OAK RIDGE NATIONAL LABORATORY

MANAGED BY UT-BATTELLE FOR THE DEPARTMENT OF ENERGY

P.O. Box 2008
Oak Ridge, TN 37831-6036
PHONE: (865) 241-5622
FAX: (865) 576-3989
E-MAIL: pracheilbm@ornl.gov

14 April 2016

Dear Dr. Costa:

Please find attached our responses to reviewer comments and revision to manuscript ID ECTX-D-15-00181 R1 entitled "Relating Fish Health and Reproductive Metrics to Contaminant Bioaccumulation at the Tennessee Valley Authority Kingston Coal Ash Spill Site". We thank the editor and the reviewers for their attention to this manuscript. We believe that addressing the comments have led to a much improved draft. We have responded point-by-point to reviewer comments.

Please don't hesitate to contact us if you require additional information or materials. We look forward to continue working with you towards publication of this manuscript.

Best regards,

Brenda M. Pracheil, Ph.D.
Aquatic Ecologist
Environmental Sciences Division
Oak Ridge National Laboratory
Email: pracheilbm@ornl.gov
Phone: 865-241-5622
Fax: 865-576-3989

TITLE: Relating Fish Health and Reproductive Metrics to Contaminant Bioaccumulation at the Tennessee Valley Authority Kingston Coal Ash Spill Site

AUTHORS: Brenda M. Pracheil[1], S. Marshall Adams[1,2], Mark S. Bevelhimer[1], Allison M. Fortner[1], Mark S. Greeley, Jr.[1], Cheryl A. Murphy[3], Teresa J. Mathews[1], and Mark J. Peterson[1]

[1]Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge, TN 37831

[2]Retired

[3] Department of Fisheries and Wildlife, Lyman Briggs College, Michigan State University, East Lansing, MI, 48824

ABSTRACT

A 4.1 million m^3 coal ash release into the Emory and Clinch rivers in December 2008 at the Tennessee Valley Authority's Kingston Fossil Plant in east Tennessee, USA, prompted a long-term, large-scale biological monitoring effort to determine if there are chronic effects of this spill on resident biota. Because of the magnitude of the ash spill and the potential for exposure to coal ash-associated contaminants (e.g., selenium (Se), arsenic (As), and mercury (Hg)) which are bioaccumulative and may present human and ecological risks, an integrative, bioindicator approach was used. Three species of fish were monitored— bluegill (*Lepomis macrochirus*), redear sunfish (*L. microlophus*), and largemouth bass (*Micropterus salmoides*)— at ash-affected and reference sites annually for five years following the spill. On the same individual fish, contaminant burdens were measured in various tissues, blood chemistry parameters as metrics of fish health, and various condition and reproduction indices. A multivariate statistical approach was then used to evaluate relationships between contaminant bioaccumulation and fish metrics to assess the chronic, sub-lethal effects of exposure to the complex mixture of coal ash-associated contaminants at and around the ash spill site. This study suggests that while fish tissue concentrations of some ash-associated contaminants are elevated at the spill site, there was no consistent evidence of compromised fish health linked with the spill. Further, although relationships between elevated fillet burdens of ash-associated contaminants and some fish metrics were found, these relationships were not indicative of exposure to coal ash or spill sites. The present study adds to the weight of evidence from prior studies suggesting that fish populations have not incurred significant biological effects from spilled ash at this site: findings that are relevant to the current national discussions on the safe disposal of coal ash waste.

INTRODUCTION

Creating strong linkages between environmental pollution exposure and health effects in wild animal populations can be difficult (Rose 2000). When there is large release of a contaminant and concomitant and conspicuous death of a large number of organisms these linkages are fairly straightforward. However, in the bulk of contaminated ecosystems linking environmental perturbations and sub-lethal population impacts often requires teasing apart multiple conflating factors. For example, creating links between a released contaminant and an animal population may require a combination of knowledge of the environmental history of the contaminated area, the environmental history of surviving organisms, controlled exposures of cell cultures or whole animals at one or multiple life stages to the released contaminant, and modelling approaches that extrapolate sub-organismal and individual effects to a population (Rose 2000). Unfortunately, controlled experimental studies aimed at mechanistically cataloguing sub-lethal effects and scaling to see if these effects have population impacts can take several years to decades to complete. As a result, many of the initial conclusions on the sub-lethal effects of environmental contamination are based on relational data from uncontrolled field and laboratory studies. These initial studies can be important for generating hypotheses for further mechanistically-focused experimentation. Perhaps more importantly, management and restoration decisions must be made on a much shorter time-scale than that allowed by controlled experimentation. Frequently, relational studies represent the best information available on a particular ecosystem for forming urgent policy decisions.

