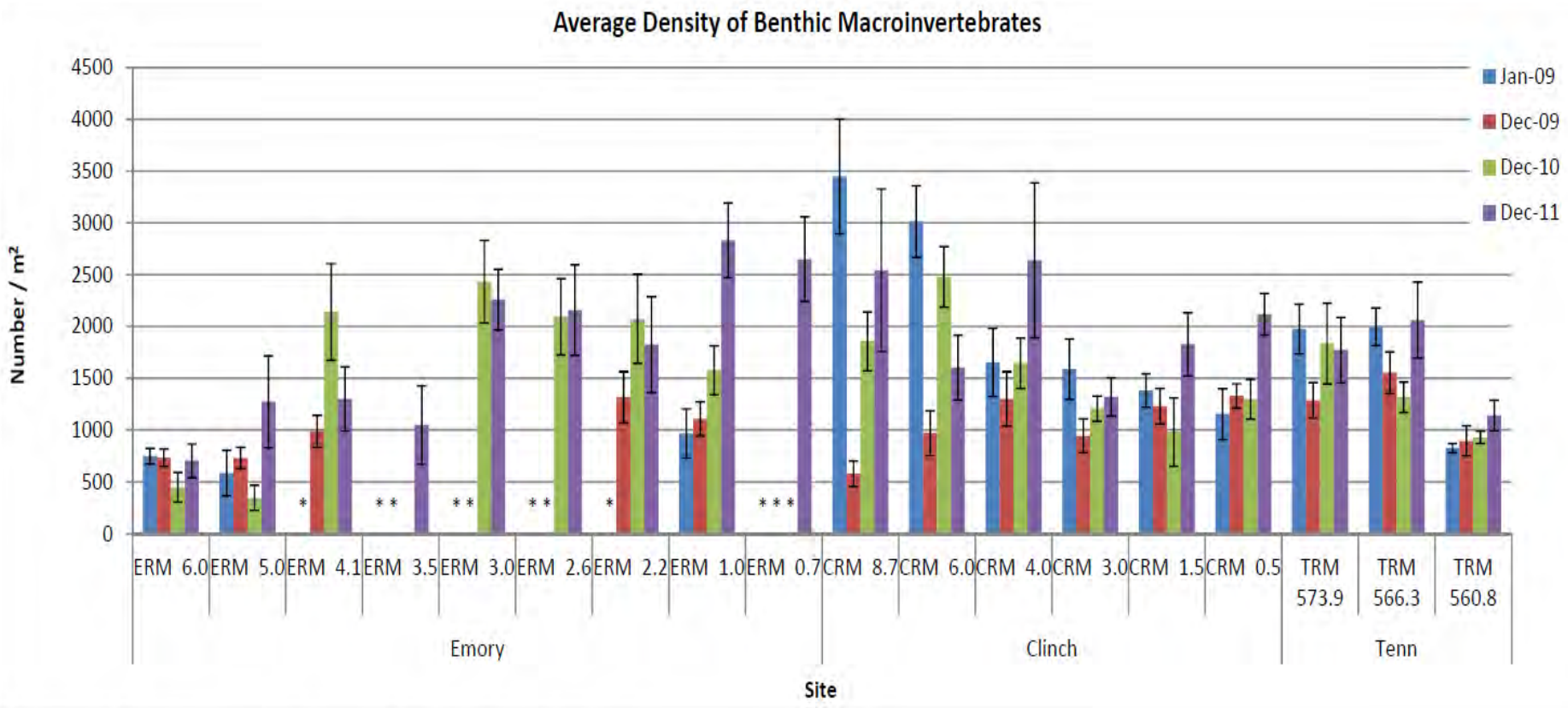
Benthic Invertebrate Community Composition
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
1



Benthic Invertebrate Community Sampling Locations
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
2



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

* Indicates sampling event(s) in which a site was not sampled.

CRM- Clinch River Mile
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Benthic Invertebrate Community Average Density
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
3

Note: Samples from the fourth sampling period were collected December 2011 – January 2012

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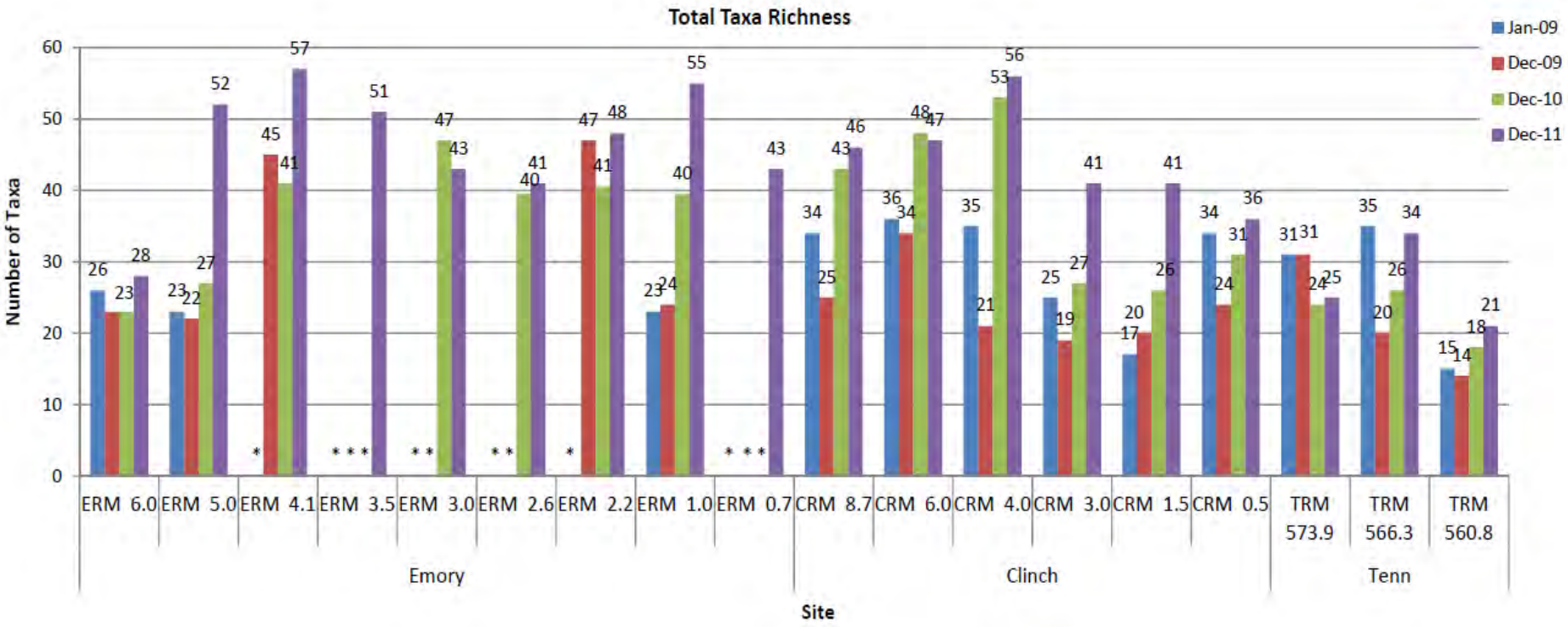
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Benthic Invertebrate Community AverageTaxa Richness
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
4



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

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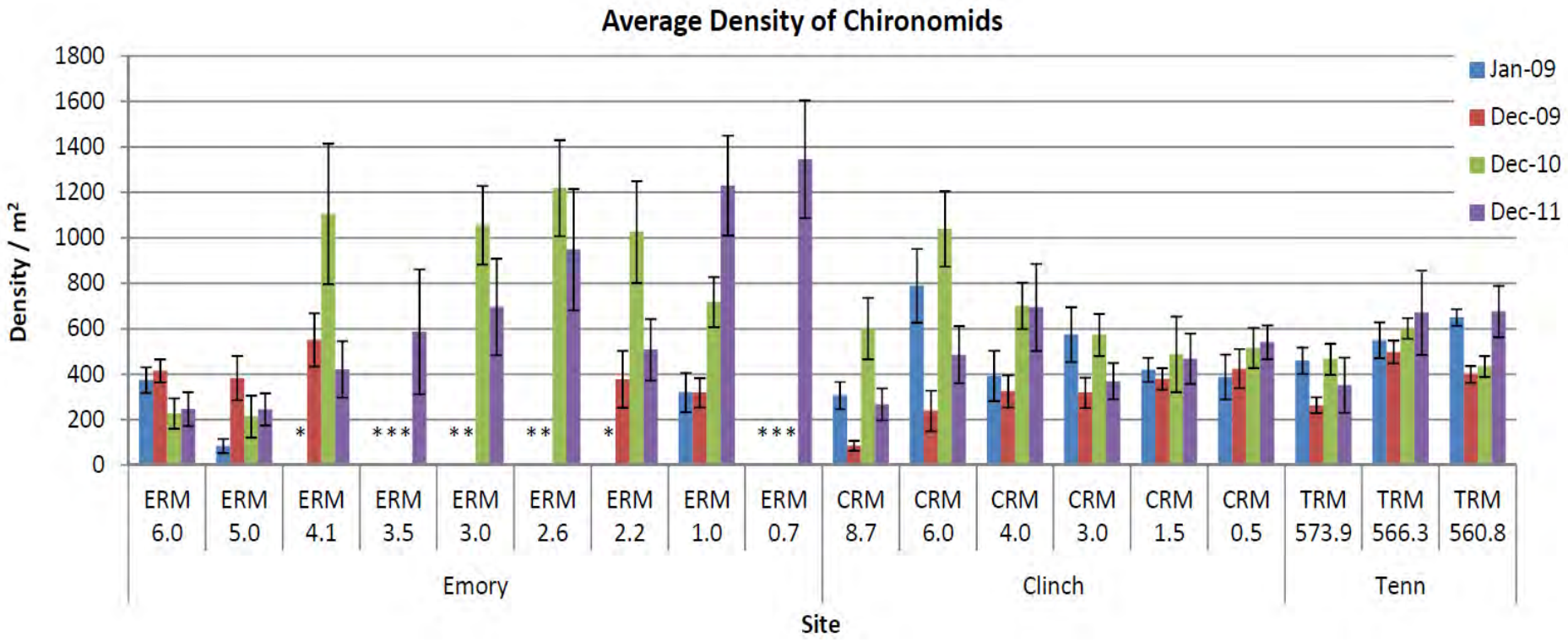
TRM – Tennessee River Mile

ARCADIS

Benthic Invertebrate Community Total Tax Richness
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
5



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

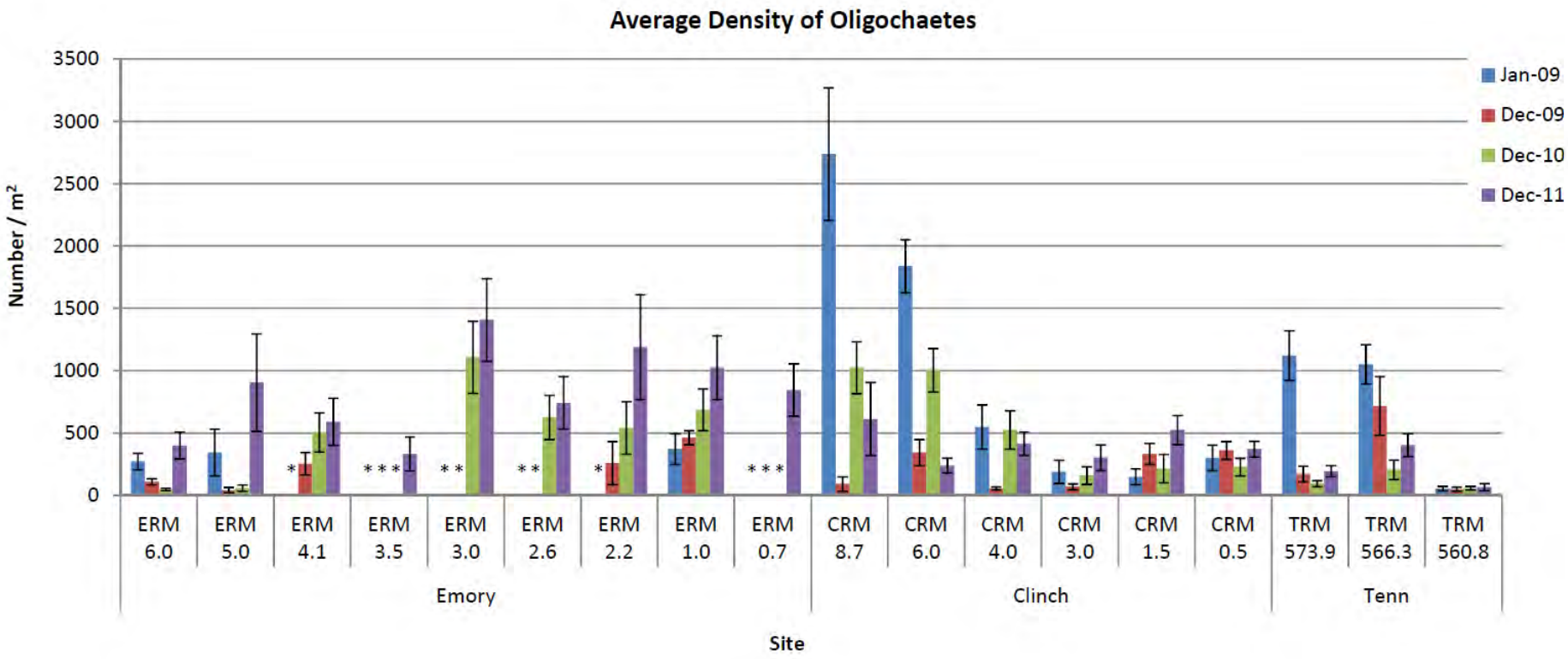
* Indicates sampling event(s) in which a site was not sampled.

CRM- Clinch River Mile
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Average Density of Chironomids from the Emory, Clinch, and Tennessee Rivers
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
6



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

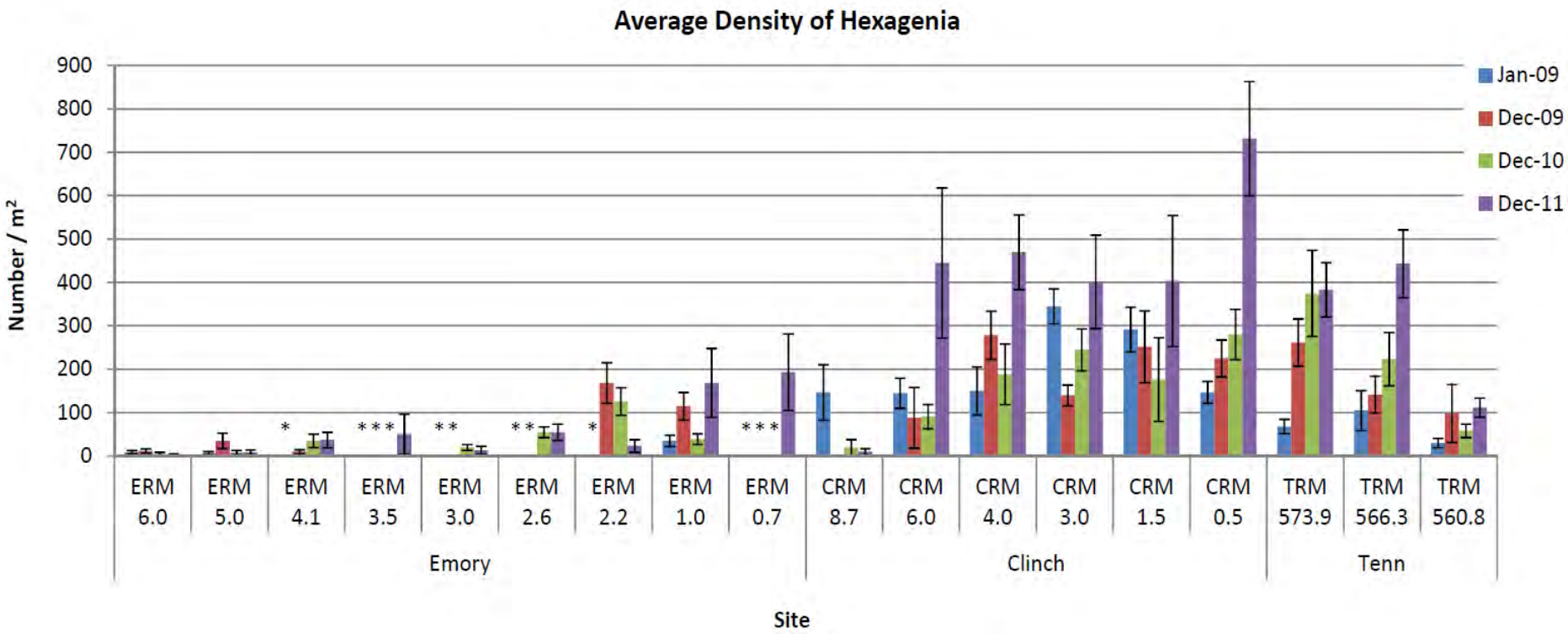
* Indicates sampling event(s) in which a site was not sampled.

CRM- Clinch River Mile
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Average Density of Oligochaetes from the Emory, Clinch, and Tennessee Rivers
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
7



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

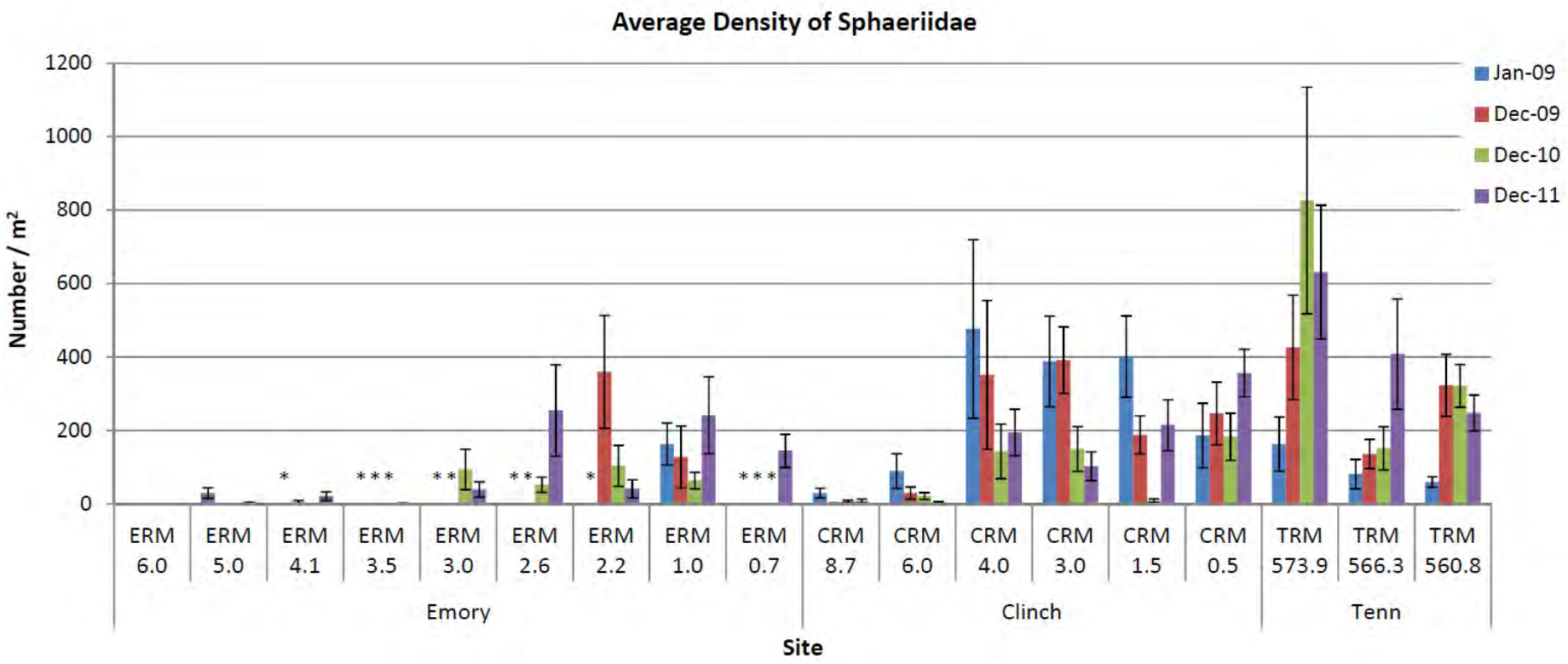
* Indicates sampling event(s) in which a site was not sampled.

CRM- Clinch River Mile
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Average Density of Hexagenia from the Emory, Clinch, and Tennessee Rivers
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
8



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

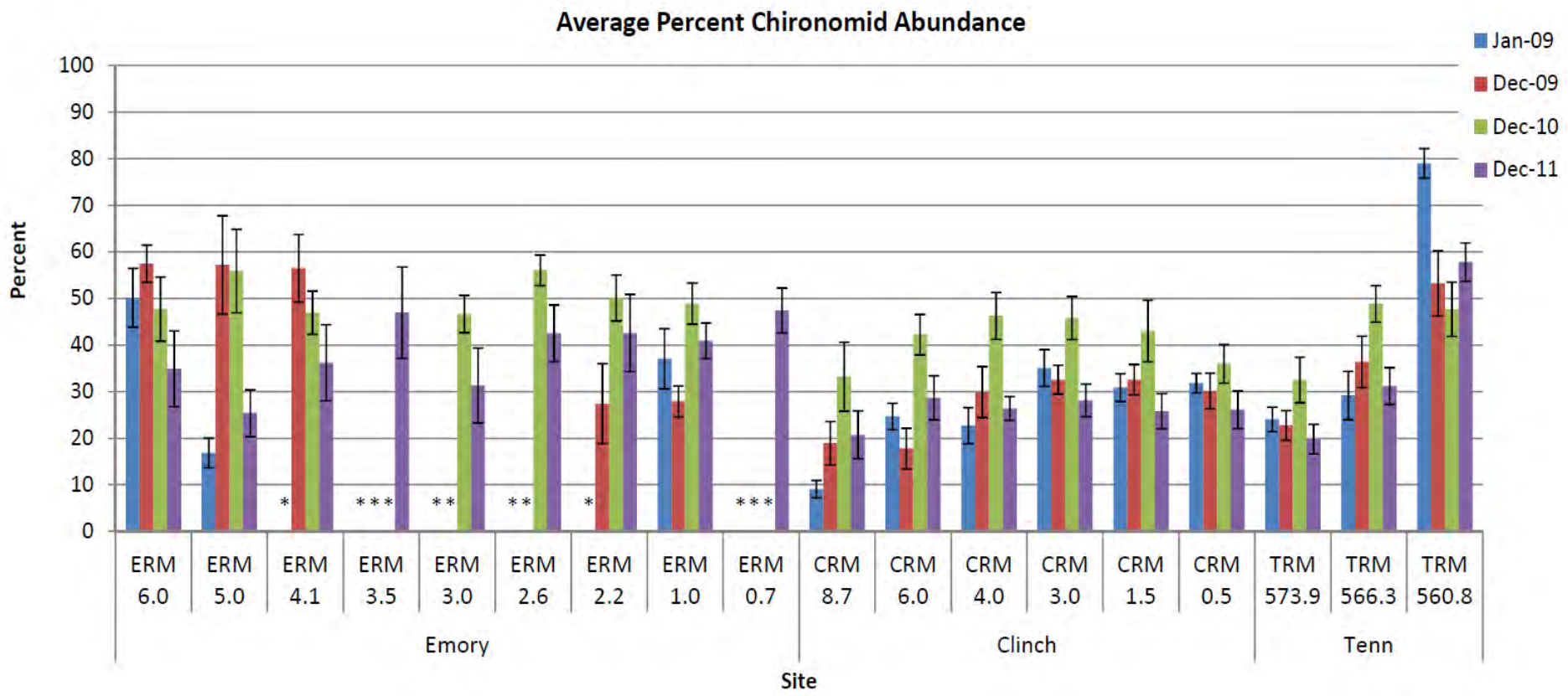
* Indicates sampling event(s) in which a site was not sampled.

CRM- Clinch River Mile
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Average Density of Sphaeriidae from the Emory, Clinch, and Tennessee Rivers
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
9



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

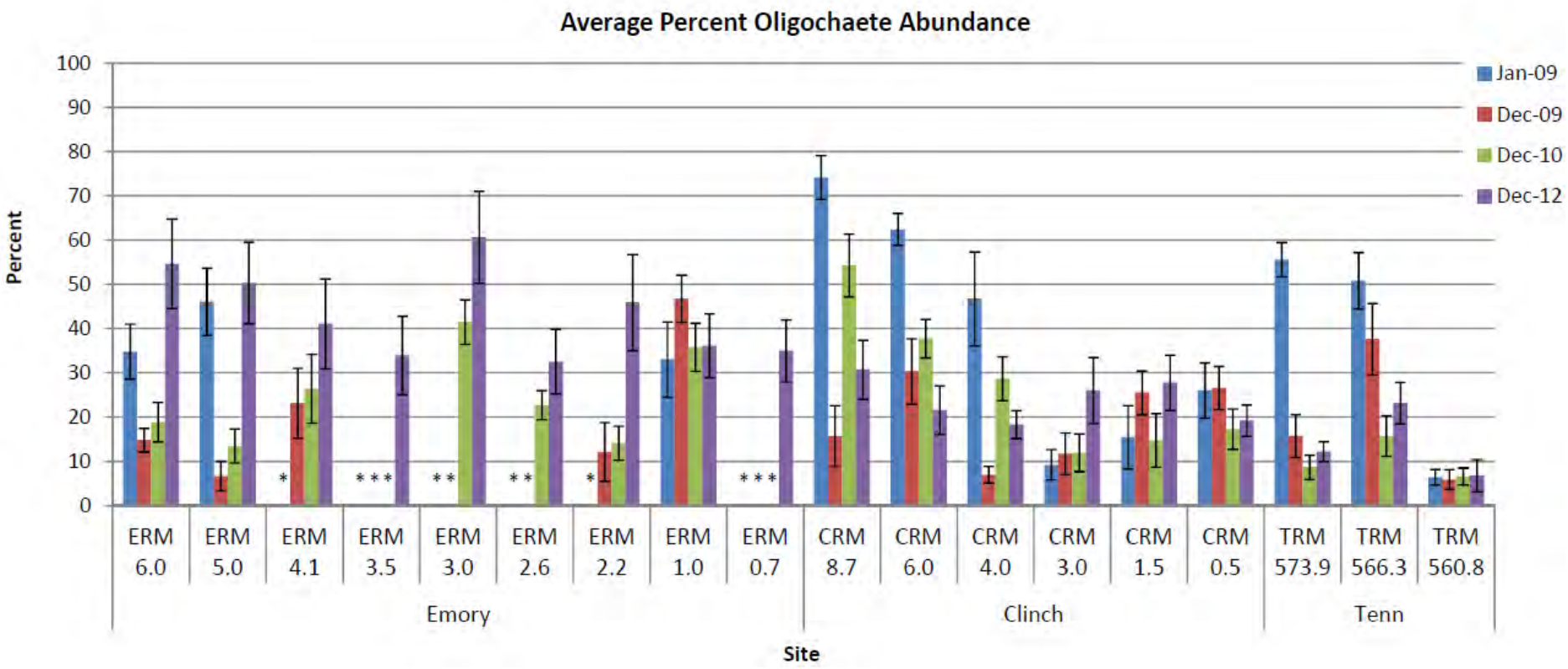
* Indicates sampling event(s) in which a site was not sampled.