The effects of coal ash, the byproduct of coal combustion, on aquatic biota has become a major concern to environmental regulators. Depending on the source of the coal used as fuel, coal ash can contain high concentrations of contaminants such as arsenic (As), mercury (Hg), and selenium (Se), which can be toxic to biota and can bioaccumulate. These contaminants can then be transferred to humans consuming fish and other aquatic organisms, thereby creating possible public health concerns. Because the chemical make-up of coal ash varies by coal source, reported effects of coal ash on biota can be varied ranging from near complete reproductive failure (Lemly 2002) to negligible and undetetable effects (Souza et al. 2013). Although the exact mechanisms and toxic constituents of coal ash are not known, coal ash spills have been linked to reduced nest success in birds (King et al. 1994) and skeletal deformities in fish (Lemly 2002).

Concerns about the effects of coal ash on aquatic organisms were again brought to the fore following the rupture of a retention pond dike in 2008 at the Tennessee Valley Authority's Kingston Fossil Plant (TVA KIF). This breach spilled coal ash into the Emory River, in east Tennessee, USA resulting in the largest disaster of its kind in US history. While acute effects of the TVA KIF coal ash spill to aquatic animals were conspicuous, including the killing of an unknown number of fish, mussels, benthic macroinvertebrates, and other aquatic organisms (Lemly and Skorupa 2013; Bryan et al. 2012; Otter et al. 2013; Souza et al. 2013), ongoing monitoring of the spill site has not suggested any major threats to humans, fish or wildlife since the initial incident: drinking water has remained safe and water quality has generally not exceeded regulatory criteria.

In the present study, the effects of coal ash contamination on biota were further explored through relating bioindicators of fish from three reference sites and three TVA KIF ash-affected sites to assess the fish response to coal ash. This approach involved relating a suite of selected biological responses including biochemical markers, condition indices and reproductive health markers (Adams and Greeley 2000) to As, Hg, and Se burdens in fishes.

Since coal ash contains a number of potentially toxic contaminants and exposure to coal ash contaminants at the KIF site has changed over time due to remediation efforts and riverine hydrologic processes, the response of fish to ash-associated contaminants is likely complex. Statistically relating fish metrics with bioaccumulation results from the KIF site can be a first step towards understanding the response to coal ash exposure over time. To this end, the TVA KIF coal ash fish health, reproduction and bioaccumulation monitoring data were used to determine if there were relationships between contaminants in fish fillets and fish metrics and, if these relationships exist, whether they were related to site and ash exposure. This study uses a statistical approach to highlight links between fish health, reproduction and selected coal ash-related contaminant burdens as an essential first step for generating hypotheses and associations that can be further explored in the future using carefully designed studies that target a mechanistic understanding of the effects of coal ash on aquatic animal health.

MATERIALS AND METHODS

Study area

The TVA KIF is located adjacent to the confluence of the Emory and Clinch rivers in east Tennessee, USA (Figure 1). Approximately 90% of the ash spilled into the Emory River, some of which was pushed up to Emory River mile 6 (TVA 2009). The remaining ~10% of the ash spilled into an embayment to the north of the TVA KIF, and downstream into the Clinch River (TVA 2009). Following the spill, fish were collected each spring for this study from 2009 to

2013 at three ash-affected sites—Emory River mile 3.0 (spill site; hereafter, S1), Emory River mile 0.9 (approx. 2 mi or 3 km downstream of the spill site; hereafter S2) and Clinch River mile 1.5 (approx. 6 mi or 10 km downstream of the spill site; hereafter S3)— and three reference sites that were unaffected by the spill— Emory River mile 8.0 (approx. 5 mi or 8 km upstream of the spill site; hereafter R1), Little Emory River mile 2.0 (approx. 2 mi or 3 km upstream of the confluence of the Emory and Little Emory rivers; hereafter R2), and Clinch River mile 8.0 (approx. 4 miles or 6 km upstream of the confluence of the Clinch and Emory rivers; hereafter R3). Ash was dredged from the ash-affected Emory River sites (S1, S2) from 2009-2010 where 65% of the 4.1 million m^3 of spilled coal ash were removed (Bartov et al. 2012). Site R1 was used in this study as a reference site; however, it has received considerable environmental contamination from a now-closed, upstream paper mill (Bartov et al. 2012). Site R3 has also received considerable environmental contamination from an upstream Department of Energy facility for many years (Bartov et al. 2012). While the reference sites chosen for this study are far from pristine, all regionally proximate, and therefore, relevant, candidate reference sites have been impacted by some form of legacy alteration or contamination. This region is one of high freshwater biodiversity and geologic heterogeneity (Pracheil et al. 2014) thereby underscoring the importance of choosing spatially proximate reference sites.

Pore-water leached from spill site sediments contained elevated levels of As, Se, Hg, boron, strontium, barium, uranium, chromium , iron, and manganese (but not lead) compared with non-impacted upstream sites (Ruhl et al. 2009; Ruhl et al. 2010; Bartov et al. 2012; Deonarine et al. 2013). Concentrations of many elements were measured in these fishes (e.g., aluminum, antimony, As, barium, beryllium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, Hg, molybdenum, nickel, potassium, Se, sodium, strontium, thallium, vanadium, zinc). However, preliminary correlations with this suite of elements showed only consistent significant trends with As, Hg, and Se, so the present study focuses on these contaminants. Also, the focus was placed on As, Hg, and Se because of their relative abundance in fillets analyzed (other contaminants had very few measurements above the LOD) and extensive documentation in the literature of these three contaminants producing biological effects on fishes and other aquatic organisms. A Hg source-apportionment study using stable isotopes conducted after dredging was completed could not conclusively determine whether Hg from sediment in ash-affected areas was a result of legacy contamination or the coal ash spill, although it is certain that at least some of the Hg was sourced by the coal ash spill (Bartov et al. 2012).

Target fish species

To assess impacts on aquatic biota, bioaccumulation and a variety of fish health metrics were monitored in bluegill (*Lepomis macrochirus*), redear sunfish (*L. microlophus*), and largemouth bass (*Micropterus salmoides*). These species were selected because they are abundant in the Emory and Clinch rivers and are commonly caught and consumed by local anglers. Furthermore, these species are relatively short-lived and generally have a limited home range (Etnier and Starnes 1994), so fillet contaminant concentrations should be representative of exposure at the site of collection (Peterson et al. 1996). These fish species also represent a variety of trophic levels: bluegill and redear sunfish feed on a varied diet of insects, crustaceans, and other zoobenthos (Etnier and Starnes 1994), but redear sunfish in this system have a preference for mollusks (Otter et al. 2013). Largemouth bass, on the other hand, are top predators eating fishes such as bluegill (Etnier and Starnes 1994). The effects of contaminants on the health of fish of different trophic levels were examined because some contaminants, such as As, Hg and Se, have been shown to biomagnify with increasing trophic position (Barwick and Maher 2003). That is, the higher the trophic level of the fish, the higher the contaminant concentration. For spatial and temporal comparability and to minimize effects of covariance between size and contaminant concentrations, fish only of sizes large enough to be caught by anglers (generally 50-150 g for sunfish, and 500-2500 g for largemouth bass, total weight) were collected for bioaccumulation and fish health studies. Fish were collected using a boat electrofisher: bluegill and largemouth bass during April-June of 2009-2013 and redear sunfish during April-June 2010-2013. Numbers of fish per year for each site and species combination generally ranged from 10-20.