CRM- Clinch River Mile
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Average Percent Chironomid Abundance for the Emory, Clinch, and Tennessee Rivers
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
10



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

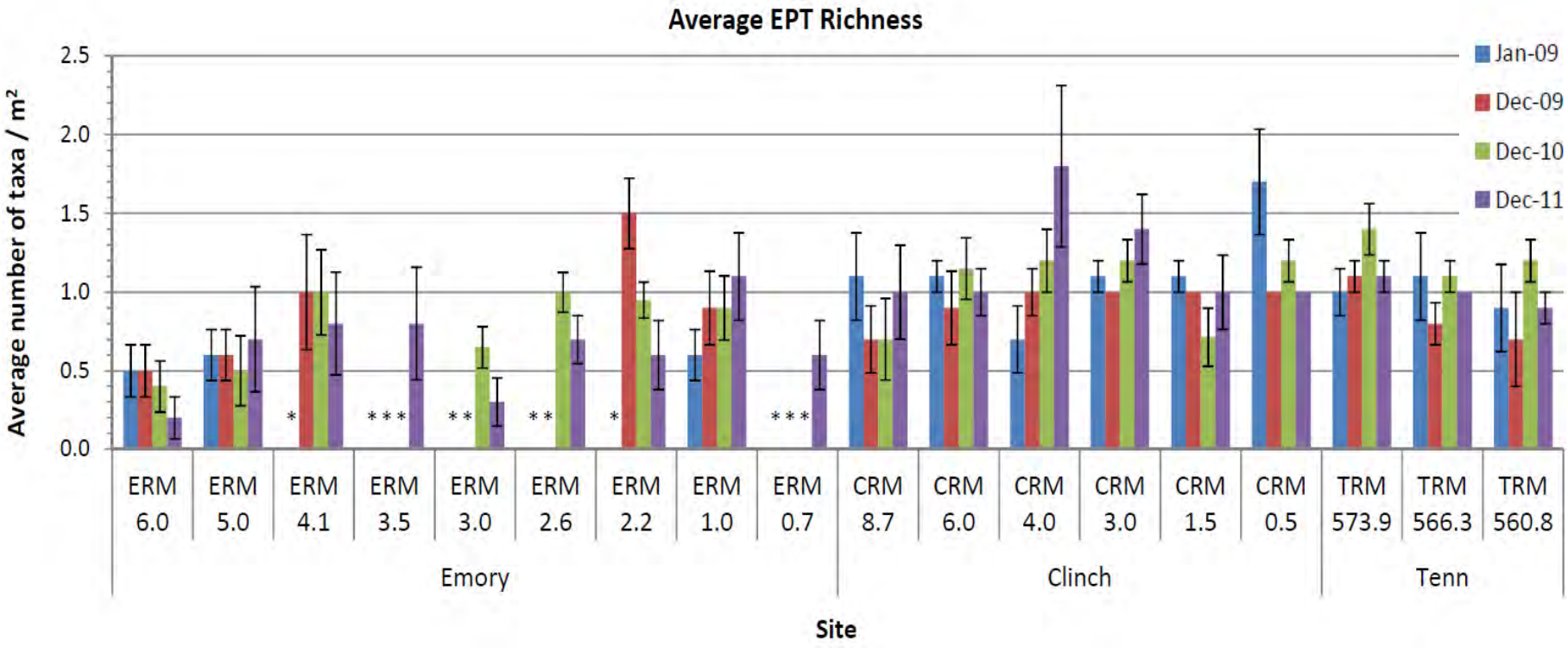
* Indicates sampling event(s) in which a site was not sampled.

CRM- Clinch River Mile
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Average Percent Oligochaete Abundance for the Emory, Clinch, and Tennessee Rivers
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
11



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

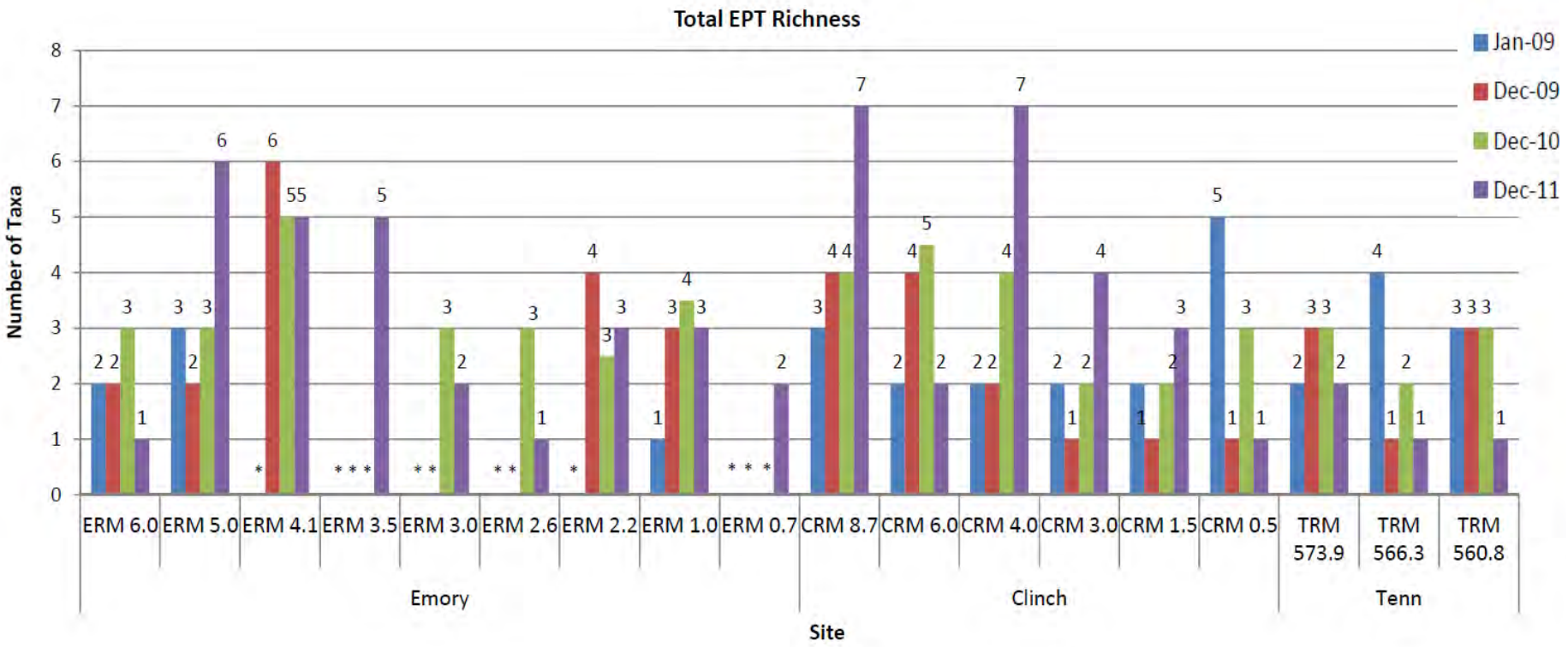
* Indicates sampling event(s) in which a site was not sampled.

CRM- Clinch River Mile
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Average EPT Richness for the Emory, Clinch, and Tennessee Rivers
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
12



Note: Samples from the fourth sampling period were collected December 2011 – January 2012

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CRM- Clinch River Mile
ERM- Emory River Mile
TRM – Tennessee River Mile

ARCADIS

Total EPT Richness for the Emory, Clinch, and Tennessee Rivers
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011
TENNESSEE VALLEY AUTHORITY
KINGSTON, TENNESSEE

FIGURE
13

Appendix F

Preliminary Evaluation of 2011 ORNL Results: Benthic Invertebrate Bioaccumulation

OAK RIDGE NATIONAL LABORATORY
MANAGED BY UT-BATTELLE FOR THE DEPARTMENT OF ENERGY

P.O. Box 2008
Oak Ridge, TN 37831-6351
Phone: (865) 576-4163
Fax: (865) 576-9938
E-mail:smithjg@ornl.gov

June 19, 2012

Dr. Neil Carriker
Program Manager, Special Projects
Tennessee Valley Authority Kingston Ash Recovery Project
Kingston, Tennessee

Dr. Carriker,

Subject: June 12, 2012 Addendum: Preliminary Evaluation of 2011 Results: *Aquatic Invertebrate Bioaccumulation*

I am pleased to submit to you this addendum to my preliminary evaluation of 2011 *Aquatic Invertebrate Bioaccumulation* Task results.

The enclosed report provides further details on the results for arsenic, selenium, and mercury in all collections of snails, mayfly nymph and mayfly adult from 2009 - 2011.

We hope this addendum is useful to you. If you have any questions or require additional information, please call me at (865) 576-4163.

Sincerely,

John G. Smith
Principal Investigator, Aquatic Invertebrate Bioaccumulation Task
Environmental Sciences Division
Oak Ridge National Laboratory

c: M. J. Peterson, ORNL
D. Jones, ARCADIS

June 12, 2012 ADDENDUM:

Preliminary Evaluation of 2011 Results: *Aquatic Invertebrate Bioaccumulation*

Introduction

This addendum to the letter report submitted on May 4, 2012, provides a more in-depth analysis and summary of spatial and temporal bioaccumulation trends for arsenic, mercury, and selenium in invertebrates collected from 2009 through 2011, for the Kingston Fossil Plant (KIF) Coal Ash Spill Project aquatic invertebrate bioaccumulation task. The target invertebrate species for this task are the silty hornsnail, *Pleurocera canaliculatum*, and the nymphs and adults of the burrowing mayfly, *Hexagenia bilineata*. Except for adult mayflies, which are collected opportunistically, the same sites were used in 2010 and 2011, while a smaller number of sites were sampled in 2009. The same sampling and analysis strategies were used in 2010 and 2011, including the collection and analysis of depurated and non-depurated snails and nymphs, and the collection and analysis of adult mayfly subgroups based on sex and adult developmental stage (i.e., subimagos and imagos); a similar strategy was followed in 2009 but on a smaller scale.

Both spatial and temporal trends in the results for all three metals were sufficiently distinct, that subjecting the data to formal hypothesis statistical tests was unnecessary. Thus, only descriptive statistics for the data are presented in summary figures and tables. Differences between the imago and subimago mayfly stages in the concentrations of arsenic, mercury, and selenium were generally subtle, and perhaps non-existent, while differences between males and females were usually more distinct. For this reason, results for the adults for imagos and subimagos of each sex were combined to generate summary graphs, but results for the four subgroups were kept separate in the summary tables of descriptive statistics.

Results

A. Arsenic

1. Concentrations of arsenic in depurated and non-depurated snails were generally similar within each site in all three years (Fig. 1, Table 1). Maximum concentrations in snails were generally found at Clinch River sites in all years.

2. Except for ERM1.0 (i.e., mile 2 from spill site), concentrations of arsenic in depurated and non-depurated mayfly nymphs also were generally similar within each site in all years (Fig. 2, Table 2). Maximum concentrations of arsenic in depurated and non-depurated nymphs were found at ERM1.0 in all years.

3. Concentrations of arsenic were generally higher in female than male mayflies (Fig. 3, Table 3), and maximum concentrations were usually found between the site of the ash spill and ERM1.0.

4. Except for ERM1.0, concentrations of arsenic in snails and mayfly nymphs were generally similar. One other exception to this trend was that concentrations of arsenic in snails from CRM6.0 were generally higher than at any site in the Emory River within each year, as were concentrations at CRM3.5 and CRM1.5, suggesting that a source of arsenic is present in the Clinch River upstream of CRM6.0.

5. Concentrations of arsenic in mayfly nymphs were generally several orders of magnitude higher than in the adults.

6. With few exceptions (i.e., female mayflies) the highest concentrations of arsenic downstream of the spill site were generally found in 2010. However, the differences between 2010 and 2011 at most sites were probably within the range of error associated with collecting, processing, and analyzing the samples, and therefore, probably does not represent a real change. However, there were some notable exceptions suggesting that lower concentrations at some sites in 2011 may have been actual declines. The mean concentrations of arsenic in depurated and non-depurated nymphs from ERM1.0 were two times lower in 2011, while differences between these 2 years at other sites were relatively small. Concentrations of arsenic in the adult mayflies were similarly lower in 2011 at ERM3.0 and ERM2, but there also appeared to be an increase from ERM1.0 downstream to CRM1.5 in female mayflies.

B. Selenium

1. Concentrations of selenium in depurated and non-depurated snails were generally similar (Fig. 4, Table 1). Highest concentrations of selenium in snails were generally found at CRM1.5. Highest concentrations in the Emory River occurred at ERM1.0 in all three years. While concentrations of selenium at ERM1.0 were higher than at the Emory River reference site, concentrations in snails from ERM2.5 were only marginally higher than at ERM6.0.

2. Concentrations of selenium in depurated and non-depurated nymphs were generally similar or slightly higher in depurated nymphs in all years (Fig. 5, Table 2). Both groups of nymphs exhibited very similar spatial trends in all years. Highest concentrations were always found at ERM1.0, and then decreased progressively with increasing distance downstream from the Emory River.

3. Concentrations of selenium were generally higher in female than male adult mayflies (Fig. 6, Table 3). The highest concentrations in adult mayflies were generally found at Emory River sites adjacent to and downstream of the ash spill site, and then progressively decreased with increasing distance from the Emory River.

4. Concentrations of selenium in non-depurated snails were similar in 2010 and 2011 (all snails were depurated in 2009).

5. Results for snails suggest that concentrations of selenium were generally highest at most sites in 2010, including the reference sites. The fact that selenium concentrations were lower at most sites in 2011 compared to 2010, including the reference sites, suggests that the differences between years at sites downstream of the spill were probably caused by the same factors contributing to the differences between years at the reference sites (e.g., natural chemical and/or physical environmental changes, sampling, processing, and/or analytical error/variation).

6. The magnitude of difference and change between sites and years in selenium concentrations in mayfly nymphs suggest that concentrations remained relatively stable at ERM4.0, CRM3.5 and CRM1.5 in 2010 and 2011 in both depurated and non-depurated nymphs. At ERM2.5 and ERM1.0, in contrast, concentrations of selenium in non-depurated nymphs were highest in 2010, while highest concentrations

in depurated nymphs were highest in 2011. This suggests a possible reduction of selenium associated with non-digestible food particles in 2011, but a higher proportion of bioavailable selenium in the food. Similar but weaker trends were apparent at ERM4.0 and TRM566.3; concentrations of selenium were highest at these sites in non-depurated nymphs in 2010, but there were no differences between years in depurated nymphs. This too would suggest the presence of a higher proportion of bioavailable selenium in 2011.

7. Concentrations of selenium were clearly much higher in 2011 than in 2010 in male and female mayflies at ERM1.0, CRM3.5, and CRM1.5. Concentrations in females from all 3 sites exceeded EPA's proposed whole body fish criterion (7.91 µg/g dry weight), and were either higher than or comparable to the proposed criterion in the males from these sites. Selenium concentrations in both sexes were lower in 2011 than in 2010 at ERM2.5. While this could possibly be a data artifact, it may also represent an actual decrease that could have been associated with dredging activities in the adjacent river channel. The mayflies at this site live in the overbank area where no dredging took place. Not only do particles from further upstream settle in this area, but it is also possible that uncontaminated clays/particles suspended in the water column during dredging could also have been deposited in the overbank area. Both of these factors could have contributed to a "dilution effect" in the sediments they built their burrows in and the food available for consumption.

C. Mercury

1. A source of mercury to the Clinch River upstream of CRM6.0 is clearly the major source of that metal to all sites in the Clinch River and possibly the lower reaches of the Emory River (Tables 1-3 and Figs. 7-9). The highest concentrations in all groups of invertebrates and years have been found at CRM6.0. Concentrations decline with distance downstream from CRM6.0, and generally remain 1.5 to 2 times higher at the lower Clinch River sites than at any of the Emory River sites. Furthermore, concentrations of mercury at ERM6.0 and LERM1.0 were generally similar to those at Emory River sites downstream of the ash spill.

2. There were no distinct temporal trends in mercury in snails or non-depurated mayfly nymphs at any site, but there appeared to be increases in depurated mayfly nymphs at most sites, including reference sites, in 2011. This latter observation would appear to be consistent with the generally higher concentrations of mercury found in adult mayflies at most sites in 2011 since the entire digestive tract and its contents are shed during the molt from the nymph to adult.

Summary and Conclusions

1. Results from adult mayflies and particularly snails provide strong evidence that at least some of the arsenic in invertebrates from some sites may be coming from a source upstream of CRM6.0. The highest concentrations of arsenic in snails were generally found at CRM6.0, but moderately higher concentrations were also at ERM1.0 and possibly ERM2.5. Concentrations of arsenic in mayfly nymphs were highest at ERM1.0 in all years. There was little difference found in the concentrations of nymphs at ERM2.5, CRM3.5, and CRM1.5, and compared with ERM1.0, concentrations were lower. The lowest

concentrations of arsenic in depurated and non-depurated mayfly nymphs were found in 2011. Even though concentrations of arsenic were considerably lower in adult mayflies than the nymphs, results for the adult mayflies provided some of the strongest evidence that some of the arsenic accumulated by invertebrates has likely come from fly ash. Some of the highest concentrations of arsenic in adult mayflies were found at ERM3.0, ERM2, and ERM1. Temporally, male and female mayflies exhibited different trends between years at some sites, but similar trends at others. Results for both groups suggest that concentrations in arsenic were much lower at ERM3 and ERM2 in 2011 compared with 2010. However, concentrations in adult females from ERM1 downstream to CRM1.5 were higher in 2011 compared with 2010, while there appeared to be no between-year differences in males from these same sites.

2. Changes appear to have occurred between 2010 and 2011 in the bioavailability of selenium to mayflies but not snails. This was demonstrated by higher concentrations of selenium in depurated nymphs and adult mayflies in the Emory River downstream of the spill site and adult mayflies at sites in the lower Clinch River. Concentrations of selenium in mayfly nymphs and adults also exceeded EPA's proposed whole body fish criterion at more sites in 2011 than in previous years. Results for snails showed no definitive evidence of temporal changes in selenium bioaccumulation. Therefore, concentrations remain somewhat elevated in snails at sites downstream of the spill site in the Emory and Clinch Rivers.

3. Results from all three groups of invertebrates provide unambiguous evidence of a major source of mercury to the Clinch River upstream of CRM6.0. Maximum concentrations of mercury in all groups of invertebrates were found at CRM6.0 in all years, with the next highest concentrations occurring at CRM3.5 and /or CRM1.5. While these results provide clear evidence of a major source of mercury upstream of CRM6.0, without historical data from the Emory River, it cannot be definitively concluded that the fly ash spill has not contributed to the mercury load downstream of the spill site.

4. The amount of spatial (within and between sites) and temporal variation exhibited during the first three years of this study shows a need to use caution in conclusions drawn from the results available to date. While bioaccumulation results for some elements have revealed relatively unambiguous spatial trends (some apparently related to fly and some not), temporal trends were more ambiguous. Temporal variation among years in data was considerable for many elements at may sites, including reference sites, thus, a data set with only three years of results will not be sufficiently robust to characterize or identify actual temporal trends with a high degree of confidence.

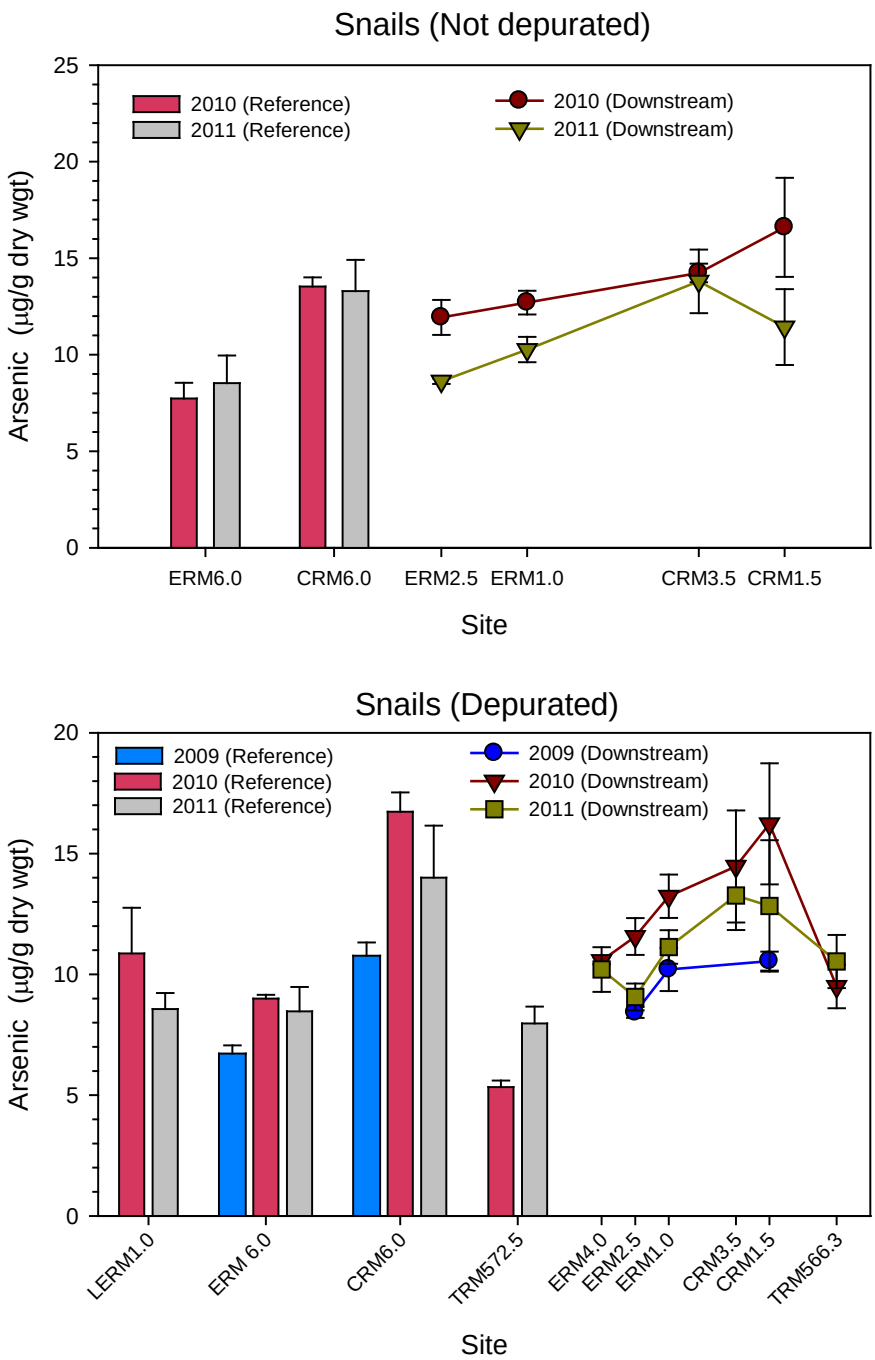
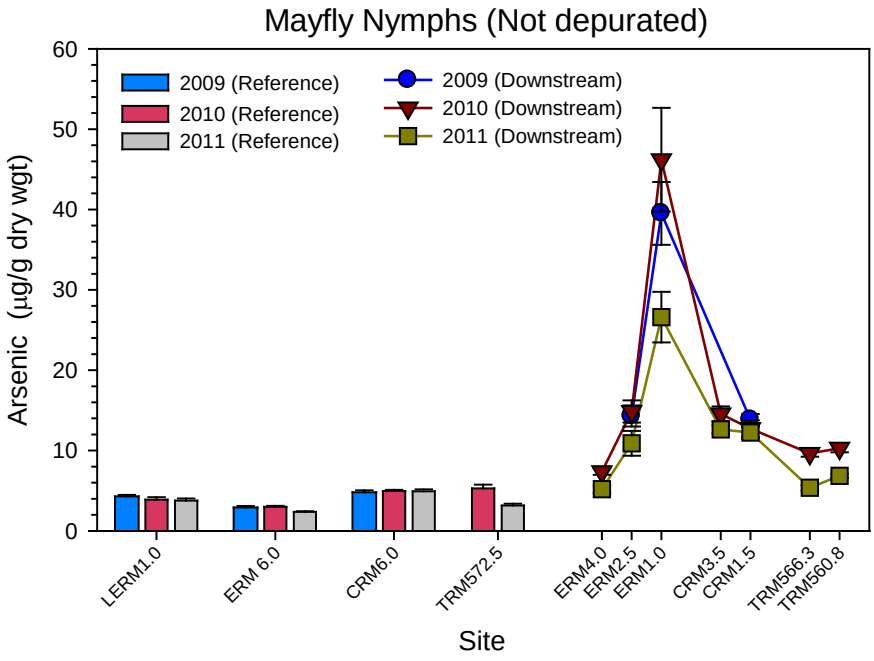


Fig. 1. Mean concentration of arsenic in non-depurated (top) and depurated (bottom) snails (*Pleurocera canaliculatum*) from sites in the Emory River, Clinch River, Tennessee River, and Little Emory River, 2009-2011. Values are means ± 1 SE.



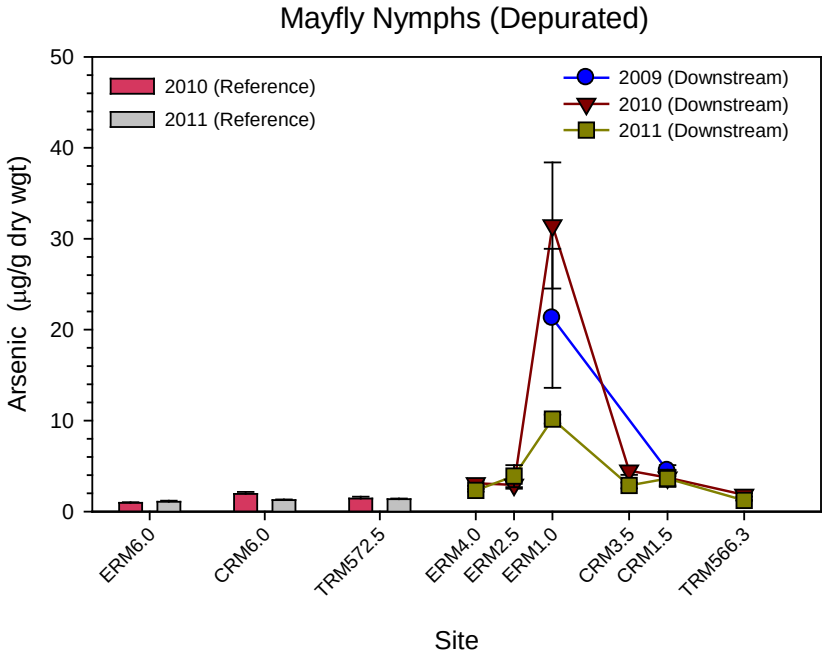


Fig. 2. Mean concentration of arsenic in non-depurated (top) and depurated (bottom) mayfly nymphs (*Hexagenia bilineata*) from sites in the Emory River, Clinch River, Tennessee River, and Little Emory River, 2009-2011. Values are means ± 1 SE.