Sample processing and calculation of health and condition metrics

Up to 1 mL of blood was collected from each fish while still in the field. Upon return to the laboratory, all fish were euthanized with MS-222. Fish were then dissected and major organs (liver, kidneys and ovaries) were removed prior to weighing. Metrics of fish health including measures of bioenergetics, hematology and immune function, carbohydrate-protein metabolism, electrolyte homeostasis, liver condition, and overall fish condition, were assessed for each fish. Several metrics of fish condition (CI) were assessed including the liver-somatic, visceral-somatic, and spleno-somatic indices and were calculated as

$$CI = \frac{M_o}{M_b} \times 100$$

where M_o= wet organ mass (g) and M_b= wet body mass (g). Overall condition factor C_f was calculated as

$$C_f = \frac{M_T}{{L_T}^3} \times 100$$

where M_T= total wet mass (g) and L_T= total length (cm).

Blood chemistry analyses

Blood hematocrit and leucocrit were determined by the standard capillary tube and centrifugation method. Fourteen blood chemistry metrics were analyzed with an Abaxis VetScan II (Abaxis, Union City, CA, USA) clinical analyzer and tested for several analytes that are known as indicators of physiological response in fish (Abaxis test rotor #500-0038). Analysis of all 14 blood chemistry metrics (Table 1) required 100 µL of blood from each fish.

Reproductive condition assessment

Representative pieces of ovarian tissue were placed in vials containing a half-strength solution of Karnovsky's Fixative for later analysis of ovary stage, oocyte (immature developing eggs) condition, and fecundity. Fish reproductive condition was quantified by sizing, staging and counting all oocytes above size thresholds for active yolk accumulation (vitellogenesis) that were contained in a weighed subsample of ovary. From these measurements, batch fecundity, numbers of vitellogenic oocytes and numbers of atretic oocytes were estimated for each ovary.

Because the fish species examined had differing life history strategies required fecundity estimates, accommodating species-specific patterns of oocyte development was a requirement. Each species can spawn multiple times during the breeding season; therefore, fecundity estimates consider only the most mature clutch of developing oocytes in pre-spawn fish, or post-ovulatory follicles in immediately post-spawn fish, following methods outlined in Greeley et al. (2012). Batch fecundities and the abundance of vitellenogenic and atretic oocytes were estimated as

$$N = \frac{N_e}{M_s} M_o$$

where N_e is the number of oocytes or post-ovulatory follicles in a clutch, the total number of vitellogenic oocytes, or the abundance of atretic oocytes in the analyzed ovary piece, M_s is the mass of the ovarian subsample, and M_o is the mass of the entire ovary.

Bioaccumulation analyses

Fish samples were shipped frozen (< -10º C) to Pace Analytical Services, Inc. (Green Bay, WI) for homogenization, moisture determination, and analysis of As, Hg, and Se. For As and Se quantification, tissue aliquots were weighed (wet) and digested in nitric acid (EPA method SW846-3050) prior to analysis using ICP-MS (EPA method SW846-6020). Tissue samples were analyzed for Hg directly (EPA method SW846-7473) using a Direct Mercury Analyzer (Milestone, Sorisole, Italy). All quality assurance procedures (e.g., blanks, matrix spikes) were conducted as specified in the analytical method.

Method detection limits (MDL) for this project were calculated based on historical blank concentrations as described by the following equation:

$$\text{Project MDL} = \text{avg} + 3\sigma$$

where avg is the mean of historical method blank concentrations and σ = standard deviation of the population of historical method blank concentrations. The MDL (prior to sample-specific adjustment to account for actual weight of the digested aliquot) was 0.0142 mg/kg for As, 0.0753 mg/kg for Se and 0.001 mg/kg for Hg. Standard reference materials (SRM) included lobster hepatopancreas (TORT-2) and dogfish liver tissue (DOLT-4) from the National Research Council in Canada (NRC) and Lake Michigan fish tissue from the National Institute of Standards and Technology (NIST-1947). The TORT-2 SRM was digested and analyzed with each batch of samples, while the DOLT-4 and NIST-1947 SRMs were alternated with every other sample batch. Acceptance criteria for Se recovery in TORT-2 were 80-120% of the certified value of 5.63 mg/kg. In addition, chicken fillets were used as the matrix for the laboratory control spike with each batch of samples, with acceptance criteria of 80-120% of the spike. All samples were analyzed and reported on a wet-weight basis. Values below detection limits were excluded from analyses. Frequencies of fillet concentrations below detection limits are shown in Appendix A (This and all appendices are provided in online Supplemental Material).