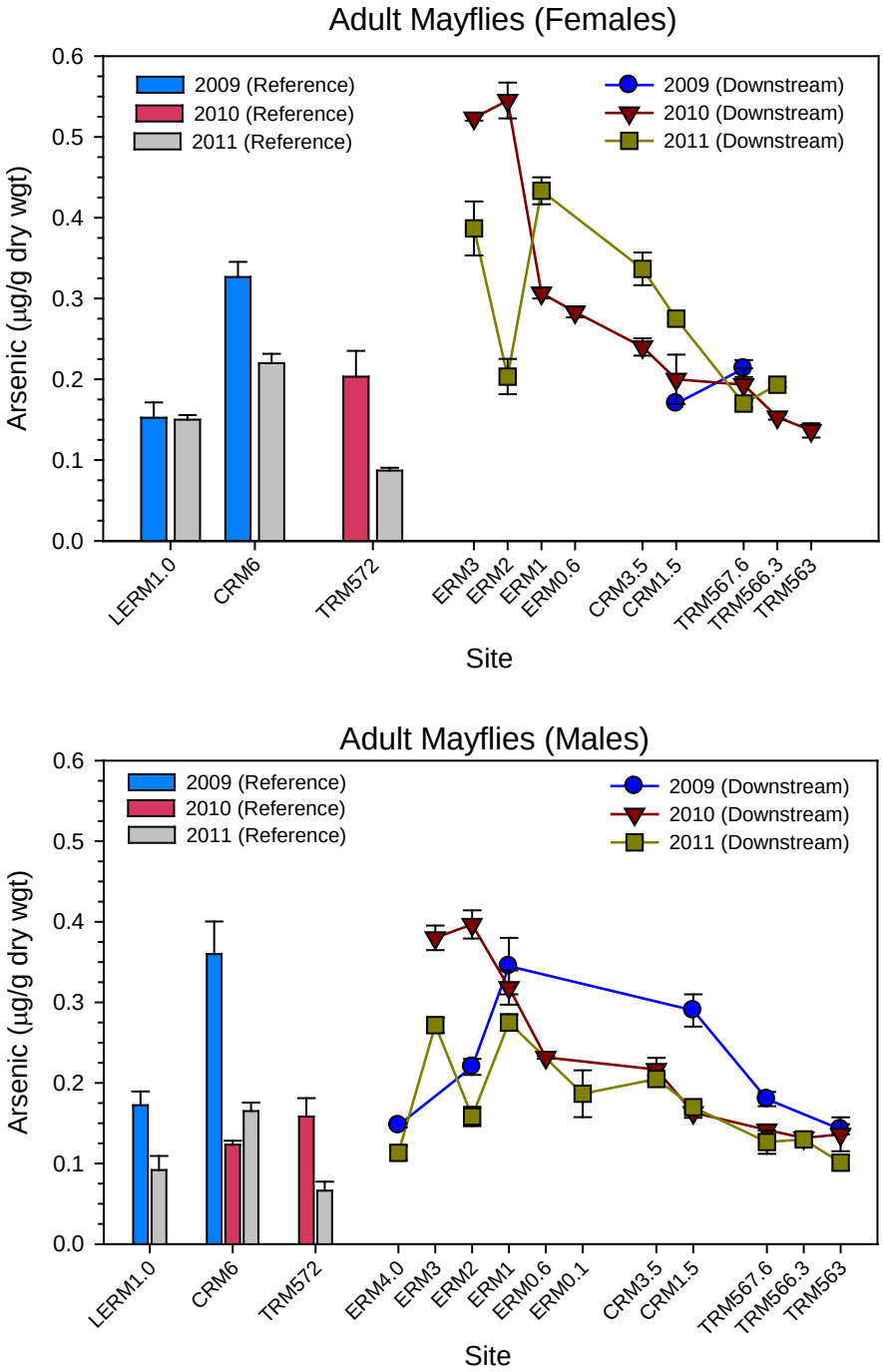


Fig. 3. Mean concentration of arsenic in female (top) and male (bottom) adult mayflies (*Hexagenia bilineata*) from sites in the Emory River, Clinch River, Tennessee River, and Little Emory River, 2009-2011. Results from imagos and subimagos have been combined for each sex. Values are means ± 1 SE.

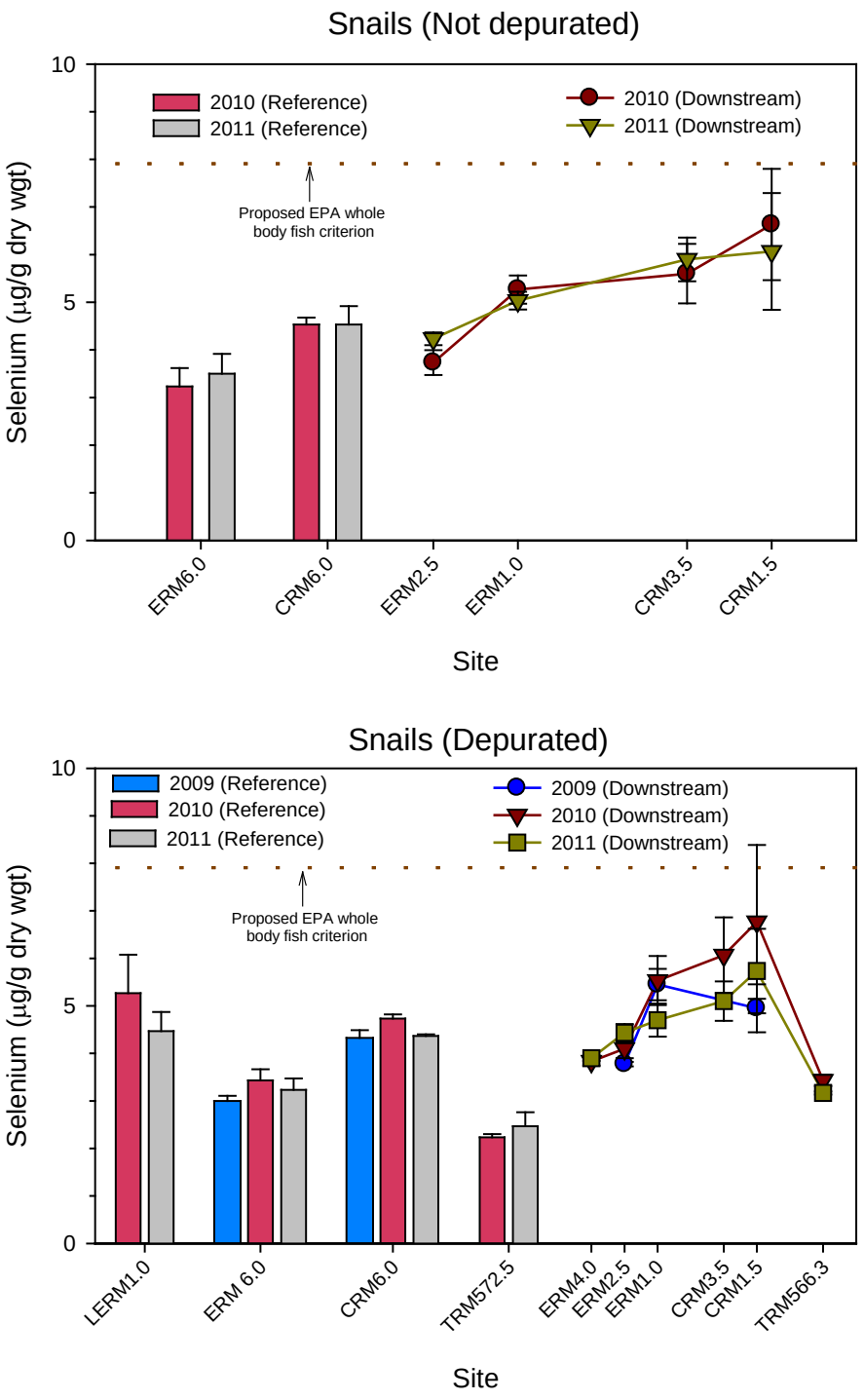


Fig. 4. Mean concentration of selenium in non-depurated (top) and depurated (bottom) snails (*Pleurocera canaliculatum*) from sites in the Emory River, Clinch River, Tennessee River, and Little Emory River, 2009-2011. Values are means ± 1 SE. The horizontal line shows the proposed EPA whole body fish criterion for selenium.

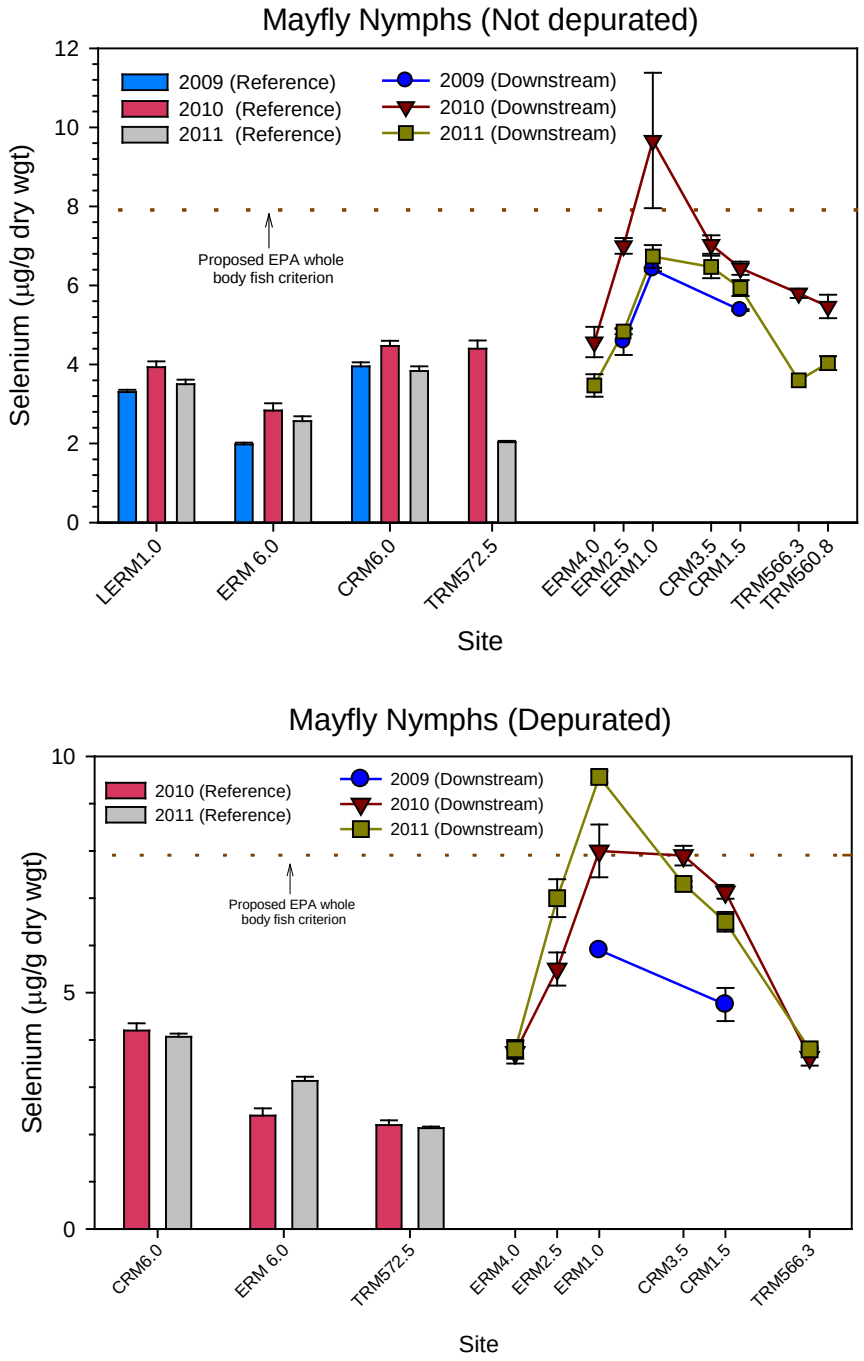
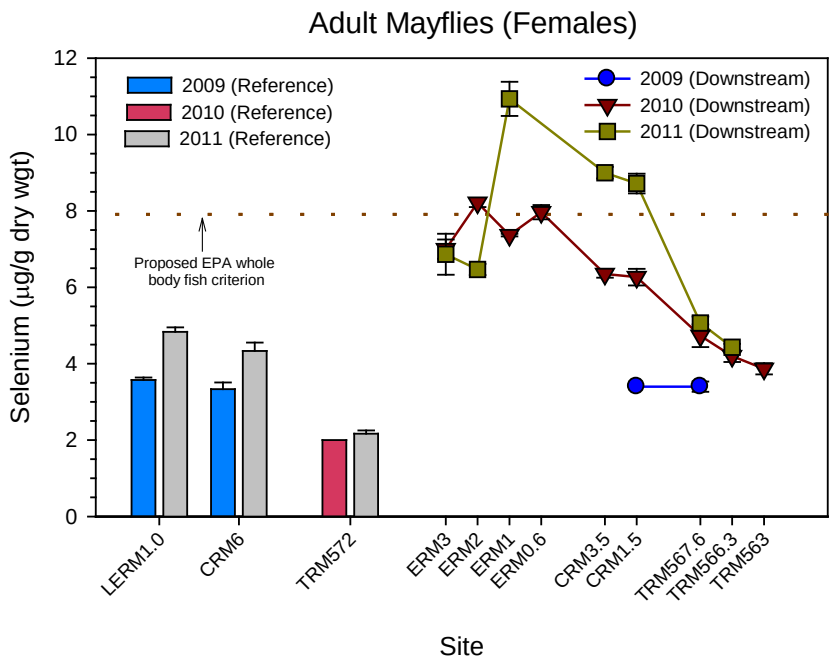


Fig. 5. Mean concentration of selenium in non-depurated (top) and depurated (bottom) mayfly nymphs (*Hexagenia bilineata*) from sites in the Emory River, Clinch River, Tennessee River, and Little Emory River, 2009-2011. Values are means ± 1 SE. The horizontal line shows the proposed EPA whole body fish criterion for selenium.



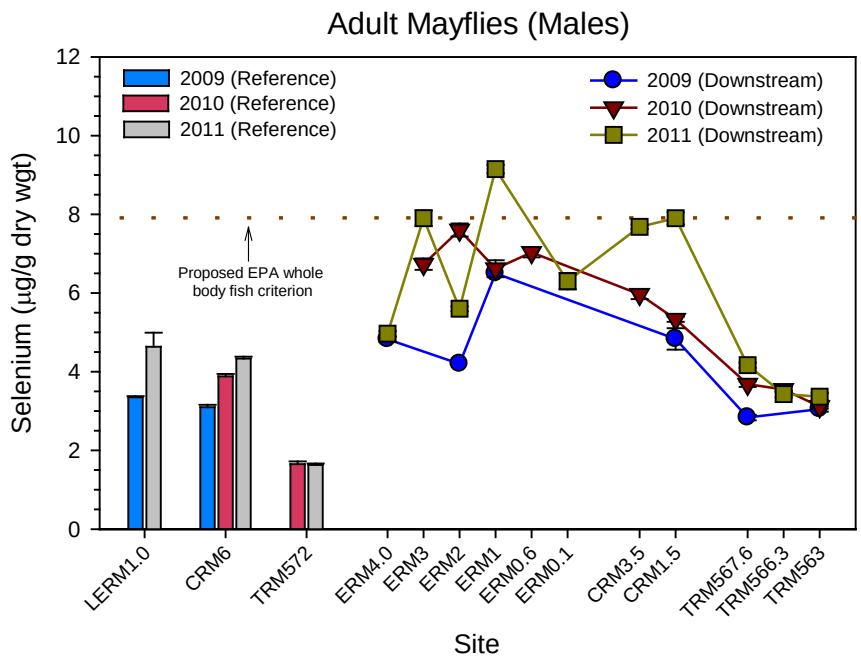


Fig. 6. Mean concentration of selenium in female (top) and male (bottom) adult mayflies (*Hexagenia bilineata*) from sites in the Emory River, Clinch River, Tennessee River, and Little Emory River, 2009-2011. Results from imagos and subimagos have been combined for each sex. Values are means ± 1 SE. The horizontal line shows the proposed EPA whole body fish criterion for selenium.

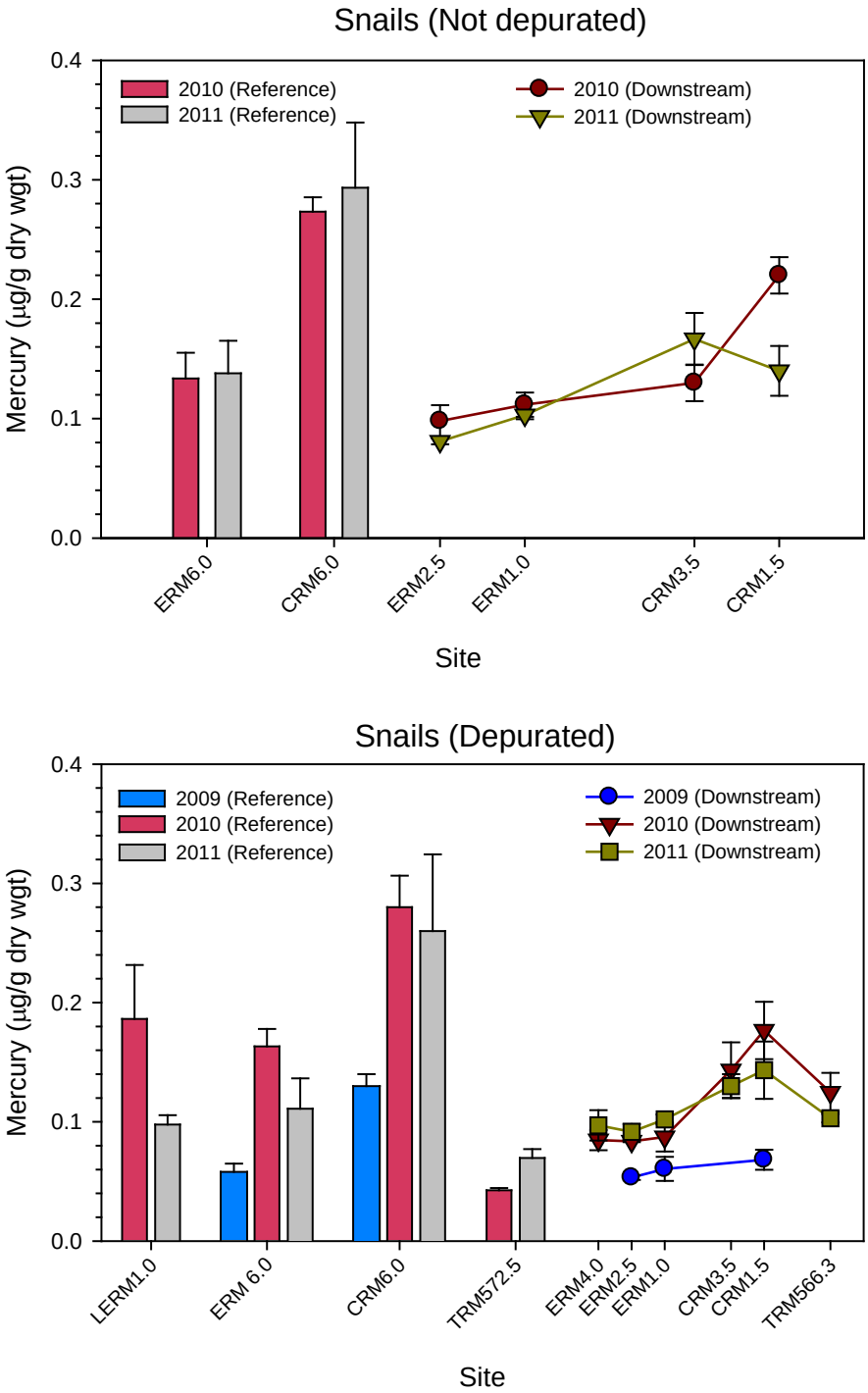
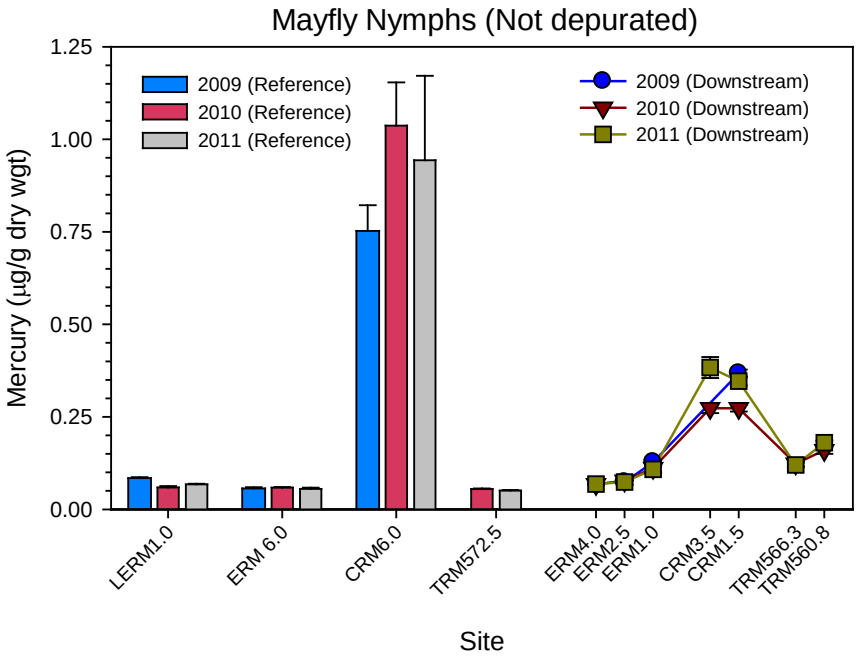


Fig. 7. Mean concentration of mercury in non-depurated (top) and depurated (bottom) snails (*Pleurocera canaliculatum*) from sites in the Emory River, Clinch River, Tennessee River, and Little Emory River, 2009-2011. Values are means ± 1 SE. Only the results from samples analyzed with EPA6020 are included.



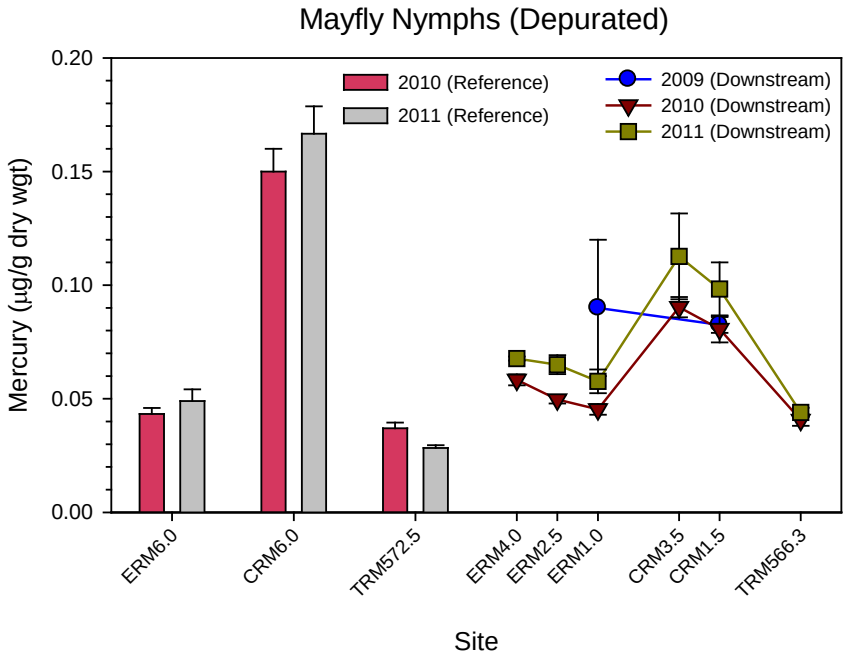


Fig. 8. Mean concentration of mercury in non-depurated (top) and depurated (bottom) mayfly nymphs (*Hexagenia bilineata*) from sites in the Emory River, Clinch River, Tennessee River, and Little Emory River, 2009-2011. Values are means ± 1 SE; mercury was not detected in either of the two samples of depurated nymphs from CRM1.5 in 2009, therefore the method detection limits for each sample were used to calculate the mean and SE. Only the results from samples analyzed with EPA6020 were included.

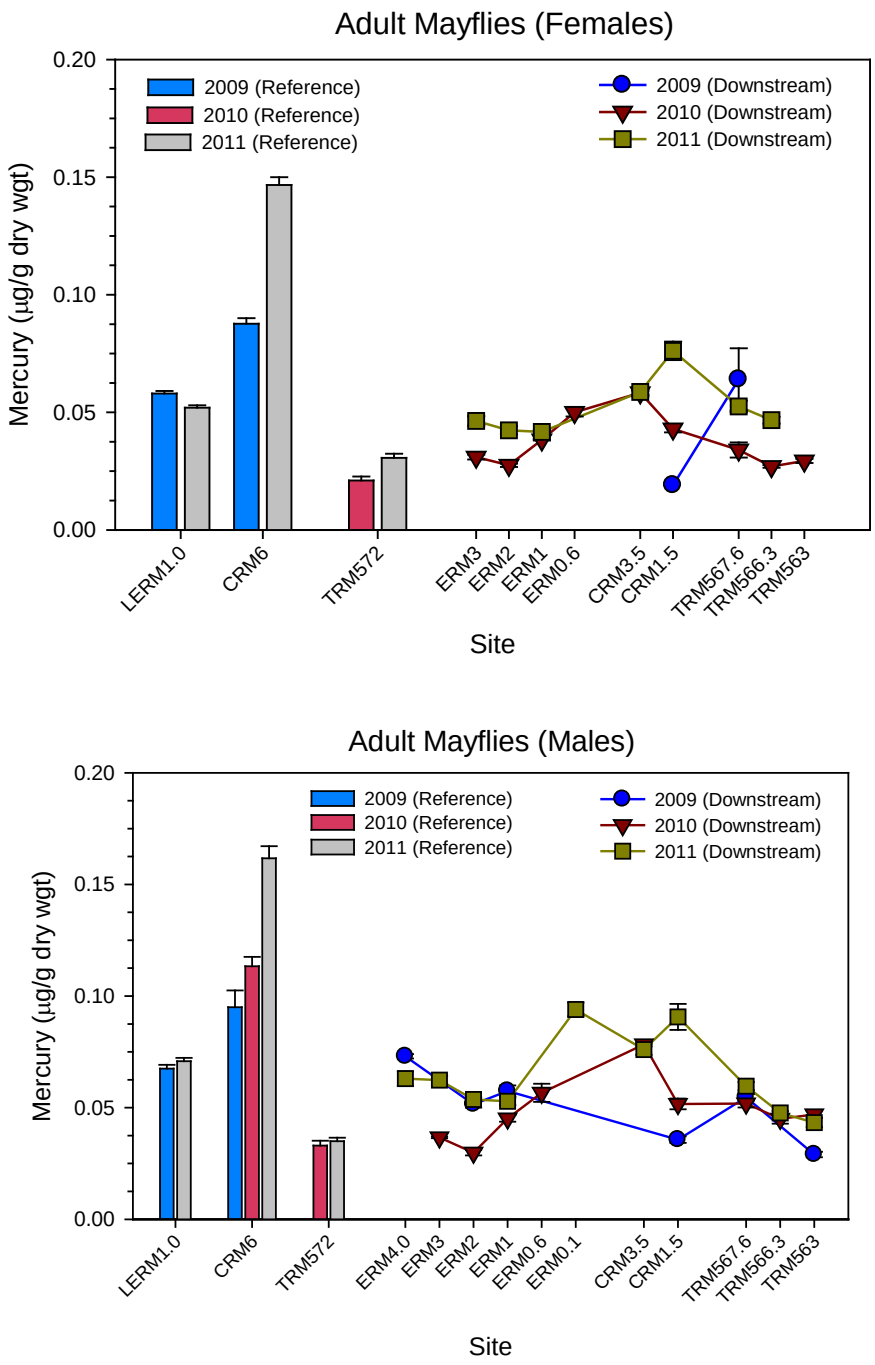


Fig. 9. Mean concentration of mercury in female (top) and male (bottom) adult mayflies (*Hexagenia bilineata*) from sites in the Emory River, Clinch River, Tennessee River, and Little Emory River, 2009-2011. Results from imagos and subimagos have been combined for each sex. Values are means ± 1 SE; mercury was not detected in 4 of 5 samples of females and 0 of 8 samples of males collected from TRM566.7 in 2009, therefore the method detection limits were used for samples from which mercury was not detected to calculate the mean and SE. Only the results from samples analyzed with EPA6020 were included.

Table 1. Descriptive statistics for arsenic, mercury, and selenium concentrations (total metal, µg/g dry weight) in snails (*Pleurocera canaliculatum*) from the Emory River, Clinch River, Little Emory River, and Tennessee River, 2009 through 2011.

			Arsenic			Mercury (EPA6020 only)			Selenium		
River/Site	Depurated[1]	Statistic[2]	2009	2010	2011	2009	2010	2011	2009	2010	2011
Emory River											
ERM6.0	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		7.7	8.5		0.134	0.138		3.23	3.50
		Median		8.5	9.2		0.150	0.160		3.4	3.7
		SD		1.42	2.47		0.0373	0.0470		0.67	0.72
		Min.		6.1	5.8		0.091	0.084		2.5	2.7
		Max.		8.6	10.6		0.160	0.170		3.8	4.1
ERM6.0	Yes	FOD	4	3	3	4	3	3	4	3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	6.7	9.0	8.5	0.058	0.163	0.111	3.00	3.43	3.23
		Median	6.6	9.1	8.0	0.056	0.160	0.120	3.0	3.2	3.1
		SD	0.68	0.26	1.75	0.0140	0.0252	0.0442	0.22	0.40	0.42
		Min.	6.1	8.7	7.0	0.046	0.140	0.063	2.8	3.2	2.9
		Max.	7.6	9.2	10.4	0.075	0.190	0.150	3.3	3.9	3.7
ERM4.0	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		10.6	10.2		0.085	0.097		3.83	3.90
		Median		10.6	10.2		0.083	0.095		3.8	3.9
		SD		0.25	1.60		0.0146	0.0221		0.15	0.10
		Min.		10.3	8.6		0.071	0.076		3.7	3.8
		Max.		10.8	11.8		0.100	0.120		4.0	4.0
ERM2.5	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		11.9	8.6		0.098	0.081		3.73	4.23
		Median		11.7	8.6		0.100	0.083		3.7	4.1
		SD		1.56	0.25		0.0231	0.0044		0.45	0.23
		Min.		10.5	8.4		0.074	0.076		3.3	4.1
		Max.		13.6	8.9		0.120	0.084		4.2	4.5

River/Site	Depurated[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
ERM2.5	Yes	FOD	4	3	3	4	3	3	4	3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	8.4	11.6	9.1	0.053	0.084	0.092	3.78	4.10	4.43
		Median	8.4	11.3	8.9	0.052	0.083	0.096	3.8	3.9	4.3
		SD	0.46	1.32	0.96	0.0039	0.0012	0.0112	0.10	0.35	0.32
		Min.	7.9	10.4	8.2	0.050	0.083	0.079	3.7	3.9	4.2
		Max.	9.0	13.0	10.1	0.059	0.085	0.100	3.9	4.5	4.8
ERM1.0	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		12.7	10.3		0.112	0.103		5.27	5.03
		Median		12.3	10.6		0.110	0.100		5.4	4.9
		SD		1.06	1.14		0.0176	0.0061		0.51	0.32
		Min.		11.9	9.0		0.095	0.099		4.7	4.8
		Max.		13.9	11.2		0.130	0.110		5.7	5.4
ERM1.0	Yes	FOD	4	3	3	4	3	3	4	3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	10.2	13.2	11.1	0.061	0.087	0.102	5.45	5.53	4.70
		Median	10.5	13.4	11.0	0.062	0.099	0.100	5.5	6.0	4.7
		SD	1.78	1.56	1.21	0.0202	0.0211	0.0072	0.66	0.90	0.60
		Min.	7.8	11.6	10.0	0.037	0.063	0.096	4.7	4.5	4.1
		Max.	12.0	14.7	12.4	0.081	0.100	0.110	6.1	6.1	5.3
Clinch River											
CRM6.0	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		13.5	13.3		0.273	0.293		4.53	4.53
		Median		13.9	12.5		0.280	0.260		4.5	4.2
		SD		0.81	2.79		0.0208	0.0945		0.25	0.67
		Min.		12.6	11.0		0.250	0.220		4.3	4.1
		Max.		14.1	16.4		0.290	0.400		4.8	5.3
CRM6.0	Yes	FOD	4	3	3	4	3	3	4	3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	10.8	16.7	14.0	0.130	0.280	0.260	4.33	4.73	4.37
		Median	11.1	17.1	13.1	0.120	0.270	0.240	4.3	4.7	4.4
		SD	1.09	1.39	3.73	0.0200	0.0458	0.1114	0.33	0.15	0.06
		Min.	9.3	15.2	10.8	0.120	0.240	0.160	4.0	4.6	4.3
		Max.	11.6	17.9	18.1	0.160	0.330	0.380	4.7	4.9	4.4

River/Site	Depurated[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
CRM3.5	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		14.2	13.8		0.130	0.167		5.60	5.90
		Median		13.8	13.2		0.140	0.150		5.3	6.2
		SD		0.84	2.85		0.0265	0.0379		1.08	0.79
		Min.		13.7	11.3		0.100	0.140		4.7	5.0
		Max.		15.2	16.9		0.150	0.210		6.8	6.5
CRM3.5	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		14.5	13.3		0.143	0.130		6.07	5.10
		Median		15.6	14.6		0.150	0.120		6.6	5.3
		SD		4.02	2.48		0.0404	0.0173		1.38	0.72
		Min.		10.0	10.4		0.100	0.120		4.5	4.3
		Max.		17.8	14.8		0.180	0.150		7.1	5.7
CRM1.5	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		16.6	11.4		0.220	0.140		6.63	6.07
		Median		18.1	10.1		0.230	0.130		7.7	5.1
		SD		4.44	3.40		0.0265	0.0361		2.02	2.12
		Min.		11.6	8.9		0.190	0.110		4.3	4.6
		Max.		20.1	15.3		0.240	0.180		7.9	8.5
CRM1.5	Yes	FOD	4	3	3		3	3		3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	10.6	16.2	12.8	0.068	0.177	0.143	4.95	6.77	5.73
		Median	10.3	17.5	10.9	0.073	0.190	0.130	4.8	7.4	5.0
		SD	0.79	4.34	4.71	0.0168	0.0416	0.0416	1.01	2.80	1.54
		Min.	9.9	11.4	9.4	0.045	0.130	0.110	3.9	3.7	4.7
		Max.	11.7	19.8	18.2	0.082	0.210	0.190	6.3	9.2	7.5
Little Emory River											
LERM1.0	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		10.9	8.6		0.186	0.098		5.27	4.47
		Median		11.9	8.6		0.210	0.100		5.7	4.4
		SD		3.27	1.15		0.0782	0.0137		1.40	0.70
		Min.		7.2	7.4		0.099	0.083		3.7	3.8
		Max.		13.5	9.7		0.250	0.110		6.4	5.2

River/Site	Depurated[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
Tennessee River											
TRM572.5	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		5.3	8.0		0.043	0.070		2.23	2.47
		Median		5.5	8.4		0.042	0.074		2.3	2.6
		SD		0.47	1.21		0.0031	0.0131		0.12	0.51
		Min.		4.8	6.6		0.040	0.055		2.1	1.9
		Max.		5.7	8.9		0.046	0.080		2.3	2.9
TRM566.7	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		10.5	9.5		0.103	0.125		3.17	3.43
		Median		10.4	8.6		0.100	0.130		3.2	3.5
		SD		1.90	1.56		0.0061	0.0284		0.06	0.21
		Min.		8.7	8.6		0.099	0.094		3.1	3.2
		Max.		12.5	11.3		0.110	0.150		3.2	3.6

[1]FOD = Frequency of detection; N = number of samples (includes only samples equal to or greater than the method detection limit); Mean = unless otherwise noted, means are based on results for samples in which the specific metal was detected; SD = standard deviation; Min. = minimum concentration detected; Max. = maximum concentration detected.

[2]The summary data provided for mercury is based only on the results from samples analyzed with EPA6020 and not EPA7473 (2011 samples only). While results from EPA6020 cannot be considered reliable for evaluating absolute concentrations, they still can be used to identify important spatial and temporal trends where they may exist.

Table 2. Descriptive statistics for arsenic, mercury, and selenium concentrations (total metal, µg/g dry weight) in mayfly nymphs (*Hexagenia bilineata*) from the Emory River, Clinch River, Little Emory River, and Tennessee River, 2009 through 2011.

			Arsenic			Mercury (EPA6020 only)[3]			Selenium		
River/Site	Depurated[1]	Statistic[2]	2009	2010	2011	2009	2010	2011	2009	2010	2011
Emory River											
ERM6.0	No	FOD	4	3	3	4	3	3	4	3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	2.93	3.00	2.37	0.057	0.059	0.055	1.98	2.83	2.57
		Median	2.95	3.00	2.40	0.059	0.060	0.053	2.0	2.7	2.5
		SD	0.350	0.200	0.153	0.0066	0.0032	0.0059	0.096	0.321	0.208
		Min.	2.50	2.80	2.20	0.047	0.055	0.051	1.9	2.6	2.4
		Max.	3.30	3.20	2.50	0.062	0.061	0.062	2.1	3.2	2.8
ERM6.0	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.95	1.08		0.043	0.049		2.40	3.13
		Median		0.90	1.00		0.043	0.052		2.3	3.1
		SD		0.132	0.193		0.0045	0.0089		0.265	0.153
		Min.		0.85	0.94		0.039	0.039		2.2	3.0
		Max.		1.10	1.30		0.048	0.056		2.7	3.3
ERM4.0	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		7.37	5.20		0.068	0.068		4.57	3.47
		Median		7.20	4.80		0.067	0.070		4.9	3.7
		SD		0.666	0.781		0.0026	0.0038		0.666	0.493
		Min.		6.80	4.70		0.066	0.064		3.8	2.9
		Max.		8.10	6.10		0.071	0.071		5.0	3.8
ERM4.0	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		3.10	2.33		0.058	0.068		3.73	3.80
		Median		3.10	2.30		0.057	0.068		3.8	3.6
		SD		0.500	0.252		0.0042	0.0015		0.404	0.346
		Min.		2.60	2.10		0.055	0.066		3.3	3.6
		Max.		3.60	2.60		0.063	0.069		4.1	4.2

River/Site	Depurated[1]	Statistic[2]	Arsenic 2009	Arsenic 2010	Arsenic 2011	Mercury (EPA6020 only)[3] 2009	Mercury (EPA6020 only)[3] 2010	Mercury (EPA6020 only)[3] 2011	Selenium 2009	Selenium 2010	Selenium 2011
ERM2.5	No	FOD	5	3	3	3	3	3	5	3	3
		N	5	3	3	5[3]	3	3	5	3	3
		Mean	14.30	14.87	10.90	0.067	0.077	0.074	4.58	7.00	4.83
		Median	14.50	13.90	12.30	0.062	0.078	0.071	4.2	7.2	4.9
		SD	2.935	2.401	2.689	0.0134	0.0012	0.0074	0.756	0.346	0.115
		Min.	9.40	13.10	7.80	0.053	0.076	0.068	3.9	6.6	4.7
		Max.	16.90	17.60	12.60	0.088	0.078	0.082	5.4	7.2	4.9
ERM2.5	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		2.93	3.90		0.050	0.065		5.50	7.00
		Median		3.30	3.40		0.049	0.063		5.8	6.6
		SD		0.723	2.095		0.0031	0.0072		0.608	0.693
		Min.		2.10	2.10		0.047	0.059		4.8	6.6
		Max.		3.40	6.20		0.053	0.073		5.9	7.8
ERM1.0	No	FOD	4	3	3	4	3	3	4	3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	39.53	46.20	26.60	0.128	0.112	0.108	6.40	9.67	6.73
		Median	40.35	50.60	26.30	0.130	0.100	0.110	6.4	10.8	6.5
		SD	7.828	11.170	5.456	0.0126	0.0247	0.0131	0.082	2.967	0.493
		Min.	29.20	33.50	21.30	0.11	0.095	0.094	6.3	6.3	6.4
		Max.	48.20	54.50	32.20	0.14	0.14	0.12	6.5	11.9	7.3
ERM1.0	Yes	FOD	2	3	3	2	3	3	2	3	3
		N	2	3	3	2	3	3	2	3	3
		Mean	21.25	31.47	10.17	0.090	0.045	0.058	5.90	8.00	9.57
		Median	21.25	34.60	10.20	0.090	0.046	0.057	5.9	8.4	9.5
		SD	10.819	12.011	0.850	0.0424	0.0040	0.0090	0.000	0.964	0.208
		Min.	13.60	18.20	9.30	0.060	0.041	0.049	5.9	6.9	9.4
		Max.	28.90	41.60	11.00	0.120	0.049	0.067	5.9	8.7	9.8
Clinch River											
CRM6.0	No	FOD	4	3	3	4	3	3	4	3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	4.80	4.97	4.93	0.753	1.037	0.943	3.95	4.47	3.83
		Median	4.75	5.00	4.80	0.735	1.100	0.720	4.0	4.6	3.9
		SD	0.497	0.252	0.416	0.1384	0.2026	0.3955	0.208	0.231	0.208
		Min.	4.30	4.70	4.60	0.62	0.81	0.71	3.7	4.2	3.6
		Max.	5.40	5.20	5.40	0.92	1.2	1.4	4.2	4.6	4.0

River/Site	Depurated[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)[3]			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
CRM6.0	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		1.93	1.27		0.150	0.167		4.20	4.07
		Median		2.10	1.20		0.160	0.160		4.1	4.0
		SD		0.379	0.115		0.0173	0.0208		0.265	0.115
		Min.		1.50	1.20		0.130	0.150		4.0	4.0
		Max.		2.20	1.40		0.160	0.190		4.5	4.2
CRM3.5	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		14.57	12.63		0.273	0.383		7.03	6.47
		Median		13.70	12.30		0.260	0.360		7.1	6.7
		SD		1.589	0.850		0.0231	0.0493		0.404	0.493
		Min.		13.60	12.00		0.26	0.35		6.6	5.9
		Max.		16.40	13.60		0.3	0.44		7.4	6.8
CRM3.5	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		4.50	2.87		0.090	0.113		7.90	7.30
		Median		4.50	2.60		0.088	0.099		7.8	7.3
		SD		0.800	0.551		0.0078	0.0327		0.361	0.100
		Min.		3.70	2.50		0.084	0.089		7.6	7.2
		Max.		5.30	3.50		0.099	0.150		8.3	7.4
CRM1.5	No	FOD	4	3	3	4	3	3	4	3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	13.83	12.73	12.23	0.368	0.273	0.347	5.38	6.43	5.93
		Median	14.05	12.60	12.40	0.365	0.270	0.340	5.4	6.6	5.9
		SD	1.434	1.804	1.258	0.0206	0.0153	0.0208	0.050	0.289	0.351
		Min.	12.00	11.00	10.90	0.35	0.26	0.33	5.3	6.1	5.6
		Max.	15.20	14.60	13.40	0.39	0.29	0.37	5.4	6.6	6.3
CRM1.5	Yes	FOD	2	3	3	0	3	3	2	3	3
		N	2	3	3	2[3]	3	3	2	3	3
		Mean	4.50	3.73	3.63	0.082	0.081	0.098	4.75	7.13	6.50
		Median	4.50	4.50	4.00	-	0.086	0.095	4.8	7.1	6.6
		SD	0.849	1.504	1.582	0.0049	0.0101	0.0202	0.495	0.252	0.361
		Min.	3.90	2.00	1.90	0.079	0.069	0.080	4.4	6.9	6.1
		Max.	5.10	4.70	5.00	0.086	0.087	0.120	5.1	7.4	6.8

River/Site	Depurated[1]	Statistic[2]	Arsenic 2009	Arsenic 2010	Arsenic 2011	Mercury (EPA6020 only)[3] 2009	Mercury (EPA6020 only)[3] 2010	Mercury (EPA6020 only)[3] 2011	Selenium 2009	Selenium 2010	Selenium 2011
Little Emory River											
LERM1.0	No	FOD	3	3	3	3	3	3	3	3	3
		N	3	3	3	3	3	3	3	3	3
		Mean	4.30	3.90	3.77	0.084	0.059	0.067	3.30	3.93	3.50
		Median	4.30	3.60	3.50	0.084	0.059	0.069	3.3	3.9	3.5
		SD	0.300	0.520	0.462	0.0045	0.0070	0.0029	0.100	0.252	0.200
		Min.	4.00	3.60	3.50	0.08	0.052	0.064	3.2	3.7	3.3
		Max.	4.60	4.50	4.30	0.089	0.066	0.069	3.4	4.2	3.7
Tennessee River											
TRM572.5	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		5.30	3.17		0.055	0.051		4.40	2.03
		Median		5.70	3.00		0.056	0.049		4.5	2.0
		SD		0.781	0.379		0.0032	0.0029		0.361	0.058
		Min.		4.40	2.90		0.051	0.049		4.0	2.0
		Max.		5.80	3.60		0.057	0.054		4.7	2.1
TRM572.5	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		1.43	1.37		0.037	0.028		2.20	2.13
		Median		1.40	1.30		0.039	0.029		2.3	2.1
		SD		0.351	0.115		0.0044	0.0021		0.173	0.058
		Min.		1.10	1.30		0.032	0.026		2.0	2.1
		Max.		1.80	1.50		0.040	0.030		2.3	2.2
TRM566.7	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		9.63	5.37		0.123	0.120		5.80	3.60
		Median		9.40	5.20		0.130	0.120		5.8	3.6
		SD		0.681	0.569		0.0115	0.0100		0.200	0.000
		Min.		9.10	4.90		0.11	0.11		5.6	3.6
		Max.		10.40	6.00		0.13	0.13		6.0	3.6

River/Site	Depurated[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)[3]			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
TRM566.7	Yes	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		1.83	1.23		0.041	0.044		3.63	3.80
		Median		1.70	1.20		0.041	0.046		3.7	3.8
		SD		0.321	0.058		0.0045	0.0035		0.306	0.100
		Min.		1.60	1.20		0.036	0.040		3.3	3.7
		Max.		2.20	1.30		0.045	0.046		3.9	3.9
TRM560.8	No	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		10.30	6.87		0.160	0.180		5.47	4.03
		Median		10.60	6.90		0.170	0.180		5.6	4.1
		SD		0.889	0.451		0.0173	0.0100		0.513	0.306
		Min.		9.30	6.40		0.14	0.17		4.9	3.7
		Max.		11.00	7.30		0.17	0.19		5.9	4.3

[1]FOD = Frequency of detection; N = number of samples (includes only samples equal to or greater than the method detection limit); Mean = unless otherwise noted, means are based on results for samples in which the specific metal was detected; SD = standard deviation; Min. = minimum concentration detected; Max. = maximum concentration detected.

[2]The summary data provided for mercury is based only on the results from samples analyzed with EPA6020 and not EPA7473 (2011 samples only). While results from EPA6020 cannot be considered reliable for evaluating absolute concentrations, they still can be used to identify important spatial and temporal trends where they may exist.

[3]For samples in which the target metal was not detected, the method detection limit was used in calculating descriptive statistics.

Table 3. Descriptive statistics for arsenic, mercury, and selenium concentrations (total metal, µg/g dry weight) in adult mayflies (*Hexagenia bilineata*) from the Emory River, Clinch River, Little Emory River, and Tennessee River, 2009 through 2011.

			Arsenic			Mercury (EPA6020 only)			Selenium		
River/Site	Sex/Adult stage[1]	Statistic[2]	2009	2010	2011	2009	2010	2011	2009	2010	2011
Emory River											
ERM4	MI	FOD	4		3	4		3	4		3
		N	4		3	4		3	4		3
		Mean	0.148		0.113	0.073		0.063	4.83		4.97
		Median	0.150		0.110	0.074		0.064	4.80		4.90
		SD	0.0050		0.0153	0.0020		0.0056	0.126		0.115
		Min.	0.140		0.100	0.070		0.057	4.70		4.90
		Max.	0.150		0.130	0.074		0.068	5.00		5.10
ERM3	FI	FOD		3			3			3	
		N		3			3			3	
		Mean		0.523			0.031			7.00	
		Median		0.520			0.032			6.80	
		SD		0.0058			0.0017			0.436	
		Min.		0.520			0.029			6.70	
		Max.		0.530			0.032			7.50	
ERM3	FS	FOD			3			3			3
		N			3			3			3
		Mean			0.387			0.046			6.87
		Median			0.420			0.046			7.30
		SD			0.0577			0.0035			0.929
		Min.			0.320			0.043			5.80
		Max.			0.420			0.050			7.50
ERM3	MI	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.380	0.253		0.037	0.064		6.73	8.27
		Median		0.390	0.250		0.037	0.064		6.70	8.20
		SD		0.0265	0.0153		0.0006	0.0055		0.252	0.306
		Min.		0.350	0.240		0.036	0.059		6.50	8.00
		Max.		0.400	0.270		0.037	0.070		7.00	8.60

River/Site	Sex/Adult stage[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
ERM3	MS	FOD			3			3			3
		N			3			3			3
		Mean			0.290			0.060			7.53
		Median			0.290			0.060			7.70
		SD			0.0100			0.0035			0.289
		Min.			0.280			0.057			7.20
		Max.			0.300			0.064			7.70
ERM2	FI	FOD		3			3			3	
		N		3			3			3	
		Mean		0.500			0.028			8.17	
		Median		0.500			0.027			8.40	
		SD		0.0000			0.0012			0.404	
		Min.		0.500			0.027			7.70	
		Max.		0.500			0.029			8.40	
ERM2	FS	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.590	0.203		0.027	0.042		8.27	6.47
		Median		0.600	0.220		0.027	0.043		8.30	6.50
		SD		0.0361	0.0379		0.0025	0.0031		0.153	0.252
		Min.		0.550	0.160		0.025	0.039		8.10	6.20
		Max.		0.620	0.230		0.030	0.045		8.40	6.70
ERM2	MI	FOD	2	3	3	2	3	3	2	3	3
		N	2	3	3	2	3	3	2	3	3
		Mean	0.220	0.363	0.133	0.052	0.031	0.053	4.20	7.63	5.50
		Median	0.220	0.360	0.140	0.052	0.032	0.053	4.20	7.70	5.50
		SD	0.0141	0.0058	0.0115	0.0021	0.0042	0.0025	0.000	0.208	0.100
		Min.	0.210	0.360	0.120	0.050	0.026	0.050	4.20	7.40	5.40
		Max.	0.230	0.370	0.140	0.053	0.034	0.055	4.20	7.80	5.60
ERM2	MS	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.430	0.183		0.029	0.055		7.57	5.70
		Median		0.450	0.190		0.029	0.055		7.70	5.70
		SD		0.0346	0.0115		0.0020	0.0015		0.611	0.100
		Min.		0.390	0.170		0.027	0.053		6.90	5.60
		Max.		0.450	0.190		0.031	0.056		8.10	5.80

River/Site	Sex/Adult stage[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
ERM1	FS	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.307	0.433		0.038	0.042		7.37	10.93
		Median		0.300	0.450		0.038	0.042		7.40	10.70
		SD		0.0115	0.0289		0.0006	0.0015		0.058	0.777
		Min.		0.300	0.400		0.038	0.040		7.30	10.30
		Max.		0.320	0.450		0.039	0.043		7.40	11.80
ERM1	MI	FOD	2	3	3	2	3	3	2	3	3
		N	2	3	3	2	3	3	2	3	3
		Mean	0.345	0.320	0.267	0.058	0.048	0.056	6.50	6.47	8.93
		Median	0.345	0.290	0.260	0.058	0.049	0.056	6.50	6.90	8.90
		SD	0.0495	0.0794	0.0115	0.0035	0.0026	0.0045	0.000	0.751	0.153
		Min.	0.310	0.260	0.260	0.055	0.045	0.051	6.50	5.60	8.80
		Max.	0.380	0.410	0.280	0.060	0.050	0.060	6.50	6.90	9.10
ERM1	MS	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.317	0.283		0.042	0.050		6.77	9.37
		Median		0.310	0.280		0.043	0.050		6.80	9.40
		SD		0.0208	0.0351		0.0012	0.0010		0.252	0.058
		Min.		0.300	0.250		0.041	0.049		6.50	9.30
		Max.		0.340	0.320		0.043	0.051		7.00	9.40
ERM0.6	FS	FOD		3			3			3	
		N		3			3			3	
		Mean		0.283			0.050			7.97	
		Median		0.290			0.050			8.10	
		SD		0.0115			0.0030			0.321	
		Min.		0.270			0.047			7.60	
		Max.		0.290			0.053			8.20	
ERM0.6	MI	FOD		3			3			3	
		N		3			3			3	
		Mean		0.233			0.048			6.87	
		Median		0.230			0.048			6.90	
		SD		0.0058			0.0020			0.252	
		Min.		0.230			0.046			6.60	
		Max.		0.240			0.050			7.10	

River/Site	Sex/Adult stage[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
ERM0.6	MS	FOD		3			3			3	
		N		3			3			3	
		Mean		0.230			0.065			7.20	
		Median		0.230			0.066			7.20	
		SD		0.0000			0.0040			0.300	
		Min.		0.230			0.061			6.90	
		Max.		0.230			0.069			7.50	
ERM0.1	MI	FOD			3			3			3
		N			3			3			3
		Mean			0.187			0.094			6.30
		Median			0.180			0.096			6.40
		SD			0.0503			0.0053			0.361
		Min.			0.140			0.088			5.90
		Max.			0.240			0.098			6.60
Clinch River											
CRM6	FS	FOD	3		3	3		3	3		3
		N	3		3	3		3	3		3
		Mean	0.327		0.220	0.088		0.147	3.33		4.33
		Median	0.340		0.220	0.089		0.150	3.40		4.50
		SD	0.0321		0.0200	0.0042		0.0058	0.306		0.379
		Min.	0.290		0.200	0.083		0.140	3.00		3.90
		Max.	0.350		0.240	0.091		0.150	3.60		4.60
CRM6	MI	FOD		6	3		6	3		6	3
		N		6	3		6	3		6	3
		Mean		0.123	0.143		0.113	0.150		3.88	4.33
		Median		0.125	0.150		0.110	0.150		3.85	4.40
		SD		0.0121	0.0115		0.0103	0.0000		0.147	0.115
		Min.		0.110	0.130		0.100	0.150		3.70	4.20
		Max.		0.140	0.150		0.130	0.150		4.10	4.40
CRM6	MS	FOD	3		3	3		3	3		3
		N	3		3	3		3	3		3
		Mean	0.360		0.187	0.095		0.173	3.10		4.33
		Median	0.360		0.180	0.088		0.170	3.10		4.30
		SD	0.0700		0.0115	0.0130		0.0058	0.100		0.153
		Min.	0.290		0.180	0.087		0.170	3.00		4.20
		Max.	0.430		0.200	0.110		0.180	3.20		4.50