Data analysis

Bioaccumulation values below the MDL were censored from all analyses. Although fillet, ovary, and liver bioaccumulation data were collected, analyses used data from fillets because that was the only appropriately-large dataset. The assumption of focusing on fillet bioaccumulation data was that it was related to concentrations found in other organs. This assumption was validated using Pearson's correlations between liver and fillet and ovary and fillet concentrations to understand how well or whether fillet bioaccumulation was related to bioaccumulation in other tissue types (Appendix B).

Fish health, reproduction, and bioaccumulation data were examined for normality by comparing the median and mean of the data set (they were considered approximately normal if they differed by a factor of < 3), and visually inspecting a normal-quantile plot of the data to see if it differed substantially from a 1:1 line. Data that were not determined to be normal were $\log_{10}$ transformed and again examined for normality using the above methods. Just number of atretic oocytes—a reproductive metric—was found to violate normality assumptions and was log-transformed.

Multivariate analyses of covariance (MANCOVA; proc mixed, SAS) were used for each species to detect differences among contaminants and fish length, site, and year of collection. Upon conducting routine examinations of the data (e.g., Pearson's correlations), it was discovered that As, Hg, and Se are correlated with each other. Due to the non-independence of these data and influence of covariates, a MANCOVA was chosen to test for differences for each species. The MANCOVA was also used for each fish species to determine relationships between 1. blood chemistry metrics and contaminants, 2. fish condition metrics and contaminants, 3. reproductive health metrics and contaminants where length was used as a covariate in each test. Post-hoc Tukey's honestly significant difference tests were used to evaluate differences between sites for all MANCOVAs.

Because there are so many possible site, year and species combinations for each measured variable (6 sites x 5 years x 3 species=90 possible combinations), individual pairwise differences were not reported although specific comparisons between sites were highlighted that help to illustrate conclusions. Instead, for measures of fish health (e.g., blood chemistry, fish condition, reproduction) the critical, and consistent comparisons made across all sites, were that made between reference sites and spill sites. These findings were presented as a summary of least-squares (LS) means for each site type with associated p-values for the difference between LS means.

RESULTS

Bioaccumulation

Across all sites, bluegill had the highest Se concentration in 2010 whereas redear sunfish had the highest Se concentrations in 2011 (Figure 2). Across all species, spill sites generally had higher Se burdens than reference sites. Fillet burdens of Se were highest in 2010 or 2011 depending on the site by species combination. Selenium concentrations in largemouth bass did not show consistent patterns with respect to site. For Hg and As, the trends were quite different. Reference sites, particularly R1 and R3 had the highest Hg values in nearly all sample periods for all species except largemouth bass.

While Hg levels rose at all sites and species through the study period, the most dramatic increases were found in largemouth bass at site R1. For largemouth bass, a post-hoc Tukey's test showed that Hg concentrations from site R1 were significantly different those from all other sites and there were no significant differences between any other sites. Post-hoc Tukey's tests also showed there were elevated concentrations of As in bluegill from spill sites in 2010 and in largemouth bass from spill sites from 2010-2011—the first years following the spill.

Nearly all main-effects and their interactions were significant in the MANCOVA examining the effect of site type (reference or ash-affected; Table 2). Year was only significant for redear sunfish, although nearly so for bluegill. Tukey's tests showed that Se was significantly higher in ash-affected sites for all three species, and largemouth bass had significantly higher Hg at reference sites than at spill sites (Table 3).

Bioaccumulation and blood chemistry functional response groups

There were no consistent patterns in blood chemistry functional response groups over years for any species (Appendix C). While all overall MANCOVA models for blood chemistry were significant for all species, no main-effects were significant for any species (Table 4). However, the interaction of main-effects were significant for largemouth bass, but not for other species. Posthoc Tukey's tests showed no differences in functional response groups between reference and spill sites (Appendix D).

Bioaccumulation and fish condition metrics

There did not appear to be distinct spatial or temporal trends in condition metric time-series data (Figure 3). Overall MANCOVA models were significant for all three fish species (Table 5). The main-effect of year as well as the interaction between condition factor type and year was significant for bluegill and redear sunfish. No main-effects were significant in the largemouth bass MANCOVA by themselves, although the interactions between year and site type and condition factor type, year and site type were significant (Table 5). Post-hoc Tukey's tests indicated that redear C_f was higher in reference sites (LS $Means_{ref}$=1.638, LS $Means_{spill}$=1.594; P-val: 0.045; Appendix E), but no other comparisons between reference and spill sites were significant.