River/Site	Sex/Adult stage[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
CRM3	FI	FOD		3			3			3	
		N		3			3			3	
		Mean		0.240			0.059			6.20	
		Median		0.240			0.058			6.30	
		SD		0.0200			0.0021			0.265	
		Min.		0.220			0.057			5.90	
		Max.		0.260			0.061			6.40	
CRM3	FS	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.240	0.337		0.058	0.059		6.50	9.00
		Median		0.230	0.340		0.057	0.060		6.50	9.10
		SD		0.0361	0.0351		0.0026	0.0032		0.100	0.265
		Min.		0.210	0.300		0.056	0.055		6.40	8.70
		Max.		0.280	0.370		0.061	0.061		6.60	9.20
CRM3	MI	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.217	0.203		0.078	0.080		5.97	7.70
		Median		0.220	0.200		0.078	0.079		5.90	7.70
		SD		0.0252	0.0153		0.0015	0.0051		0.208	0.200
		Min.		0.190	0.190		0.077	0.076		5.80	7.50
		Max.		0.240	0.220		0.080	0.086		6.20	7.90
CRM3	MS	FOD			3			3			3
		N			3			3			3
		Mean			0.207			0.072			7.67
		Median			0.210			0.071			7.70
		SD			0.0058			0.0012			0.058
		Min.			0.200			0.071			7.60
		Max.			0.210			0.073			7.70
CRM1.5	FI	FOD	1	3	3	1	3	3	1	3	3
		N	1	3	3	1	3	3	1	3	3
		Mean	0.170	0.200	0.277	0.019	0.043	0.076	3.40	6.27	8.87
		Median	0.170	0.180	0.270	0.019	0.042	0.075	3.40	6.10	8.80
		SD		0.0529	0.0115		0.0026	0.0046		0.379	0.306
		Min.	0.170	0.160	0.270	0.019	0.041	0.072	3.40	6.00	8.60
		Max.	0.170	0.260	0.290	0.019	0.046	0.081	3.40	6.70	9.20

River/Site	Sex/Adult stage[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
CRM1.5	FS	FOD			3			3			3
		N			3			3			3
		Mean			0.273			0.076			8.57
		Median			0.270			0.068			8.70
		SD			0.0153			0.0144			0.907
		Min.			0.260			0.068			7.60
		Max.			0.290			0.093			9.40
CRM1.5	MI	FOD	3	3	3	3	3	3	3	3	3
		N	3	3	3	3	3	3	3	3	3
		Mean	0.290	0.163	0.170	0.036	0.052	0.091	4.83	5.33	7.90
		Median	0.310	0.170	0.170	0.036	0.053	0.092	5.00	5.40	8.00
		SD	0.0346	0.0115	0.0100	0.0025	0.0042	0.0101	0.473	0.115	0.265
		Min.	0.250	0.150	0.160	0.033	0.047	0.080	4.30	5.20	7.60
		Max.	0.310	0.170	0.180	0.038	0.055	0.100	5.20	5.40	8.10
Little Emory River											
LERM1.0	FI	FOD	4			4			4		
		N	4			4			4		
		Mean	0.153			0.058			3.58		
		Median	0.150			0.058			3.60		
		SD	0.0377			0.0022			0.126		
		Min.	0.110			0.056			3.40		
		Max.	0.200			0.061			3.70		
LERM1.0	FS	FOD			3			3			3
		N			3			3			3
		Mean			0.150			0.052			4.83
		Median			0.150			0.051			4.90
		SD			0.0100			0.0017			0.208
		Min.			0.140			0.051			4.60
		Max.			0.160			0.054			5.00
LERM1.0	MI	FOD	4		3	4		3	4		3
		N	4		3	4		3	4		3
		Mean	0.173		0.054	0.068		0.074	3.35		5.43
		Median	0.165		0.050	0.069		0.074	3.35		5.50
		SD	0.0340		0.0106	0.0033		0.0025	0.058		0.115
		Min.	0.140		0.046	0.063		0.071	3.30		5.30
		Max.	0.220		0.066	0.070		0.076	3.40		5.50

River/Site	Sex/Adult stage[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
LERM1.0	MS	FOD			3			3			3
		N			3			3			3
		Mean			0.130			0.068			3.83
		Median			0.130			0.069			3.90
		SD			0.0100			0.0017			0.115
		Min.			0.120			0.066			3.70
		Max.			0.140			0.069			3.90
Tennessee River											
TRM572.5	FI	FOD			3			3			3
		N			3			3			3
		Mean			0.087			0.031			2.17
		Median			0.090			0.030			2.20
		SD			0.0061			0.0031			0.153
		Min.			0.080			0.028			2.00
		Max.			0.091			0.034			2.30
TRM572.5	FS	FOD		3			3			3	
		N		3			3			3	
		Mean		0.203			0.021			2.00	
		Median		0.200			0.021			2.00	
		SD		0.0551			0.0030			0.000	
		Min.		0.150			0.018			2.00	
		Max.		0.260			0.024			2.00	
TRM572.5	MI	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.110	0.066		0.037	0.035		1.50	1.63
		Median		0.100	0.075		0.038	0.034		1.50	1.60
		SD		0.0265	0.0195		0.0031	0.0026		0.000	0.058
		Min.		0.090	0.044		0.034	0.033		1.50	1.60
		Max.		0.140	0.080		0.040	0.038		1.50	1.70
TRM572.5	MS	FOD		3			3			3	
		N		3			3			3	
		Mean		0.207			0.029			1.80	
		Median		0.200			0.029			1.80	
		SD		0.0115			0.0025			0.100	
		Min.		0.200			0.026			1.70	
		Max.		0.220			0.031			1.90	

River/Site	Sex/Adult stage[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
TRM566.7	FI	FOD	2		3	0		3	2		3
		N	2		3	2[4]		3	2		3
		Mean	0.190		0.170	0.05		0.054	3.80		5.17
		Median	0.190		0.170	0.05		0.053	3.80		5.50
		SD	0.0283		0.0100	0		0.0066	0.141		0.577
		Min.	0.170		0.160	0.05		0.048	3.70		4.50
		Max.	0.210		0.180	0.05		0.061	3.90		5.50
TRM566.7	FS	FOD	4	3	3	1	3	3	4	3	3
		N	4	3	3	4[4]	3	3	4	3	3
		Mean	0.225	0.193	0.170	0.071	0.034	0.051	3.20	4.73	4.97
		Median	0.225	0.190	0.180	0.0525	0.035	0.053	3.20	4.60	5.00
		SD	0.0173	0.0351	0.0173	0.0395	0.0056	0.0062	0.115	0.513	0.252
		Min.	0.210	0.160	0.150	0.049	0.028	0.044	3.10	4.30	4.70
		Max.	0.240	0.230	0.180	0.13	0.039	0.056	3.30	5.30	5.20
TRM566.7	MI	FOD	4	3	3	0	3	3	4	3	3
		N	4	3	3	4[4]	3	3	4	3	3
		Mean	0.165	0.130	0.127	0.0545	0.055	0.060	2.88	3.63	4.17
		Median	0.160	0.130	0.130	0.052	0.056	0.059	2.80	3.60	4.10
		SD	0.0173	0.0100	0.0252	0.00568	0.0032	0.0031	0.222	0.153	0.115
		Min.	0.150	0.120	0.100	0.051	0.051	0.057	2.70	3.50	4.10
		Max.	0.190	0.140	0.150	0.063	0.057	0.063	3.20	3.80	4.30
TRM566.7	MS	FOD	4	3		0	3		4	3	
		N	4	3		4[4]	3		4	3	
		Mean	0.195	0.153		0.0548	0.049		2.80	3.73	
		Median	0.195	0.150		0.0545	0.047		2.70	3.80	
		SD	0.0238	0.0058		0.0021	0.0035		0.200	0.208	
		Min.	0.170	0.150		0.053	0.047		2.70	3.50	
		Max.	0.220	0.160		0.057	0.053		3.10	3.90	
TRM566.3	FI	FOD			3			3			3
		N			3			3			3
		Mean			0.193			0.047			4.43
		Median			0.190			0.047			4.40
		SD			0.0058			0.0025			0.153
		Min.			0.190			0.044			4.30
		Max.			0.200			0.049			4.60

River/Site	Sex/Adult stage[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
TRM566.3	FS	FOD		3			3			3	
		N		3			3			3	
		Mean		0.153			0.027			4.20	
		Median		0.150			0.027			4.10	
		SD		0.0058			0.0010			0.265	
		Min.		0.150			0.026			4.00	
		Max.		0.160			0.028			4.50	
TRM566.3	MI	FOD		3	3		3	3		3	3
		N		3	3		3	3		3	3
		Mean		0.127	0.130		0.050	0.048		3.47	3.43
		Median		0.130	0.130		0.051	0.048		3.50	3.40
		SD		0.0153	0.0100		0.0023	0.0015		0.153	0.153
		Min.		0.110	0.120		0.047	0.046		3.30	3.30
		Max.		0.140	0.140		0.051	0.049		3.60	3.60
TRM566.3	MS	FOD		3			3			3	
		N		3			3			3	
		Mean		0.137			0.040			3.63	
		Median		0.140			0.041			3.60	
		SD		0.0058			0.0012			0.058	
		Min.		0.130			0.039			3.60	
		Max.		0.140			0.041			3.70	
TRM563	FS	FOD		3			3			3	
		N		3			3			3	
		Mean		0.137			0.029			3.87	
		Median		0.140			0.029			3.90	
		SD		0.0153			0.0015			0.252	
		Min.		0.120			0.028			3.60	
		Max.		0.150			0.031			4.10	
TRM563	MI	FOD	4	3	3	4	3	3	4	3	3
		N	4	3	3	4	3	3	4	3	3
		Mean	0.143	0.093	0.101	0.029	0.049	0.043	3.05	3.13	3.37
		Median	0.140	0.090	0.095	0.029	0.048	0.043	3.05	3.10	3.40
		SD	0.0126	0.0055	0.0168	0.0024	0.0040	0.0035	0.129	0.058	0.153
		Min.	0.130	0.089	0.088	0.027	0.045	0.040	2.90	3.10	3.20
		Max.	0.160	0.099	0.120	0.032	0.053	0.047	3.20	3.20	3.50

River/Site	Sex/Adult stage[1]	Statistic[2]	Arsenic			Mercury (EPA6020 only)			Selenium		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
TRM563	MS	FOD		3			3			3	
		N		3			3			3	
		Mean		0.180			0.045			3.10	
		Median		0.180			0.046			3.10	
		SD		0.0300			0.0031			0.200	
		Min.		0.150			0.042			2.90	
		Max.		0.210			0.048			3.30	

[1]FI = Female imago; FS = female subimago; MI = male imago; MS = male subimago.

[2]FOD = Frequency of detection; N = number of samples (includes only samples equal to or greater than the method detection limit); Mean = unless otherwise noted, means are based on results for samples in which the specific metal was detected; SD = standard deviation; Min. = minimum concentration detected; Max. = maximum concentration detected.

[3]The summary data provided for mercury is based only on the results from samples analyzed with EPA6020 and not EPA7473 (2011 samples only). While results from EPA6020 cannot be considered reliable for evaluating absolute concentrations, they still can be used to identify important spatial and temporal trends where they may exist.

[4]For samples in which the target metal was not detected, the method detection limit was used in calculating descriptive statistics.

Appendix G

Preliminary Evaluation of 2011 ARCADIS Results: Great Blue Heron Egg Bioaccumulation

Preliminary Evaluation of 2011 Results:
Great Blue Heron Egg Bioaccumulation

June 2012

Introduction

The Tennessee Valley Authority (TVA) Kingston Fossil Plant (KIF), one of TVA's larger fossil plants, is located at the confluence of the Emory and Clinch Rivers on Watts Bar Reservoir in Roane County, Tennessee. Ash, a by-product of a coal-fired power plant, is stored in unlined containment areas, including a former Dredge Cell. Failure of the Dredge Cell dike released about 5.4 million cubic yards (cy) of coal ash covering approximately 300 acres. Fly ash also entered the channel and overbank areas of the riverine section of the Emory River. While the released fly ash itself is primarily composed of fine silica particles very similar to sand, it also contains trace amounts of arsenic, chromium, copper, lead, mercury, nickel, selenium, thallium, vanadium, zinc, and other metals which occur naturally in the coal.

Evaluations of the spatial extent of ash deposition indicate that ash may have traveled upstream as far as Emory River mile (ERM) 6.0, and as far downstream as Tennessee River mile (TRM) 566 (Jacobs 2010). In the upstream direction, the thickness of ash appeared to diminish quickly beyond about ERM 3.5. In the downstream direction, ash deposition generally diminishes quickly below about ERM 1.0, with pockets of greater depth occurring in depositional areas in the lower Emory River and Clinch River. Downstream of Clinch River mile (CRM) 2.0, ash deposits generally vary from trace amounts to 2 inches. Ash deposition of 0.5 to 1 inch was observed in the Tennessee River at TRM 566 (south of the Clinch River). Only trace amounts of ash have been observed further downstream (Jacobs 2010).

Dredging efforts in the Emory River began on March 20, 2009 and continued until May 29, 2010, and was completed in several phases. Hydraulic dredging in the river began during the first phase, which was an initial dredging pilot program on March 20, 2009. This pilot study continued until July 20, 2009 (during the time-critical removal action). Phase I production dredging began in August 2009 and focused on removing the greatest volume of ash in the quickest time frame to reduce the potential for upstream flooding by clearing the river channel and to minimize downriver migration risk. At the end of the pilot and Phase I dredging, approximately 1.96 million cy of ash had been removed from the river. Phase II dredging began in February 2010 in order to further minimize the potential future ash migration down river. This period of dredging was considered "precision" dredging and was focused on returning the river channel to its original (pre-release) depths while minimizing disturbance of legacy sediment. An estimated 780,000 cy of ash was removed during the Phase II dredging. During dredging operations, turbidity was expected to increase in the immediate area of the dredging. Engineering controls (silt curtains) and operational controls (i.e., reduce cutter head speed, reduce rate of advance, and reverse cutter head rotation) were implemented to minimize suspending solids during the dredging operations.

Great Blue Heron (*Ardea herodias*, hereafter: heron) were selected as a representative aquatic-feeding bird species for the site. Heron are commonly found in Tennessee and are present year-round. They are consumers of primarily fish and invertebrates, but also consume small birds, mammals, amphibians and reptiles. Because of their higher position in the food web, heron have the potential for bioaccumulation of metals or metalloids from their prey items or incidental ingestion of water and sediments. Heron serve as

a useful receptor in order to understand exposure and potential effects of these constituents on the aquatic-feeding bird community.

The main study objectives were to 1) determine the extent of maternal transfer of metals and metalloids to the eggs from an impacted area and a reference area and 2) assess risk to heron by comparing concentrations measured at the study sites with literature-derived effects values, when available.

Study Methods

Collections

A detailed description of methods is available in *Trace Element Concentrations in Great Blue Heron and Osprey: 2009 – 2010* (ARCADIS 2012). Heron occupied two colonies near the site during the 2009, 2010, and 2011 collection periods (Figure 1). These colonies were situated in tree tops at ERM 3.0 and on a tall, steel transmission line structure at TRM 569.5. The ERM 3.0 colony has approximately 50 nests, and the TRM 569.5 colony has approximately 25 nests. A total of 20 eggs were collected in 2011 and were analyzed for 26 metals and metalloids. A summary of the heron egg collections for 2009, 2010, and 2011 are presented below (Exhibit 1).

Exhibit 1. Great Blue Heron Egg Collection Summary

Sampling Location	2009	2010	2011
ERM 3.0	5	9	10
TRM 569.5*	5	4	10

* - Reference location

Summary statistics were calculated (sample size, frequency of detection, minimum and maximum detection and detection limits) for each year and location (Table 1). Spatial trends were evaluated using a parametric, one-sided Dunnett's test for 2011 data. Spatial and temporal trends for 2010 and 2011 data were evaluated using a two-way Analysis of variance (ANOVA) test (Statistical Analysis Software [SAS] version 9.3). Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05.

Each constituent was evaluated for frequency of detection. Constituents with a low frequency of detection were excluded for statistical testing. For all constituents detected in goose eggs with normal or lognormal distribution, two-way ANOVA tests were conducted (SAS version 9.3), evaluating years and location. Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05.

Results and Discussion

Summary statistics were generated for arsenic, mercury, and selenium concentrations measured in heron eggs (Table 1). Arsenic was not detected in any samples and therefore could not be evaluated statistically.

Selenium and mercury were both found above detection limits in all samples. Results for selenium and mercury are discussed below.

In 2011, concentrations of selenium in eggs at ERM 3.0 were higher than in reference eggs ($p < 0.0001$). When comparing 2009, 2010, and 2011 selenium concentrations in eggs, there were statistically significant spatial and temporal differences in egg concentrations collected at ERM 3.0 compared to TRM 569.5 (reference) (Table 2, Figure 2). Mean concentrations of selenium in impacted eggs on the Emory River were higher than mean concentrations of selenium found in eggs at the reference. Mean concentrations were highest in 2010 (3.64 milligrams per kilogram [mg/kg]), but decreased in 2011 (3.46 mg/kg).

Selenium is an essential nutrient at low doses; however, it readily accumulates in birds and can be transferred to developing offspring (Heinz 1996). When accumulated above the required amount, studies have shown effects of selenium on bird reproduction through teratogenesis and offspring mortality, as well as other kinds of sublethal effects. Teratogenic deformities in birds include anophthalmy (missing eyes), spinal and bill deformations, defects in internal organs, and histological abnormalities (Harding et al. 2005; Heinz 1996; Hoffman 2002). Selenium has been evaluated in various field and laboratory studies. Maternal transfer of selenium to avian offspring has been well documented in various species of wild aquatic birds from both Kesterson National Wildlife Refuge (Ohlendorf et al. 1986) and Martins Reservoir (King 1988; King et al. 1994). These field studies have also been corroborated by several laboratory studies using dietary exposures of selenium-dosed food in mallards (Heinz et al. 1987 and 1989).

Selenium concentrations known to cause reproductive or teratogenic effects in birds vary, possibly due to differences in each species' ability to maternally transfer selenium to their young or from species-specific differences in responses to maternally transferred selenium (Fairbrother et al. 1999; Ohlendorf et al. 1986). While selenium has been studied for a number of years and there are many reviews in relation to exposure and effects of selenium in birds, the most recent compilation of selenium effects data in avian eggs (Ohlendorf and Heinz 2011) was used in this evaluation of tissue concentrations. Ohlendorf et al. (1986) found that selenium concentrations of 5 mg/kg dry weight (dw) in black-necked stilt (Himantopus mexicanus) eggs resulted in 20 percent mortality or deformity in embryos, but a re-analysis of the stilt field data indicated only 10 percent mortality results at 21 to 31 mg/kg (Adams et al. 2003) and • 5 mg/kg is useful for estimating background levels but not adverse effects. Studies on other species recently have been reviewed and suggest threshold effects (EC10) concentrations ranging from 7.7 to 60 mg/kg dw in various species of avian eggs (Janz et al. 2010).

Mean concentrations of selenium in impacted eggs in 2009, 2010, and 2011 ranged from 2.9 to 3.64 mg/kg dw (Table 1). These mean selenium concentrations were all below the conservative threshold of 7.7 mg/kg selenium in mallards. Furthermore, all individual selenium concentrations in eggs were also below this threshold.

Mercury data from 2009 and 2010 was measured using ICP-MS (EPA SW-846 6020). In 2011, a more precise method was chosen (EPA SW-846 7473), and a correction factor for the 6020 method results is currently under development. Consequently, only results for 2011 mercury concentrations are discussed. In 2011, concentrations of mercury in eggs on the Emory River were higher than in reference eggs ($p < 0.0001$). Mercury effects have been documented in many literature studies. Ranges of concentrations causing effects have been reviewed and summarized by Thompson et al. (1996), who concluded that mercury concentrations in eggs up to 2.5 mg/kg dw appear to have little detrimental effect on reproduction. The mean concentrations

of mercury in heron eggs collected from ERM 3.0 in 2011 was 0.37 mg/kg dw, and the maximum concentration was 0.56 mg/kg dw. Both of these concentrations for mercury were below this literature-derived effects value.

Conclusions

While concentrations of selenium and mercury were both above detection limits in all samples and indicated spatial differences, with higher concentrations in impacted eggs than reference eggs, both constituents were below their corresponding literature-based thresholds for adverse effects values. Furthermore, temporal evaluations of selenium indicate a potential decrease in selenium concentrations from 2010 to 2011. Arsenic was not detected in any samples, indicating little or no exposure for heron. In summary, the available suggests it is unlikely that adverse effects related to the ash-release at either the individual or population level are occurring for heron.

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ARCADIS

Table 1. Great Blue Heron Egg Summary Statistics
Tennessee Valley Authority Kingston, Tennessee

Analyte	Location	Year	Number of Detects	Number of Samples	Mean[1] ± SD	Range
Arsenic	Reference	2009	0	5	NC ± NC	0.34 - 0.55
	Reference	2010	0	4	NC ± NC	0.1 - 0.16
	Reference	2011	0	10	NC ± NC	0.14 - 0.19
	ERM3.0	2009	0	5	NC ± NC	0.51 - 0.67
	ERM3.0	2010	0	9	NC ± NC	0.13 - 0.18
	ERM3.0	2011	0	10	NC ± NC	0.12 - 0.18
Mercury	Reference	2009	5	5	0.35 ± 0.37	0.11 - 0.99
	Reference	2010	4	4	0.43 ± 0.36	0.21 - 0.96
	Reference	2011	10	10	0.28 ± 0.18	0.11 - 0.59
	ERM3.0	2009	5	5	0.41 ± 0.13	0.26 - 0.6
	ERM3.0	2010	9	9	0.79 ± 0.54	0.2 - 1.99
	ERM3.0	2011	10	10	0.37 ± 0.14	0.17 - 0.56
Selenium	Reference	2009	5	5	2.52 ± 0.61	1.6 - 3.2
	Reference	2010	4	4	2.78 ± 0.17	2.6 - 3
	Reference	2011	10	10	2.12 ± 0.76	1.27 - 3.55
	ERM3.0	2009	5	5	2.9 ± 0.34	2.4 - 3.3
	ERM3.0	2010	9	9	3.64 ± 0.69	2.6 - 5
	ERM3.0	2011	10	10	3.46 ± 0.42	2.54 - 4.09

[1] Mean calculations include reporting limits substituted for non-detects; concentrations presented in milligrams per kilogram dry weight (mg/kg dw).

NA- Not applicable

NC - Not calculated because all samples were non-detects; range represents reporting limits only

Reference - Includes samples collected from Tennessee River Mile 569.5.

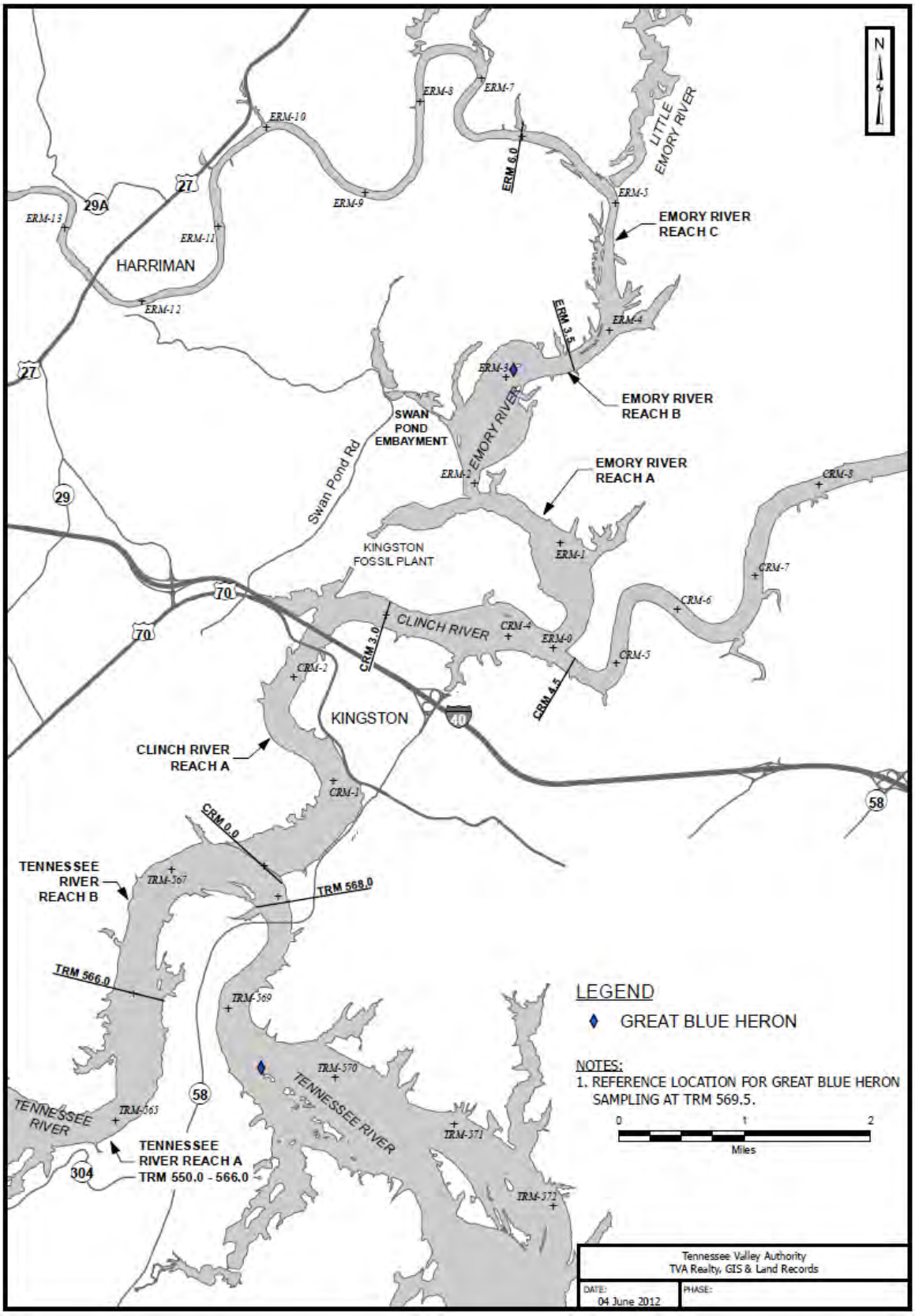
SD- standard deviation

ARCADIS

Table 2. Great Blue Heron Egg ANOVAs - Comparisons by Year and Location
Tennessee Valley Authority Kingston, Tennessee

Analyte	Number of Samples	R^2	Year	Location	Year*Location	Post-hoc Comparisons[1]
Arsenic	NA	NA	NA	NA	NA	NA
Mercury[2]	43	0.31	0.08	0.01	0.86	ERM 2010 > TRM 2011 p = 0.006
Selenium	43	0.54	0.11	< 0.0001	0.11	ERM 2011 > REF 2011; p = 0.0001 ERM 2010 > REF 2011; p < 0.0001 ERM 2010 > REF 2009; p = 0.02

[1] Significant differences from the post-hoc Tukey-Kramer two-tailed test.
[2] Analyte results were log transformed.
ERM - Emory River mile.
NA - Not applicable; constituent was not detected with enough frequency to evaluate statistically.
REF - Reference location; includes samples collected at Tennessee River mile 569.5.
R^2 - Correlation coefficient.



ARCADIS

Great Blue Heron Egg Sampling Locations

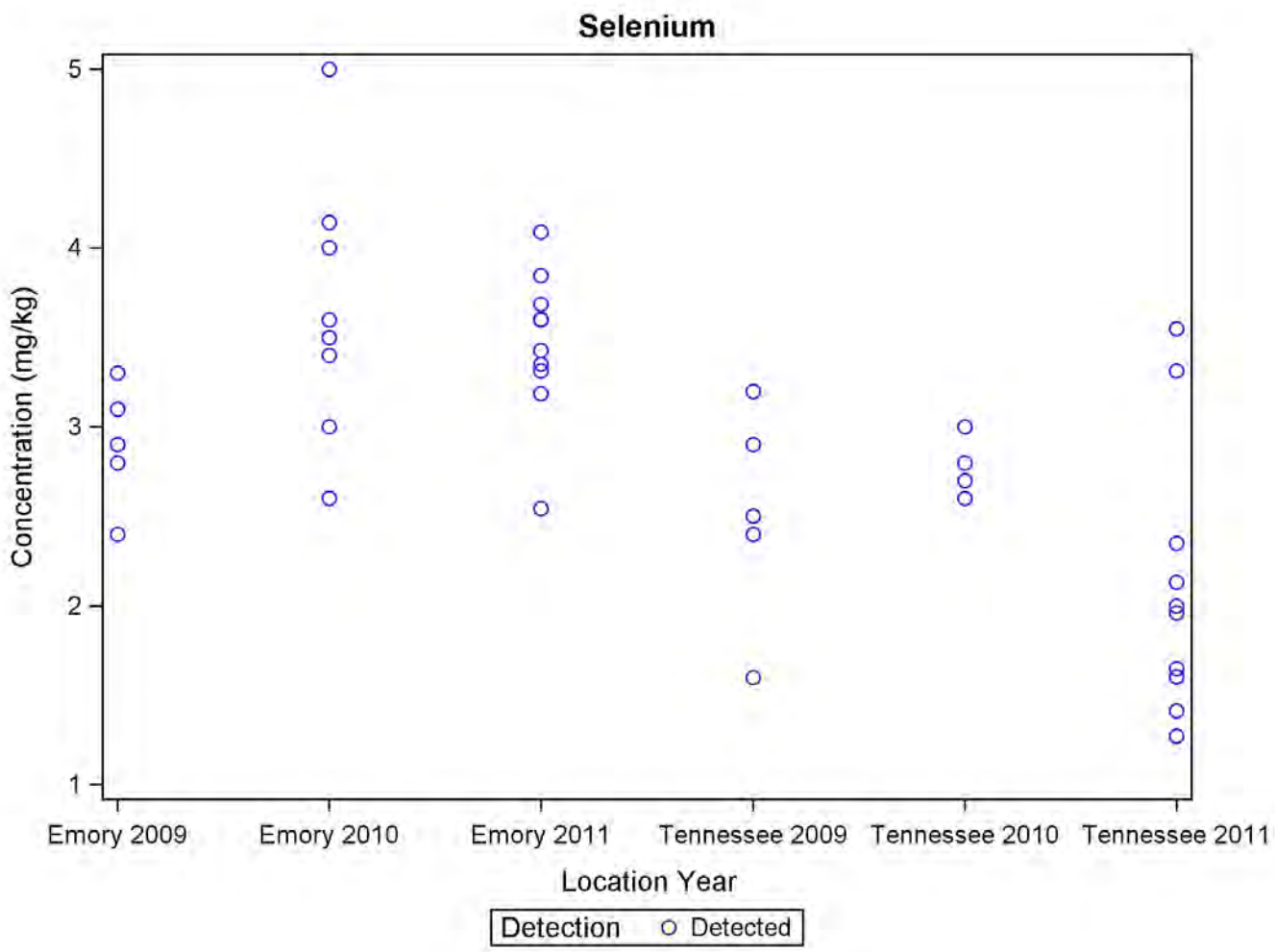
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

TENNESSEE VALLEY AUTHORITY

KINGSTON, TENNESSEE

FIGURE

1



Note: All concentrations presented in milligrams per kilogram dry weight (mg/kg dw).

Reference – Includes samples collected at Tennessee River Mile 569.5.

ARCADIS

Selenium Concentrations in Great Blue Heron Eggs

Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

TENNESSEE VALLEY AUTHORITY

KINGSTON, TENNESSEE

FIGURE

2

Appendix H

Preliminary Evaluation of 2011 ARCADIS Results: Canada Goose Egg Bioaccumulation

Preliminary Evaluation of 2011 Results: Canada Goose Egg Bioaccumulation

June 2012

Introduction

The Tennessee Valley Authority (TVA) Kingston Fossil Plant (KIF), one of TVA's larger fossil plants, is located at the confluence of the Emory and Clinch Rivers on Watts Bar Reservoir in Roane County, Tennessee. Ash, a by-product of a coal-fired power plant, is stored in unlined containment areas, including a former Dredge Cell. Failure of the Dredge Cell dike released about 5.4 million cubic yards (cy) of coal ash covering approximately 300 acres. Fly ash also entered the channel and overbank areas of the riverine section of the Emory River. While the released fly ash itself is primarily composed of fine silica particles very similar to sand, it also contains trace amounts of arsenic, chromium, copper, lead, mercury, nickel, selenium, thallium, vanadium, zinc, and other metals which occur naturally in the coal.

Evaluations of the spatial extent of ash deposition indicate that ash may have traveled upstream as far as Emory River mile (ERM) 6.0, and as far downstream as Tennessee River mile (TRM) 566 (Jacobs 2010). In the upstream direction, the thickness of ash appeared to diminish quickly beyond about ERM 3.5. In the downstream direction, ash deposition generally diminishes quickly below about ERM 1.0, with pockets of greater depth occurring in depositional areas in the lower Emory River and Clinch River. Downstream of Clinch River mile (CRM) 2.0, ash deposits generally vary from trace amounts to 2 inches. Ash deposition of 0.5 to 1 inch was observed in the Tennessee River at TRM 566 (south of the Clinch River). Only trace amounts of ash have been observed further downstream (Jacobs 2010).

Dredging efforts in the Emory River began on March 20, 2009 and continued until May 29, 2010, and was completed in several phases. Hydraulic dredging in the river began during the first phase, which was an initial dredging pilot program on March 20, 2009. This pilot study continued until July 20, 2009 (during the time-critical removal action). Phase I production dredging began in August 2009 and focused on removing the greatest volume of ash in the quickest time frame to reduce the potential for upstream flooding by clearing the river channel and to minimize downriver migration risk. At the end of the pilot and Phase I dredging, approximately 1.96 million cy of ash had been removed from the river. Phase II dredging began in February 2010 in order to further minimize the potential future ash migration down river. This period of dredging was considered "precision" dredging and was focused on returning the river channel to its original (pre-spill) depths while minimizing disturbance of legacy sediment. An estimated 780,000 cy of ash was removed during the Phase II dredging. During dredging operations, turbidity was expected to increase in the immediate area of the dredging. Engineering controls (silt curtains) and operational controls (i.e., reduce cutter head speed, reduce rate of advance, and reverse cutter head rotation) were implemented to minimize suspending solids during the dredging operations.

Canada geese (*Branta canadensis*) were not included as part of the Baseline Ecological Risk Assessment; however, they were observed nesting near KIF and seen consuming vegetation cover established on areas containing residual ash. Canada geese are year-round residents in Tennessee, and they forage by dabbling in the water or grazing on vegetation in fields. Canada geese have the potential for bioaccumulation of metals and metalloids from their prey items or incidental ingestion of water and sediments.

The main study objectives were to 1) determine the extent of maternal transfer of metals and metalloids to the eggs from an impacted area and a reference area and 2) assess risk to Canada geese by comparing concentrations measured at the study sites with literature-derived effects values, when available.

Study Methods

Collections

A detailed description of methods is available in *Trace Element Concentrations and Dietary Exposure Modeling in Canada Geese: 2009 – 2010* (ARCADIS 2012). Canada goose eggs were collected from similar locations sampled in previous years (Figure 1) on the Emory, Clinch, and Tennessee Rivers, following the same sampling procedures from previous years (ARCADIS 2012). A total of 23 eggs were collected in 2011 and were analyzed for 26 metals and metalloids. A summary of the Canada goose egg collections for 2009, 2010, and 2011 are presented below (Exhibit 1).

Exhibit 1. Canada Goose Egg Collection Summary

Sampling Location	2009	2010	2011
ERM 2.0		1	
ERM 2.5			1
ERM 3.0	4	11	9
CRM 2.0			1
CRM 2.5		3	3
CRM 4.0		1	1
TRM 569.5*		5	
TRM 570*			2
TRM 571.5*		5	6

* - Reference location

Summary statistics were calculated (sample size, frequency of detection, minimum and maximum detection and detection limits) for each year and location (Table 1). Spatial trends were evaluated using a parametric, one-sided Dunnett's test for 2011 data. Spatial and temporal trends for 2010 and 2011 data were evaluated using a two-way analysis of variance (ANOVA) test (Statistical Analysis Software [SAS] version 9.3). Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05.

Each constituent was evaluated for frequency of detection. Constituents with a low frequency of detection were excluded for statistical testing. For all constituents detected in goose eggs with normal or lognormal distribution, two-way ANOVA tests were conducted (SAS version 9.3), evaluating years and location. Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05.

Results and Discussion

Summary statistics were generated for arsenic, mercury, and selenium concentrations measured in Canada goose eggs (Table 1). Arsenic was not detected in any samples and mercury was only detected in two out of 50 samples (less than 1 percent detection); as a result, these constituents could not be evaluated statistically. Selenium was found above detection limits in all samples. Results of selenium are discussed below.

In 2011, concentrations of selenium in eggs on the Emory River were higher than in reference eggs ($p < 0.0001$). When comparing 2010 and 2011 selenium concentrations in eggs, there were statistically significant spatial and temporal differences in egg concentrations collected on both the Emory and Clinch Rivers compared to the Tennessee River (reference) and to each other (Table 2, Figure 2). Mean concentrations of selenium in impacted eggs on the Emory River were higher than mean concentrations of selenium found in eggs on the Clinch River and at the reference. Mean concentrations in 2010 were 2.52 milligrams per kilogram (mg/kg) and increased to 4.34 mg/kg in 2011.

Selenium is an essential nutrient at low doses; however, it readily accumulates in birds and can be transferred to developing offspring (Heinz 1996). When accumulated above the required amount, studies have shown effects of selenium on bird reproduction through teratogenesis and offspring mortality, as well as other kinds of sublethal effects. Teratogenic deformities in birds include anophthalmy (missing eyes), spinal and bill deformations, defects in internal organs, and histological abnormalities (Harding et al. 2005; Heinz 1996; Hoffman 2002). Selenium has been evaluated in various field and laboratory studies. Maternal transfer of selenium to avian offspring has been well documented in various species of wild aquatic birds from both Kesterson National Wildlife Refuge (Ohlendorf et al. 1986) and Martins Reservoir (King 1988; King et al. 1994). These field studies have also been corroborated by several laboratory studies using dietary exposures of selenium-dosed food in mallards (Heinz et al. 1987 and 1989).

Selenium concentrations known to cause reproductive or teratogenic effects in birds vary, possibly due to differences in each species' ability to maternally transfer selenium to their young or from species-specific differences in responses to maternally transferred selenium (Fairbrother et al. 1999; Ohlendorf et al. 1986). While selenium has been studied for a number of years and there are many reviews in relation to exposure and effects of selenium in birds, the most recent compilation of selenium effects data in avian eggs (Ohlendorf and Heinz 2011) was used in this evaluation of tissue concentrations. Ohlendorf et al. (1986) found that selenium concentrations of 5 mg/kg dry weight (dw) in black-necked stilt (Himantopus mexicanus) eggs resulted in 20 percent mortality or deformity in embryos, but a re-analysis of the stilt field data indicated only 10 percent mortality results at 21 to 31 mg/kg (Adams et al. 2003) and • 5 mg/kg is useful for estimating background levels but not adverse effects. Studies on other species recently have been reviewed and suggest threshold effects concentrations ranging from 7.7 to 60 mg/kg dw in various species of avian eggs (Janz et al. 2010).

Mean concentrations of selenium in impacted eggs in 2010 and 2011 were 2.52 and 4.34 mg/kg dw, respectively (Table 1). These mean selenium concentrations were below the conservative threshold of 7.7 mg/kg selenium in mallards. Only one sample from the 2011 Emory River collections was greater than this threshold, with a concentration of 8.28 mg/kg dw. Overall, this egg represents approximately 3 percent of all eggs evaluated in the impacted reaches.

Conclusions

While maternal transfer of selenium has been reported in various species of birds (Janz et al. 2010), the past 3 years of monitoring found only one egg from the 2011 Emory River with just 0.5 mg/kg selenium above a threshold value of 7.7 mg/kg dw. At an individual basis and relative to reference sites, geese may have accumulated levels of ash-related selenium in 2009 through 2011. However, it is unlikely that individual egg concentrations have translated to adverse effects related to the ash-release at either the individual or population level. The mean selenium concentrations documented in 2011 also remain below threshold concentrations. Although selenium concentrations in eggs were higher in 2011 than 2009 or 2010, these differences were not statistically significant. In contrast, the majority of the other measured constituents indicate a decreasing trend in exposure may be occurring over time.

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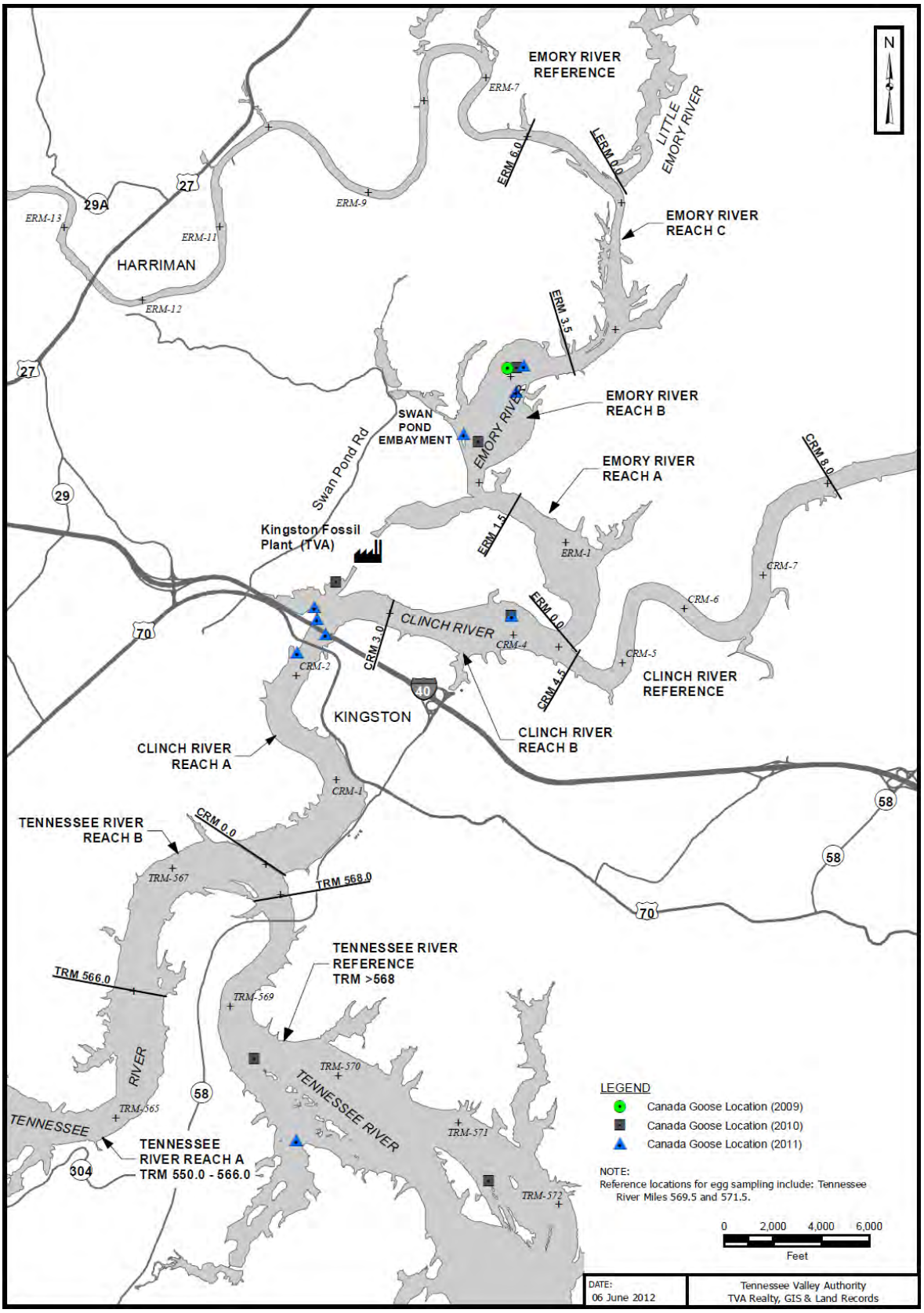
Table 1. Canada Goose Egg Summary Statistics
Tennessee Valley Authority Kingston, Tennessee

Analyte	Location	Year	Number of Detects	Number of Samples	Mean[1] ± SD	Range
Arsenic	Reference	2010	0	10	NC ± NC	0.15 - 0.21
	Reference	2011	0	8	NC ± NC	0.08 - 0.10
	Emory River	2009	0	4	NC ± NC	0.25 - 0.29
	Emory River	2010	0	11	NC ± NC	0.07 - 0.10
	Emory River	2011	0	10	NC ± NC	0.07 - 0.09
	Clinch River	2010	0	4	NC ± NC	0.09 - 0.19
	Clinch River	2011	0	5	NC ± NC	0.09 - 0.09
Mercury	Reference	2010	2	10	0.04 ± 0.003	0.03 - 0.04
	Reference	2011	0	8	NC ± NC	0.03 - 0.04
	Emory River	2009	0	4	NC ± NC	0.05 - 0.06
	Emory River	2010	0	11	NC ± NC	0.02 - 0.08
	Emory River	2011	0	10	NC ± NC	0.03 - 0.04
	Clinch River	2010	0	4	NC ± NC	0.03 - 0.08
	Clinch River	2011	0	5	NC ± NC	0.04 - 0.04
Selenium	Reference	2010	10	10	0.62 ± 0.13	0.42 - 0.86
	Reference	2011	8	8	0.72 ± 0.30	0.47 - 1.39
	Emory River	2009	4	4	2.04 ± 2.31	0.80 - 5.50
	Emory River	2010	11	11	2.52 ± 1.04	0.54 - 4.10
	Emory River	2011	10	10	4.34 ± 2.11	0.68 - 8.28
	Clinch River	2010	4	4	1.10 ± 0.29	0.80 - 1.50
	Clinch River	2011	5	5	0.93 ± 0.25	0.68 - 1.30

[1] Mean calculations include reporting limits substituted for non-detects; concentrations presented in milligrams per kilogram dry weight (mg/kg dw).
NA - Not applicable
NC - Not calculated because all samples were non-detects; range represents reporting limits only
Reference - Includes samples collected upstream of Tennessee River mile 568.0
SD - Standard deviation



ARCADIS

Canada Goose Egg Sampling Locations

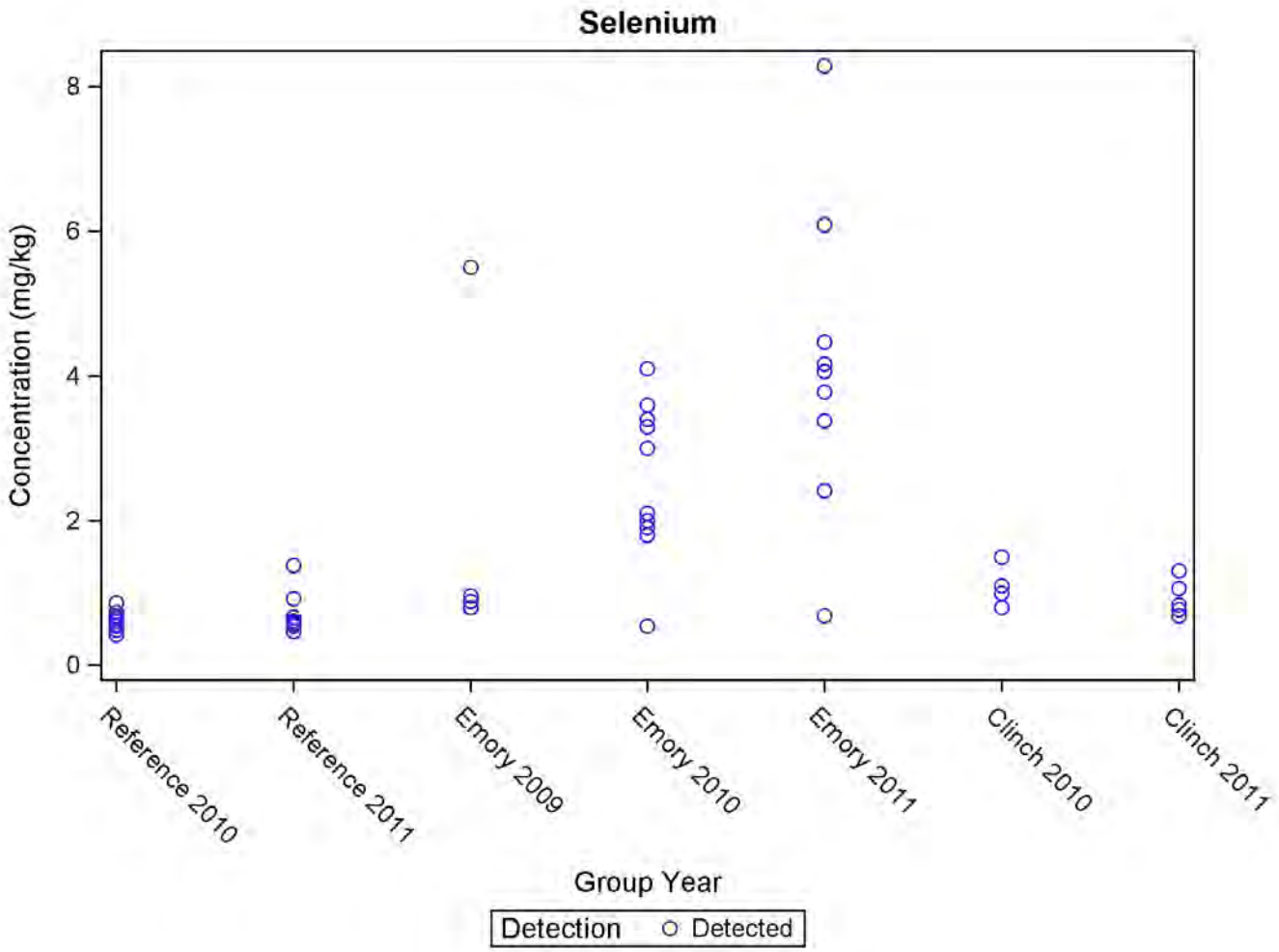
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

TENNESSEE VALLEY AUTHORITY

KINGSTON, TENNESSEE

FIGURE

1



Note: All concentrations presented in milligrams per kilogram dry weight (mg/kg dw).

Reference – Includes samples collected upstream of Tennessee River Mile 568.0

ARCADIS

Selenium Concentrations in Canada Goose Eggs

Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

TENNESSEE VALLEY AUTHORITY

KINGSTON, TENNESSEE

FIGURE

2

Appendix I

Preliminary Evaluation of 2011 ARCADIS Results: Amphibian Whole Body Bioaccumulation

Preliminary Evaluation of 2011 Results:
Amphibian Whole Body Bioaccumulation

June 2012

Introduction

The Tennessee Valley Authority (TVA) Kingston Fossil Plant (KIF), one of TVA's larger fossil plants, is located at the confluence of the Emory and Clinch Rivers on Watts Bar Reservoir in Roane County, Tennessee. Ash, a by-product of a coal-fired power plant, is stored in unlined containment areas, including a former Dredge Cell. Failure of the Dredge Cell dike released about 5.4 million cubic yards (cy) of coal ash covering approximately 300 acres. Fly ash also entered the channel and overbank areas of the riverine section of the Emory River. While the released fly ash itself is primarily composed of fine silica particles very similar to sand, it also contains trace amounts of arsenic, chromium, copper, lead, mercury, nickel, selenium, thallium, vanadium, zinc, and other metals which occur naturally in the coal.

Various groups of organisms were exposed to ash after the spill. Amphibians are of particular concern because 2.51 acres of wetland habitats in the embayments were covered with ash (Jacobs 2009). Amphibians have been considered a "sensitive sentinel to environmental change" due to their unique life cycle which exposes them to aquatic dissolved metals, as well as metals sequestered to sediments (Hopkins and Roe as cited in: Sparling, et al. 2010). Not only are dermal and ingestion exposure pathways to contaminants significant in wetlands, but amphibians are also exposed to contaminants in a variety of their life stages. As a result, elevated concentrations of metals and metalloids have the potential to elicit adverse effects in amphibians.

Accumulation of these ash-related metals and metalloids may start early on for anurans (frogs and toads), as literature studies have suggested maternal transfer of some contaminants into the egg. Shortly after an egg is deposited into the aquatic environment, aqueous uptake of contaminants into the egg can also occur. Both larval and adult anurans experience dermal and respiratory uptake pathways (Birge et al. 2000, Kadokami, et al. 2002, 2004, Hopkins, et al. 2006 as cited in: Sparling, et al. 2010). In addition, for metals such as mercury and selenium, exposure from food sources and sediment is more substantial than concentrations found in the aqueous phase. Other metals such as aluminum and copper are typically taken up via exposures to the water (Pickhardt, et al. 2006 as cited in: Sparling, et al. 2010).

The American toad (*Bufo americanus*), spring peeper (*Pseudacris crucifer*), and the upland chorus frog (*Pseudacris feriarum*) were selected as representative amphibian species for the site, where they are commonly found. They are consumers of small insects, and they are consumed by fish, snakes, birds, turtles, and mammals (Dorcas and Gibbons 2008). Because they consume aquatic and riparian insects and are also consumed by a wide range of receptors, frogs and toads may bioaccumulate metals and metalloids from their prey and transfer concentrations to their consumers. Frogs and toads are a useful receptor in order to understand exposures and potential effects of these constituents on the amphibian community.