Bioaccumulation and reproductive health metrics

Both redear and bluegill sunfish had decreases in atretic oocytes from 2010 to 2011 at ash-affected sites (Figure 4). In these fish, the highest mean atretic oocytes were found at spill sites in the years following the spill (2010 being the first year data were collected following the spill for redear sunfish) and during/ immediately after dredging, although site S1 also had the highest fecundity and the largest number of vitellogenic oocytes reported. Redear sunfish showed some additional spatial and temporal trends in reproductive metrics by site and year. While overall MANCOVA models and main-effects of length were significant for all species, the main-effect of Se was significant for both bluegill and redear as well as the main-effect of As for redear (Table 6). Interaction effects of year and site type were additionally significant for bluegill and largemouth bass and the three-way interaction of metric type, year and site type for bluegill. No post-hoc Tukey's tests for differences in reproductive metrics between reference and ash-affected sites were significant at the α=0.05 level, although there was a significant difference between reference and ash-affected sites in vitellenogenic oocytes of redear sunfish at the α=0.10 level (LS Means_{ref}=17,694, LS Means_{spill}=14,740; P-value=0.0694; Appendix F).

DISCUSSION

This is the first study to explicitly relate fish bioaccumulation to biological endpoints at the TVA KIF spill site, relating contaminant bioaccumulation data and a variety of fish biological endpoints. Most importantly, this is the first study from the TVA KIF that draws quantitative links between contaminants and reproductive endpoints: an essential component for scaling biological effects to the population level. Even after looking at these data using multiple approaches both in this study and in prior studies, the weight of findings suggests there is little evidence of major long-term fish health effects of the TVA KIF coal ash spill. For example, Bevelhimer et al. (2014) did not find associations between fish blood chemistry metrics and sites of collection beyond the first years after the spill, finding that variation among years was greater than variation among sites. In most cases, while site was often a significant effect in MANCOVAs examining relationships between contaminant concentration and site (Table 2), these differences did not translate to detectable differences between reference and spill sites. Greeley et al. (2014) looked at the effects of coal ash contaminated sediments on fathead minnow (*Pimephales promelas*) embryos and larvae and found no adverse effects of coal ash on survival, incidence of developmental abnormalities, or hatching success. Studies of bird nesting around the spill site similarly showed little evidence of physiological impairments, although they have somewhat elevated levels of Se (Beck et al. 2014).

Among the foremost concerns with this ash spill was that long-term, elevated levels of Se may cause reproductive failures or reduced fecundity in aquatic organisms as reported from other Se-contaminated sites (Gillespie and Baumann 1986; Lemly 2002). Fillet Se concentrations were higher at spill sites than reference sites for all three fish species, but this study found little evidence of impending reproductive failure in fishes although there is some evidence of that ash-affected sites experienced some short-term reproductive impairments following the spill. For example, this study reports very low numbers of vitellenogenic oocytes in bluegill the spring immediately following the spill at site S1 and higher numbers of atretic oocytes in redear sunfish at sites S2 and S3 in the years following dredging (2010-2011; Figure 4). Also, there was not a consistent pattern with respect to reference and spill sites with GSI (Table 5; Figure 3) or significant differences in reproductive metrics at the $\alpha=0.05$ level for any species (Table 6; Figure 4), further suggesting that long-term reproductive effects of this coal ash spill were negligible.

It has been documented that coal ash from the TVA KIF spill contains a number of other elements (N=23, in addition to As, Hg and Se) that can potentially produce toxic effects in fishes such as cobalt, cadmium, and lead. However, due to the high frequency of non-detects and quantities below detection limits, this study focuses solely on As, Hg and Se. Legacy environmental contamination from now-defunct paper mills and other industry in the area has potentially left a variety of other contaminants in the ecosystem that have the potential to influence the fish health response and interact with the class of toxicants that were monitored. Unfortunately, data on non-metal/ non-metalloid contaminants in fishes were not assessed as part of the TVA KIF monitoring and assessment plan.