Study objectives were to 1) quantify tissue concentrations of metals in three different species for among site and year comparisons, and 2) assess risk to amphibians by relating concentrations measured at the study sites to reference area concentrations and literature derived effects values, when available.

Study Methods

Collections

A detailed description of methods is available in *Trace Element Concentrations in Amphibians: 2009 – 2010* (ARCADIS 2012). Frogs and toads were collected from impacted sites (West and North Embayments) and locations north of the ash release that were identified as unimpacted (Dawson Farm and Rocky Top Farm). Additionally, during the spring 2009 and 2010 collection periods, frogs and toads were sampled from a reference pond at the Timberlake subdivision in Knox County, Tennessee. This reference location was replaced for the spring 2011 collection period with agricultural and forested wetland ditches located on a residential property in Roane County, Tennessee approximately 9 miles northeast of the site (Figure 1). For comparative statistical purposes, Dawson Farm, Rocky Top Farm, and Timberlake or Walls were grouped and referred hereafter as the reference location. A total of 150 amphibians were collected in 2011.

Summary statistics were calculated (sample size, frequency of detection, minimum and maximum detection, and detection limits) for each year and location (Table 1). Spatial trends were evaluated using a parametric, one-sided Dunnett's test for 2011 data. Spatial and temporal trends for 2010 and 2011 data were evaluated using a two-way analysis of variance (ANOVA) test (Statistical Analysis Software [SAS] version 9.3). Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05.

Each constituent was evaluated for frequency of detection. Constituents with a low frequency of detection were excluded for statistical testing. For all constituents detected in amphibians with normal or lognormal distribution, two-way ANOVA tests were conducted (SAS version 9.3), evaluating years and location. Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05.

Results and Discussion

Summary statistics were generated for arsenic, mercury, and selenium concentrations measured in each amphibian species (Tables 1, 2, and 3). Results for each constituent are discussed below.

Selenium was found above detection limits in all samples for all species (Tables 1, 2, and 3; Figures 2, 3, and 4). Selenium concentrations were statistically significantly greater in the West Embayment in 2011 compared to the reference locations for all species. When evaluating spatial trends for all years of data, all species indicated statistically significant differences in concentrations of selenium by locations (Tables 4, 5, and 6). While there were some differences in selenium concentrations between years, these differences were identified between ash-impacted and reference locations from different years and are unlikely to provide a true temporal comparison.

While effects values are limited for amphibians, there are reported selenium control and impacted concentration ranges. The literature ranges for amphibians at control sites ranged from 1.44 to 2.19 milligrams per kilogram (mg/kg) dry weight (dw) selenium, and the impacted areas ranged from 17.40 to 42.40 mg/kg dw selenium (Exhibit 1). In this study, spring peepers and upland chorus frogs all had selenium concentrations

within the reference/control site ranges found in Exhibit 1. This suggests that significant bioaccumulation of selenium has not occurred and that the statistical differences observed may be a reflection of natural variability. Only American toads from ash-impacted locations had reported selenium concentrations comparable to literature ranges for impacted sites. Concentrations of selenium at all sites for all species were lower than those concentrations of selenium known to be embryotoxic to some fish and birds at 4 to 16 mg/kg dw (Lemly 1996; Ohlendorf 2003). As with most field studies collection of mobile species such as American toads, which can migrate at least 0.5 mile during the spring breeding months, are associated with much uncertainty.

Exhibit 1. Literature-Derived Concentrations of Selenium in Anurans

Analyte	Species/ Life Stage	Literature Concentrations (mg/kg dw)	Source
Selenium	*R. clamitans* Metamorphs	Control- 1.44 Impacted- 20.88	Snodgrass et al. 2004
Selenium	*B. terrestris* Adult male	Control- 2.19 Impacted- 17.40	Hopkins et al. 1998
Selenium	*G. carolinensis* Postovopositional	Control-1.85 Impacted- 42.40	Hopkins et al. 2006

Arsenic was not detected with enough frequency to evaluate statistically in spring peepers or upland chorus frogs (less than 30 percent detection). Although some spatial differences were identified in American toad arsenic concentrations (Table 2), temporal comparisons suggest that concentrations of these constituents may be decreasing over time. Arsenic concentrations in toads from the North Embayment in 2011 were statistically significantly higher than concentrations in toads from the reference. However, when comparing arsenic concentrations across years, the concentrations in both embayments in 2011 were lower than corresponding concentrations in 2010 (Table 1).

Mercury was found above detection limits in most samples for all species in 2011 (between 70 and 80 percent detection). Only spatial comparisons could be made for mercury, showing some statistically significantly higher concentrations in mercury concentrations from American toads and spring peepers in the West Embayment compared to the reference ($p = 0.04$ and $p = 0.05$, respectively).

Literature studies have documented mercury effects to amphibian populations, including reduced survival, growth, and development (Gerstenberger and Pearson 2002). Studies have found mercury concentrations to be elevated in anurans from mercuric sulfate impacted sites (Bergeron et al. 2011) and historical mining areas (Hothem et al. 2010). The lowest literature effects level for anurans is 0.24 mg/kg total mercury tissue concentrations, from leopard frog larvae (*Rana sphenocephala*), where they experienced decreased metamorphic success (Unrine et al. 2004). In comparison to the larval effects level, which is the more sensitive life-stage, adult anurans from the embayments and reference sites all had less than 0.24 mg/kg dw. Additionally, typical state health advisories for fish and game consumption typically set the mercury concentration to 0.5 mg/kg and federal regulations are set at 1 mg/kg (Gerstenberger and Pearson 2002).

Conclusions

Some spatial and temporal differences were identified for selenium, arsenic, and mercury in each of the amphibian species. While no literature-based adverse effects values have been established for selenium, concentrations of selenium in amphibians are within literature ranges of control sites with the exception of the American toad. This species of amphibians is known to migrate at least 0.5 mile during the spring breeding months; as a result, interpretation of these data is associated with uncertainty. Similarly, literature-based effects values are lacking for arsenic; however, concentrations of arsenic in amphibians appeared to be decreasing in 2011 compared to 2010. Concentrations of mercury found in amphibians were all below literature-based adverse effects values. Consequently, it is unlikely that adverse effects related to the ash-release at either the individual or population level are occurring for amphibians.

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Table 1. American Toad Whole Body Summary Statistics
Tennessee Valley Authority Kingston, Tennessee

Analyte	Location	Year	Number of Detects	Number of Samples	Mean[1] ± SD	Range
Arsenic	Reference	2009	10	10	2.38 ± 1.68	0.71 – 6.48
	Reference	2010	6	13	0.40 ± 0.27	0.22 – 1.1
	Reference	2011	18	30	0.27 ± 0.35	0.1145 – 1.698
	North Embayment	2009	0	10	NC ± NC	0.36 – 0.47
	North Embayment	2010	10	10	3.45 ± 7.90	0.27 – 25.9
	North Embayment	2011	9	10	0.76 ± 1.05	0.134 – 3.381
	West Embayment	2009	0	11	NC ± NC	0.39 – 0.74
	West Embayment	2010	6	10	0.31 ± 0.13	0.22 – 0.65
	West Embayment	2011	9	10	0.22 ± 0.06	0.1198 – 0.278
Mercury	Reference	2009	1	10	0.09 ± 0.01	0.079 – 0.119
	Reference	2010	1	13	0.10 ± 0.009	0.09 – 0.12
	Reference	2011	23	30	0.10 ± 0.05	0.05106 – 0.2347
	North Embayment	2009	5	10	0.09 ± 0.01	0.072 – 0.11
	North Embayment	2010	1	10	0.11 ± 0.01	0.085 – 0.13
	North Embayment	2011	7	10	0.08 ± 0.03	0.04825 – 0.1476
	West Embayment	2009	6	11	0.11 ± 0.03	0.081 – 0.18
	West Embayment	2010	2	10	0.11 ± 0.02	0.091 – 0.14
	West Embayment	2011	10	10	0.12 ± 0.03	0.08205 – 0.1525
Selenium	Reference	2009	10	10	1.73 ± 0.44	0.893 – 2.3
	Reference	2010	13	13	1.60 ± 0.29	1.1 – 2.248
	Reference	2011	30	30	1.74 ± 0.56	0.766 – 3.136
	North Embayment	2009	10	10	1.40 ± 0.46	0.93 – 2.3
	North Embayment	2010	10	10	2.01 ± 0.54	0.92 – 2.9
	North Embayment	2011	10	10	2.22 ± 0.86	1.195 – 4.279
	West Embayment	2009	11	11	2.03 ± 0.56	1.176 – 3.3
	West Embayment	2010	10	10	2.11 ± 0.45	1.4 – 3
	West Embayment	2011	10	10	2.66 ± 0.77	1.641 – 4.286

[1] Mean calculations include reporting limits substituted for non-detects; concentrations presented in milligrams per kilogram dry weight (mg/kg dw).
NA - Not applicable.
NC - Not calculated because all samples were non-detects; range represents reporting limits only
Reference - Includes Dawson Farm, Rocky Top Farm, Timberlake Pond (2009, 2010), and Walls' Property (2011).
SD - Standard deviation.

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Table 2. Spring Peeper Whole Body Summary Statistics
Tennessee Valley Authority Kingston, Tennessee

Analyte	Location	Year	Number of Detects	Number of Samples	Mean[1] ± SD	Range
Arsenic	Reference	2009	1	23	0.45 ± 0.05	0.43 – 0.66288
	Reference	2010	2	31	0.25 ± 0.03	0.18 – 0.39039
	Reference	2011	4	34	0.11 ± 0.02	0.08651 – 0.1861
	North Embayment	2009	NA	NA	NA ± NA	NA – NA
	North Embayment	2010	0	5	NC ± NC	0.227 – 0.253
	North Embayment	2011	3	12	0.11 ± 0.02	0.0814 – 0.1527
	West Embayment	2009	0	10	NC ± NC	0.3 – 0.441
	West Embayment	2010	0	9	NC ± NC	0.226 – 0.257
	West Embayment	2011	1	10	0.11 ± 0.009	0.09123 – 0.119
Mercury	Reference	2009	5	23	0.09 ± 0.009	0.086 – 0.12
	Reference	2010	0	31	NC ± NC	0.076 – 0.109
	Reference	2011	28	34	0.07 ± 0.04	0.03806 – 0.2172
	North Embayment	2009	NA	NA	NA ± NA	NA – NA
	North Embayment	2010	0	5	NC ± NC	0.093 – 0.104
	North Embayment	2011	4	12	0.05 ± 0.01	0.03488 – 0.07983
	West Embayment	2009	5	10	0.10 ± 0.04	0.06 – 0.179
	West Embayment	2010	2	9	0.12 ± 0.04	0.095 – 0.199
	West Embayment	2011	8	10	0.09 ± 0.05	0.0386 – 0.2022
Selenium	Reference	2009	23	23	1.54 ± 0.35	0.9607 – 2.2
	Reference	2010	31	31	1.39 ± 0.32	0.86463 – 2.32314
	Reference	2011	34	34	1.33 ± 0.28	0.8462 – 2.165
	North Embayment	2009	NA	NA	NA ± NA	NA – NA
	North Embayment	2010	5	5	1.55 ± 0.40	0.996 – 2.049
	North Embayment	2011	12	12	1.43 ± 0.29	1.098 – 1.985
	West Embayment	2009	10	10	2.13 ± 0.57	1.2 – 2.893
	West Embayment	2010	9	9	1.81 ± 0.86	1.061 – 3.858
	West Embayment	2011	10	10	1.77 ± 0.42	0.8421 – 2.143

[1] Mean calculations include reporting limits substituted for non-detects; concentrations presented in milligrams per kilogram dry weight (mg/kg dw).
NA - Not applicable.
NC - Not calculated because all samples were non-detects; range represents reporting limits only.
Reference - Includes Dawson Farm, Rocky Top Farm, Timberlake Pond (2009, 2010), and Walls' Property (2011).
SD - Standard deviation.

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Table 3. Upland Chorus Frog Whole Body Summary Statistics
Tennessee Valley Authority Kingston, Tennessee

Analyte	Location	Year	Number of Detects	Number of Samples	Mean[1] ± SD	Range
Arsenic	Reference	2009	NA	NA	NA ± NA	NA – NA
	Reference	2010	5	12	0.26 ± 0.02	0.236 – 0.318
	Reference	2011	6	30	0.11 ± 0.03	0.06959 – 0.2132
	North Embayment	2009	5	9	0.70 ± 0.43	0.42 – 1.6441
	North Embayment	2010	2	11	0.41 ± 0.19	0.225676856 – 0.81642
	North Embayment	2011	6	10	0.14 ± 0.03	0.08966 – 0.1971
	West Embayment	2009	1	2	1.09 ± 0.93	0.433886463 – 1.74498
	West Embayment	2010	0	11	NC ± NC	0.215502183 – 0.90611
	West Embayment	2011	7	10	0.15 ± 0.07	0.06527 – 0.318
Mercury	Reference	2009	NA	NA	NA ± NA	NA – NA
	Reference	2010	0	12	NC ± NC	0.096 – 0.104
	Reference	2011	24	30	0.07 ± 0.03	0.03351 – 0.1502
	North Embayment	2009	0	9	NC ± NC	0.068 – 0.08803
	North Embayment	2010	0	11	NC ± NC	0.089161572 – 0.11
	North Embayment	2011	5	10	0.05 ± 0.01	0.03534 – 0.07527
	West Embayment	2009	0	2	NC ± NC	0.087248908 – 0.08798
	West Embayment	2010	3	11	0.11 ± 0.02	0.088253275 – 0.15284
	West Embayment	2011	9	10	0.07 ± 0.03	0.03394 – 0.1301
Selenium	Reference	2009	NA	NA	NA ± NA	NA – NA
	Reference	2010	12	12	1.37 ± 0.38	0.987 – 2.157
	Reference	2011	30	30	1.15 ± 0.34	0.507 – 1.818
	North Embayment	2009	9	9	1.54 ± 0.23	1.243231441 – 1.86332
	North Embayment	2010	11	11	1.72 ± 0.43	1.1 – 2.70917
	North Embayment	2011	10	10	1.32 ± 0.16	1.131 – 1.547
	West Embayment	2009	2	2	1.85 ± 0.06	1.807860262 – 1.89039
	West Embayment	2010	11	11	1.96 ± 0.53	0.868558952 – 2.51092
	West Embayment	2011	10	10	1.49 ± 0.43	0.6789 – 2.416

[1] Mean calculations include reporting limits substituted for non-detects; concentrations presented in milligrams per kilogram dry weight (mg/kg dw).
NA - Not applicable.
NC - Not calculated because all samples were non-detects; range represents reporting limits only.
Reference - Includes Dawson Farm, Rocky Top Farm, Timberlake Pond (2009, 2010), and Walls' Property (2011).
SD - Standard deviation.

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Table 4. American Toad Whole Body ANOVAs - Comparisons by Year and Location

Tennessee Valley Authority **Kingston, Tennessee**

Analyte	Number of Samples	R^2	Year	Location	Year*Location	Post-hoc Comparisons[1]
Arsenic	114	0.58	< 0.0001	0.0004	< 0.0001	NE 2011 > NE 2010 p = 0.01 NE 2011 > REF 2011 p = 0.03 REF 2011 < NE 2009, WE 2009, REF 2009 p = 0.04; p = 0.007; p < 0.0001 NE 2011 < REF 2009 p < 0.0001 WE 2011 < REF 2009, NE 2010 p < 0.0001
Mercury	114	0.16	0.15	0.007	0.37	–
Selenium	114	0.26	0.006	< 0.0001	0.05	NE 2011 > NE 2009 p = 0.03 WE 2011 > REF 2011 p = 0.003 WE 2011 > REF 2010 p = 0.004 WE 2011 > NE 2009 p = 0.0001

Note: All analyte results were log transformed.

[1] Significant differences from the post-hoc Tukey-Kramer two-tailed test.

Shading denotes statistically significant difference with ($p < 0.05$).

– - No individual comparisons identified.

NA - Not applicable; constituent was not detected with enough frequency to evaluate statistically.

NE - North Embayment.

REF - Reference; includes Dawson Farm, Rocky Top Farm, Timberlake Pond (2009, 2010), and Walls' Property (2011).

R^2 - Correlation coefficient.

WE - West Embayment

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Table 5. Spring Peeper Whole Body ANOVAs - Comparisons by Year and Location

Tennessee Valley Authority **Kingston, Tennessee**

Analyte	Number of Samples	R^2	Year	Location	Year*Location	Post-hoc Comparisons[1]
Arsenic	NA	NA	NA	NA	NA	NA
Mercury	NA	NA	NA	NA	NA	NA
Selenium	101	0.14	0.62	0.001	0.85	WE 2011 > REF 2011 p = 0.03

Note: All analyte results were log transformed.

[1] Significant differences from the post-hoc Tukey-Kramer two-tailed test.

Shading denotes statistically s Shading denotes statistically significant difference with ($p < 0.05$).

NA - Not applicable; constituent was not detected with enough frequency to evaluate statistically.

REF - Reference; includes Dawson Farm, Rocky Top Farm, Timberlake Pond (2009, 2010), and Walls' Property (2011).

R^2 - Correlation coefficient.

WE - West Embayment.

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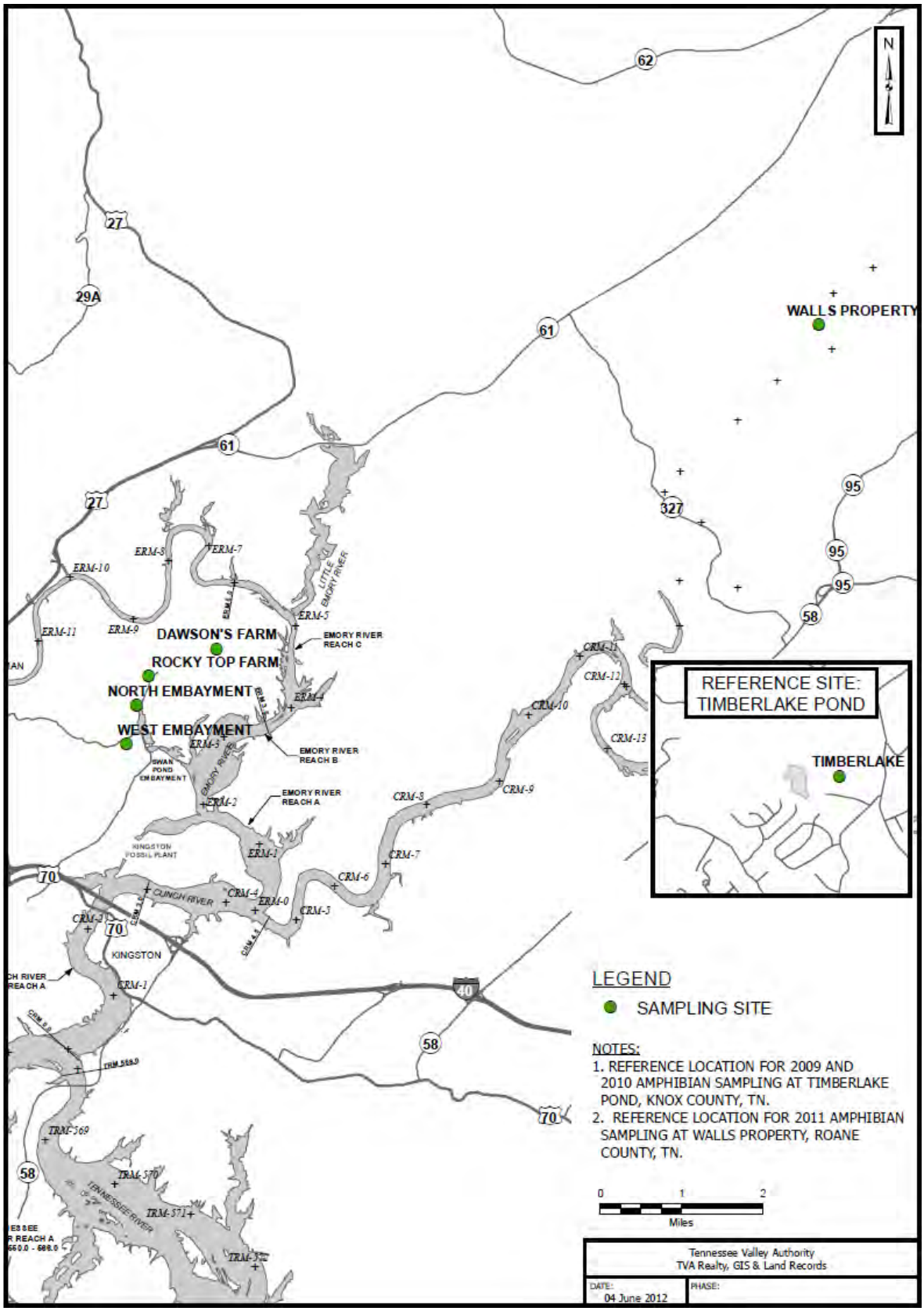
Table 6. Upland Chorus Frog Whole Body ANOVAs - Comparisons by Year and Location
Tennessee Valley Authority Kingston, Tennessee

Analyte	Number of Samples	R^2	Year	Location	Year*Location	Post-hoc Comparisons[1]
Arsenic	NA	NA	NA	NA	NA	NA
Mercury	NA	NA	NA	NA	NA	NA
Selenium	84	0.32	0.0008	0.0006	0.88	REF 2011 < NE 2010, WE 2010 p = 0.001; p < 0.0001

Note: All analyte results were log transformed.
[1] Significant differences from the post-hoc Tukey-Kramer two-tailed test.
Shading denotes statistically si Shading denotes statistically significant difference with ($p < 0.05$).
NA - Not applicable; constituent was not detected with enough frequency to evaluate statistically.
NE - North Embayment.
REF - Reference; includes Dawson Farm, Rocky Top Farm, Timberlake Pond (2009, 2010), and Walls' Property (2011).
R^2 - Correlation coefficient.
WE - West Embayment.



ARCADIS

Amphibian Sampling Locations

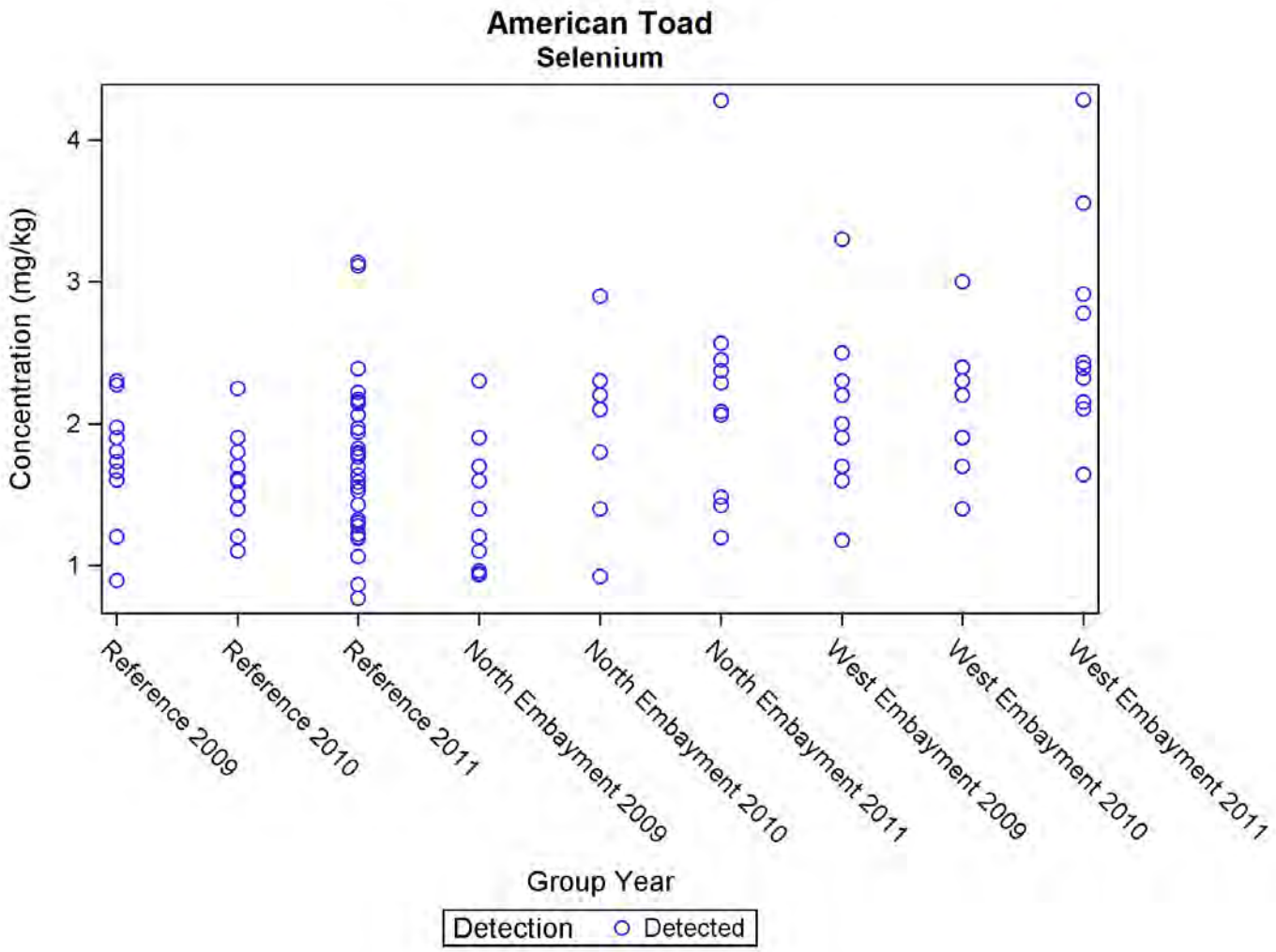
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

TENNESSEE VALLEY AUTHORITY

KINGSTON, TENNESSEE

FIGURE

1



Note: All concentrations presented in milligrams per kilogram dry weight (mg/kg dw).

Reference – Includes collections from Dawson Farm, Rocky Top Farm, Timperlake Pond (2009, 2010), and Walls' Property (2011).

ARCADIS

Selenium Concentrations in American Toads

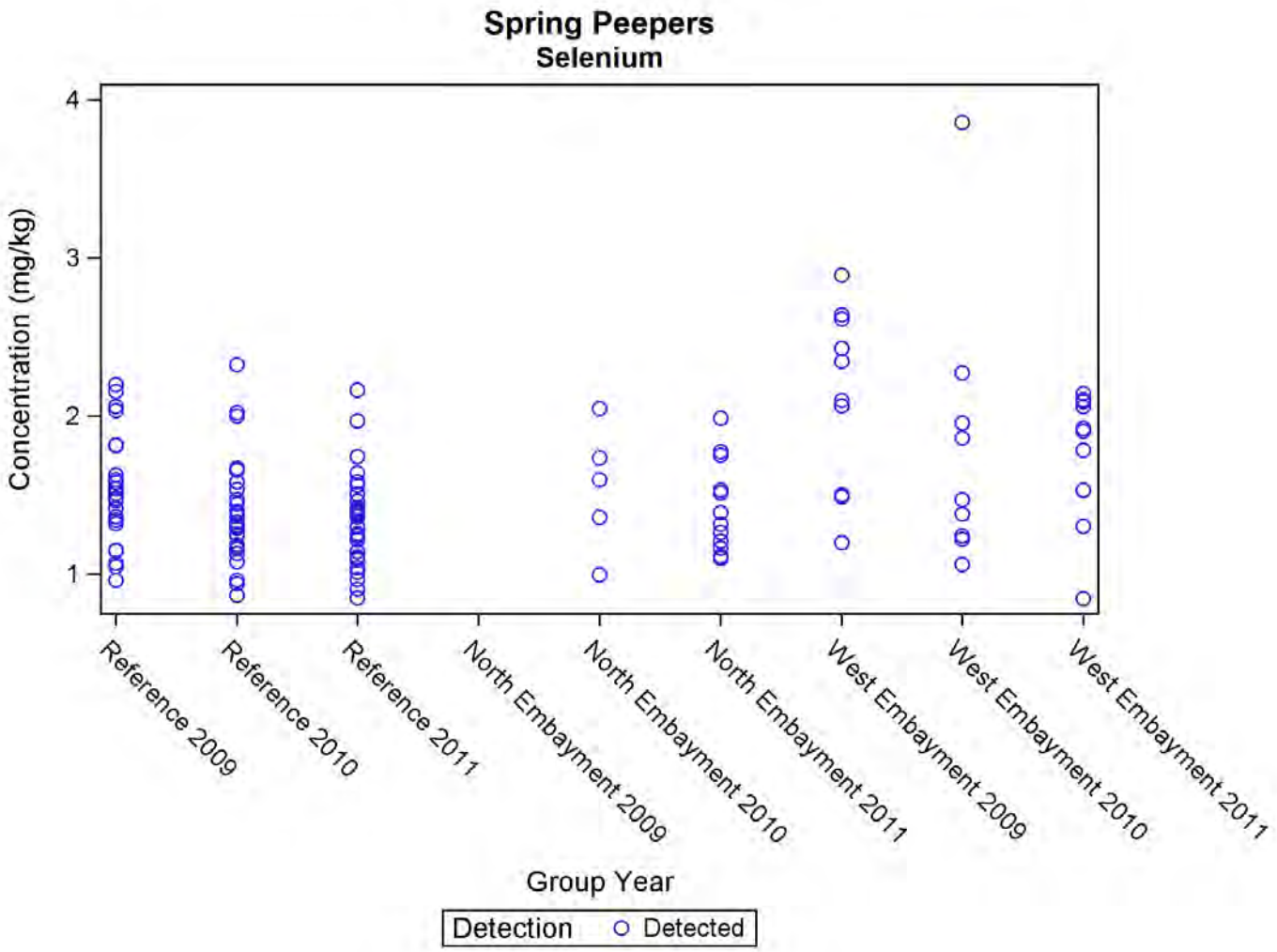
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

TENNESSEE VALLEY AUTHORITY

KINGSTON, TENNESSEE

FIGURE

2



Note: All concentrations presented in milligrams per kilogram dry weight (mg/kg dw).

Reference – Includes collections from Dawson Farm, Rocky Top Farm, Timperlake Pond (2009, 2010), and Walls' Property (2011).

ARCADIS

Selenium Concentrations in Spring Peepers

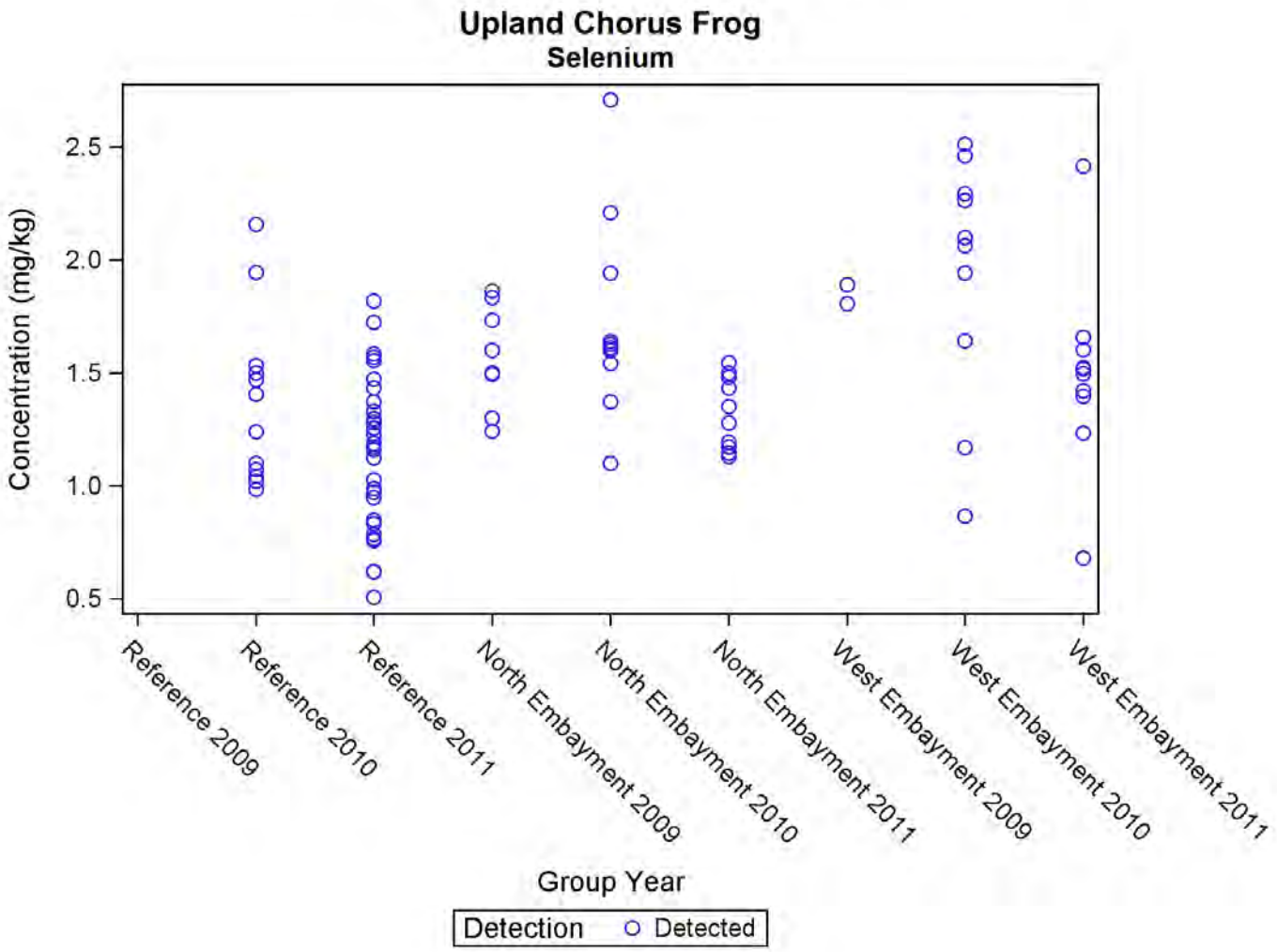
Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

TENNESSEE VALLEY AUTHORITY

KINGSTON, TENNESSEE

FIGURE

3



Note: All concentrations presented in milligrams per kilogram dry weight (mg/kg dw).

Reference – Includes collections from Dawson Farm, Rocky Top Farm, Timperlake Pond (2009, 2010), and Walls' Property (2011).

ARCADIS

Selenium Concentrations in Upland Chorus Frogs

Updated Data Analysis and Temporal Trend Evaluations in Biota: 2009 – 2011

TENNESSEE VALLEY AUTHORITY

KINGSTON, TENNESSEE

FIGURE

4

Appendix J

Preliminary Evaluation of 2011 ARCADIS Results: Turtle Blood Bioaccumulation

Preliminary Evaluation of 2011 Results:
Turtle Blood Bioaccumulation

June 2012

Introduction

The Tennessee Valley Authority (TVA) Kingston Fossil Plant (KIF), one of TVA's larger fossil plants, is located at the confluence of the Emory and Clinch Rivers on Watts Bar Reservoir in Roane County, Tennessee. Ash, a by-product of a coal-fired power plant, is stored in unlined containment areas, including a former Dredge Cell. Failure of the Dredge Cell dike released about 5.4 million cubic yards (cy) of coal ash covering approximately 300 acres. Fly ash also entered the channel and overbank areas of the riverine section of the Emory River. While the released fly ash itself is primarily composed of fine silica particles very similar to sand, it also contains trace amounts of arsenic, chromium, copper, lead, mercury, nickel, selenium, thallium, vanadium, zinc, and other metals which occur naturally in the coal.

Evaluations of the spatial extent of ash deposition indicate that ash may have traveled upstream as far as Emory River mile (ERM) 6.0, and as far downstream as Tennessee River mile (TRM) 566 (Jacobs 2010). In the upstream direction, the thickness of ash appeared to diminish quickly beyond about ERM 3.5. In the downstream direction, ash deposition generally diminishes quickly below about ERM 1.0, with pockets of greater depth occurring in depositional areas in the lower Emory River and Clinch River. Downstream of Clinch River mile (CRM) 2.0, ash deposits generally vary from trace amounts to 2 inches. Ash deposition of 0.5 to 1 inch was observed in the Tennessee River at TRM 566 (south of the Clinch River). Only trace amounts of ash have been observed further downstream (Jacobs 2010).

Dredging efforts in the Emory River began on March 20, 2009 and continued until May 29, 2010, and was completed in several phases. Hydraulic dredging in the river began during the first phase, which was an initial dredging pilot program on March 20, 2009. This pilot study continued until July 20, 2009 (during the time-critical removal action). Phase I production dredging began in August 2009 and focused on removing the greatest volume of ash in the quickest time frame to reduce the potential for upstream flooding by clearing the river channel and to minimize downriver migration risk. At the end of the pilot and Phase I dredging, approximately 1.96 million cy of ash had been removed from the river. Phase II dredging began in February 2010 in order to further minimize the potential future ash migration down river. This period of dredging was considered "precision" dredging and was focused on returning the river channel to its original (pre-spill) depths while minimizing disturbance of legacy sediment. An estimated 780,000 cy of ash was removed during the Phase II dredging. During dredging operations, turbidity was expected to increase in the immediate area of the dredging. Engineering controls (silt curtains) and operational controls (i.e., reduce cutter head speed, reduce rate of advance, and reverse cutter head rotation) were implemented to minimize suspending solids during the dredging operations.

Aquatic turtles were selected as a representative reptile species for the site. Turtles can occupy various levels in the food web, and can have the potential for bioaccumulation of metals and metalloids. Turtles serve as a useful receptor in order to understand exposure and potential effects of these constituents on turtle populations and provide insight to the health of the reptile community (ARCADIS 2012).

The main study objectives were to 1) compare community metric results among locations and across years; 2) compare concentrations of metals and metalloids in different species of turtle tissues among locations and years; 3) evaluate turtle reproductive condition among locations and years; and 4) relate concentrations measured at the study sites to reference area concentrations and literature derived effects values, when available.

Study Methods

Collections

In 2011, turtle monitoring was conducted by Virginia Polytechnic Institute and State University (VA Tech). Detailed descriptions of study methods are available in *Effect of the Remediated Coal Ash Spill in Kingston, TN on Aquatic and Terrestrial Consumers* (Hopkins et al. 2012). Turtles were trapped on the Emory, Clinch, and Tennessee Rivers. Several species, including the common musk turtle (Sternotherus odoratus), common snapping turtle (Chelydra serpentina), and Eastern spiny softshell turtle (Apalone spinifera spinifera), were identified as target species. Blood, claw, and carapace samples (softshell turtles only) were collected from each target species and analyzed for 26 metals and metalloids. In addition, eggs and hatchings were also collected and a subset of these tissues was also analyzed for metals and metalloids. Data are not yet available for claws, carapace, eggs, or hatchlings; therefore, only blood data have been included for this evaluation. In addition, only data from species collected in previous years were evaluated for spatial and temporal trends in this update.

Only results for selenium, arsenic, and mercury concentrations in blood are discussed in this evaluation. Summary statistics were calculated (sample size, frequency of detection, minimum and maximum detection and detection limits) for each year and location (Tables 1, 2, and 3). Each constituent was evaluated for frequency of detection. Constituents with a low frequency of detection were excluded for statistical testing. Spatial trends were evaluated using a parametric, one-sided Dunnett's test for 2011 data. For all constituents detected in turtle blood with normal or lognormal distribution, two-way analysis of variance (ANOVA) tests were conducted (Statistical Analysis Software [SAS] version 9.3), evaluating years and location. Lognormal variables were transformed before the analyses. ANOVAs were then followed by a post-hoc test using Tukey-Kramer for unequal sample sizes. For all statistical tests, the null hypothesis was rejected when p-values were less than 0.05.

A preliminary analysis of the spatial and temporal trends for selenium, arsenic, and mercury (spatial trends only) concentrations in turtle blood tissues was conducted for this report. A more detailed evaluation will be published by VA Tech in March 2013.

Results and Discussion

Summary statistics were generated for arsenic, mercury, and selenium concentrations measured in turtle blood for each species (Tables 1, 2, and 3). Results for each constituent are discussed below.

Selenium

Selenium was found above detection limits in all or most samples for each species. In 2011, selenium concentrations in common musk and softshell turtles collected from the Emory and Clinch Rivers were

found to be statistically significantly higher than concentrations of selenium in turtles from the reference location (upstream Tennessee River). When evaluating 2010 and 2011 concentrations, there were some statistically significant differences between years (Tables 4, 5, and 6). However, these differences were identified between ash-impacted and reference locations from different years and are unlikely to provide a true temporal comparison. Only selenium concentrations in blood collected from common musk turtles in the Clinch River were statistically significantly different when comparing 2010 and 2011 (Table 4); however, given the distance of the Clinch River from the ash release and the lower levels of selenium in impacted sites found closer to the release, it is unlikely that the increases in selenium in musk turtle blood are attributed to potential effects of the ash release and could possibly be caused by a historical source or a source unrelated to the ash spill.

Arsenic

Arsenic was found above detection limits in approximately half of the samples for each species (between 50 and 65 percent detection). Arsenic concentrations in common musk and softshell turtles collected from the Emory and Clinch Rivers in 2011 were found to be statistically significantly higher than concentrations of arsenic in turtles from the reference location (upstream Tennessee River). When evaluating 2010 and 2011 concentrations, there were some statistically significant differences between years and locations for all three species (Tables 4, 5, and 6). However, these differences were identified between ash-impacted and reference locations from different years and are unlikely to provide a true temporal comparison. Furthermore, concentrations of arsenic decreased between 2010 and 2011 in all cases for the musk turtle. Concentrations of arsenic in snapping turtles collected on the Clinch River were statistically significantly higher than reference concentrations (Table 5, $p = 0.04$). Similarly, arsenic concentrations in softshell turtles collected on the Emory and Clinch Rivers were statistically significantly higher than reference locations in 2011 (Table 6). However, temporal trends for both snapping turtles and softshell turtles indicate that while there were some differences between 2010 and 2011 locations, arsenic concentrations decreased from 2010 to 2011.

Mercury

Mercury was found above detection limits in most samples for all species (between 70 and 98 percent detection). No significant differences were identified for 2011 mercury concentrations in any turtle species in any of the rivers. While location was identified as statistically significant in the two-way ANOVA for common musk (Table 4), no individual comparisons were identified as being statistically significant using the post-hoc Tukey-Kramer comparisons. There were no temporal comparisons identified as being significantly different for snapping and softshell turtles.

Different analytical methods were used for mercury in 2010 and 2011. In 2010, the ICP-MS acid digestion method was used by Pace to analyze for mercury, whereas samples from 2011 collections were analyzed using an ICP-MS isotope dilution method in the Environmental Analytical Chemistry Lab at Dartmouth College. There has not been a comparison between methods used to analyze turtle blood for mercury. However, it is unlikely that differences between these analytical methods would have masked significant temporal trends.

Conclusions

Preliminary conclusions of selenium, arsenic, and mercury concentrations in turtle blood indicate that there may be some spatial trends connected to the ash release, with higher concentrations of selenium and arsenic in turtle blood from impacted rivers compared to reference locations. However, concentrations of selenium and arsenic also appear to be decreasing from 2010 to 2011 in all locations for all three species. Comparisons of selenium concentrations in musk turtles collected on the Emory River were the only location where 2011 concentrations were marginally higher than 2010 concentrations.

References

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Table 1. Common Musk Turtle Blood Summary Statistics
Tennessee Valley Authority Kingston, Tennessee

Analyte	Location	Year	Number of Detects	Number of Samples	Mean[1] ± SD	Range
Arsenic	Reference	2010	1	20	0.04 ± 0.01	0.025 – 0.058
	Reference	2011	28	29	0.01 ± 0.005	0.0029 – 0.025
	Emory River	2010	0	20	NC ± NC	0.024 – 0.058
	Emory River	2011	35	36	0.03 ± 0.02	0.0024 – 0.1005
	Clinch River	2010	27	41	0.05 ± 0.02	0.024 – 0.11
	Clinch River	2011	27	27	0.02 ± 0.01	0.007 – 0.0693
	TN River	2010	5	22	0.03 ± 0.002	0.025 – 0.036
	TN River	2011	NA	NA	NA ± NA	NA – NA
Mercury	Reference	2010	13	20	0.01 ± 0.003	0.011 – 0.019
	Reference	2011	27	29	0.01 ± 0.01	0.005 – 0.0476
	Emory River	2010	8	20	0.02 ± 0.03	0.011 – 0.17
	Emory River	2011	35	36	0.02 ± 0.02	0.0052 – 0.0788
	Clinch River	2010	21	41	0.01 ± 0.004	0.01 – 0.029
	Clinch River	2011	25	27	0.02 ± 0.01	0.0039 – 0.062
	TN River	2010	6	22	0.01 ± 0.001	0.01 – 0.017
	TN River	2011	NA	NA	NA ± NA	NA – NA
Selenium	Reference	2010	20	20	0.42 ± 0.12	0.22 – 0.73
	Reference	2011	29	29	0.36 ± 0.13	0.11 – 0.634
	Emory River	2010	20	20	0.46 ± 0.17	0.18 – 0.92
	Emory River	2011	36	36	0.50 ± 0.23	0.1152 – 1.081
	Clinch River	2010	41	41	0.92 ± 0.29	0.36 – 1.6
	Clinch River	2011	27	27	0.65 ± 0.21	0.1317 – 1.0497
	TN River	2010	22	22	0.51 ± 0.10	0.33 – 0.69
	TN River	2011	NA	NA	NA ± NA	NA – NA

[1] Mean calculations include reporting limits substituted for non-detects; concentrations presented in milligrams per kilogram wet weight (mg/kg ww).
NC - Not calculated because all samples were non-detects; range represents reporting limits only.
NA - Not applicable.
Reference - Includes samples collected upstream of Tennessee River Mile 568.0.
SD - Standard deviation.

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Table 2. Common Snapping Turtle Blood Summary Statistics
Tennessee Valley Authority Kingston, Tennessee

Analyte	Location	Year	Number of Detects	Number of Samples	Mean[1] ± SD	Range
Arsenic	Reference	2010	1	21	0.04 ± 0.01	0.025 – 0.057
	Reference	2011	28	29	0.01 ± 0.008	0.003 – 0.0347
	Emory River	2010	0	8	NC ± NC	0.027 – 0.058
	Emory River	2011	26	26	0.01 ± 0.01	0.0044 – 0.0678
	Clinch River	2010	0	5	NC ± NC	0.026 – 0.053
	Clinch River	2011	22	22	0.02 ± 0.02	0.0027 – 0.0798
	TN River	2010	0	17	NC ± NC	0.025 – 0.052
	TN River	2011	NA	NA	NA ± NA	NA – NA
Mercury	Reference	2010	21	21	0.07 ± 0.04	0.017 – 0.16
	Reference	2011	28	29	0.08 ± 0.06	0.0041 – 0.235
	Emory River	2010	7	8	0.07 ± 0.06	0.012 – 0.17
	Emory River	2011	25	26	0.10 ± 0.06	0.002 – 0.2224
	Clinch River	2010	5	5	0.10 ± 0.02	0.073 – 0.12
	Clinch River	2011	22	22	0.12 ± 0.09	0.0066 – 0.284
	TN River	2010	17	17	0.07 ± 0.03	0.037 – 0.11
	TN River	2011	NA	NA	NA ± NA	NA – NA
Selenium	Reference	2010	21	21	0.35 ± 0.05	0.24 – 0.44
	Reference	2011	29	29	0.33 ± 0.09	0.1598 – 0.4833
	Emory River	2010	8	8	0.34 ± 0.11	0.15 – 0.45
	Emory River	2011	24	26	0.39 ± 0.17	0.01 – 0.652
	Clinch River	2010	5	5	0.38 ± 0.06	0.29 – 0.45
	Clinch River	2011	22	22	0.43 ± 0.35	0.1175 – 1.8324
	TN River	2010	17	17	0.39 ± 0.06	0.27 – 0.5
	TN River	2011	NA	NA	NA ± NA	NA – NA

[1] Mean calculations include reporting limits substituted for non-detects; concentrations presented in milligrams per kilogram wet weight (mg/kg ww).
NC - Not calculated because all samples were non-detects; range represents reporting limits only.
NA - Not applicable.
Reference - Includes samples collected upstream of Tennessee River Mile 568.0.
SD - Standard deviation.

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Table 3. Eastern Spiny Softshell Turtle Blood Summary Statistics
Tennessee Valley Authority Kingston, Tennessee

Analyte	Location	Year	Number of Detects	Number of Samples	Mean[1] ± SD	Range
Arsenic	Reference	2010	0	10	NC ± NC	0.025 – 0.054
	Reference	2011	17	30	0.004 ± 0.003	0.0011 – 0.0179
	Emory River	2010	7	20	0.06 ± 0.06	0.025 – 0.24
	Emory River	2011	30	35	0.03 ± 0.05	0.0011 – 0.1898
	Clinch River	2010	6	16	0.05 ± 0.02	0.025 – 0.09
	Clinch River	2011	14	15	0.01 ± 0.009	0.0022 – 0.0402
	TN River	2010	0	18	NC ± NC	0.022 – 0.056
	TN River	2011	NA	NA	NA ± NA	NA – NA
Mercury	Reference	2010	4	10	0.01 ± 0.004	0.01 – 0.023
	Reference	2011	26	30	0.01 ± 0.008	0.004 – 0.0381
	Emory River	2010	10	20	0.02 ± 0.03	0.0099 – 0.12
	Emory River	2011	32	35	0.02 ± 0.03	0.0034 – 0.143
	Clinch River	2010	12	16	0.02 ± 0.01	0.01 – 0.054
	Clinch River	2011	15	15	0.02 ± 0.01	0.0048 – 0.0497
	TN River	2010	7	18	0.01 ± 0.004	0.0091 – 0.027
	TN River	2011	NA	NA	NA ± NA	NA – NA
Selenium	Reference	2010	9	10	0.39 ± 0.24	0.062 – 0.83
	Reference	2011	30	30	0.20 ± 0.17	0.0182 – 0.598
	Emory River	2010	19	20	0.57 ± 0.46	0.063 – 1.4
	Emory River	2011	35	35	0.55 ± 0.50	0.0525 – 1.6336
	Clinch River	2010	16	16	0.73 ± 0.47	0.11 – 1.5
	Clinch River	2011	15	15	0.52 ± 0.38	0.068 – 1.2701
	TN River	2010	15	18	0.35 ± 0.20	0.06 – 0.65
	TN River	2011	NA	NA	NA ± NA	NA – NA

[1] Mean calculations include reporting limits substituted for non-detects; concentrations presented in milligrams per kilogram wet weight (mg/kg ww).
NC - Not calculated because all samples were non-detects; range represents reporting limits only.
NA - Not applicable.
Reference - Includes samples collected upstream of Tennessee River mile 568.0.
SD - Standard deviation.

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Table 4. Common Musk Turtle Blood ANOVAs - Comparisons by Year and Location
Tennessee Valley Authority Kingston, Tennessee

Analyte	Number of Samples	R^2	Year	Location	Year*Location	Post-hoc Comparisons[1]
Arsenic	173	0.52	< 0.0001	0.0004	0.005	CR 2011 < CR 2010, REF 2010 p < 0.0001, p = 0.0003 ER 2011 < CR 2010, REF 2010 p < 0.0001 REF 2011 < REF 2010, CR 2010 p < 0.0001 CR 2011 > REF 2011 p < 0.0001 ER 2011 > REF 2011 p < 0.0001
Mercury[2]	173	0.052	0.54	0.02	0.64	–
Selenium	173	0.42	0.01	< 0.0001	0.03	CR 2011 > REF 2011 p < 0.0001 CR 2011 > ER 2011 p = 0.04

[1] Significant differences from the post-hoc Tukey-Kramer two-tailed test.
[2] Analyte results were log transformed.
– No differences.
Shading denotes statistically significant difference with ($p < 0.05$).
ERM - Emory River mile.
REF - Includes samples collected upstream of Tennessee River mile 568.0.
R^2 - Correlation coefficient.

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Table 5. Common Snapping Turtle Blood ANOVAs - Comparisons by Year and Location
Tennessee Valley Authority Kingston, Tennessee

Analyte	Number of Samples	R^2	Year	Location	Year*Location	Post-hoc Comparisons[1]
Arsenic	111	0.55	< 0.0001	0.25	0.28	CR 2011 > REF 2011; p = 0.04 CR 2011 < REF 2010 p < 0.0001 ER 2011 < REF 2010; p < 0.0001 REF 2011 < REF 2010 p < 0.0001
Mercury[2]	111	0.04	0.95	0.29	0.56	–
Selenium	111	0.01	0.67	0.73	0.98	NA

[1] Significant differences from the post-hoc Tukey-Kramer two-tailed test.
[2] Analyte results were log transformed.
– No differences.
Shading denotes statistically significant difference with ($p < 0.05$).
ERM - Emory River mile.
NA - Not applicable; constituent was not detected with enough frequency to evaluate statistically.
REF - Includes samples collected upstream of Tennessee River mile 568.0.
R^2 - Correlation coefficient.

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Table 6. Eastern Spiny Softshell Turtle Blood ANOVAs - Comparisons by Year and Location
Tennessee Valley Authority Kingston, Tennessee

Analyte	Number of Samples	R^2	Year	Location	Year*Location	Post-hoc Comparisons[1]
Arsenic	126	0.52	< 0.0001	0.0004	0.113	ER 2011 > REF 2011 p < 0.0001 CR 2011 > REF 2011 p = 0.0008 CR 2011 < CR 2010, ER 2010 p = 0.003, p = 0.0008 ER 2011 < ER 2010, CR 2010 p < 0.0001, p = 0.0002 REF 2011 < ER 2010, CR 2010 p < 0.0001
Mercury[2]	126	0.03983	0.71	0.20	0.74	–
Selenium	126	0.19	0.03	0.006	0.28	REF 2011 < ER 2010, 2011 p = 0.01, p = 0.006 REF 2011 < CR 2010, 2011 p = 0.0001, p = 0.02

[1] Significant differences from the post-hoc Tukey-Kramer two-tailed test.
[2] Analyte results were log transformed.
– No differences.
Shading denotes statistically significant difference with ($p < 0.05$).
ERM - Emory River mile.
REF - Includes samples collected upstream of Tennessee River mile 568.0.
R^2 - Correlation coefficient.