The lack of long-term effects of Se on fishes in the current study does not necessarily point to an absence of fish health impacts of the TVA KIF coal ash spill as after effects can take a decade or more to manifest. Selenium accumulates through the food chain, and it may take several years to determine actual trends and effects. For instance, developmental deformities due to Se contamination in Belews Lake, NC did not manifest for 10 years following contamination by coal ash waste inputs as Se was transferred up the food chain from producers to consumers (Lemly 1993). While sediment samples have lower Se than those in Belews Lake, aqueous concentrations were similar between the two systems (Mathews et al. 2014); therefore it’s possible that harmful effects of Se have yet to come to light in the Emory and Clinch rivers. However, hydrological differences between the TVA KIF spill area (a lotic ecosystem) and Belews Lake (a lentic ecosystem) may be helping to mitigate effects of the coal ash spill at the TVA KIF plant by continually moving coal ash sediments downstream. It is not possible at this time to know whether the lack of demonstrated fish health impacts from the TVA KIF ash spill is due to insufficient passage of time or to hydrological or other effects thus underscoring the need for continued fish health monitoring in the TVA KIF spill area.

Another hypothesis warranting further examination in this system is that Se concentrations and subsequent health effects have been much lower in this coal ash spill due to the reported antagonistic interaction between Se and Hg and the abundance of Hg in the study area (Southworth et al. 2000; Sackett et al. 2010). Mercury is a common constituent of coal ash, but compared to legacy sources, there is not conclusive evidence that the ash spill is the major source of Hg (Ruhl et al. 2009). Such interactions may also help to explain why fish tissue concentrations of Se observed near the spill site are substantially lower than those reported following other coal ash spills (e.g., Lohner et al. 2001; Lemly 2002; Lemly 2014).

Mercury contamination was generally higher at reference sites than ash-affected sites. In particular, largemouth bass collected from site R1 had levels of Hg that were higher than all other sites (Figure 2). Potential sources of this Hg at reference sites include a now-closed paper mill upstream of R1 (USEPA 2012) in addition to nearby coal combustion at the TVA KIF site. It is unclear how much of the Hg at ash-affected sites is sourced from the coal-ash spill itself. Bartov et al. (2012) were not able to assign the percentage of Hg from the coal ash spill with certainty from a sediment Hg isotope-speciation study. While some of the Hg at ash-affected sites appears to be from the coal ash, the exact fraction is unclear.

Multigenerational effects of contaminant contaminants in other organisms and systems have been shown, so it is possible that adverse effects of the TVA KIF spill have yet to manifest. In particular, long-term studies examining possible effects beyond embryonic and larval stages have not been conducted. There is a growing body of literature showing that effects of chronic metal and metalloid contaminant exposure can vary over time whereby organisms can become increasingly sensitive with successive generations (Stewart et al. 2010; Völker et al. 2013; Jacobasch et al. 2014). For instance, the influence of Hg exposure has been shown to be passed on to subsequent generations (Tsui and Wang 2005; Hammerschmidt and Sandheinrich 2005). The three to five years of post-coal ash spill data for a fish species presented in this study may therefore not be enough time to see multigenerational effects. Also, given that the highest contaminant concentrations were recorded in 2010 or 2011 after dredging, when there was only two years of data after the highest contaminant concentrations were observed in fish in 2010 (Figure 2). It is therefore possible that some of the most important fish health and reproductive impacts of the coal ash spill have not yet become apparent or are of sufficiently low level that they are not yet detectible, thus underscoring the importance for continued fish monitoring in the study area.

CONCLUSIONS

These types of monitoring studies highlight the desperate need for a more mechanistic understanding surrounding the impact of contaminants on biological systems. Relational studies, as demonstrated here, are a good starting point, but can quickly be overwhelmed by the sheer number of potential combinations and numbers of metrics. Also, as alluded to in this study, effects could be masked by antagonistic interactions that occur at the molecular level which cannot be teased-apart by statistics alone. While there was some suggestion that there may be reproductive effects in redear sunfish, the relatively small sample size and the high degree of individual variability reduced the ability of analyses to detect these differences with higher certainty. Due to this uncertainty and the relational nature of this study, there is a need to follow up this work with carefully designed experiments to determine how adverse effects measured at sub-organism level translate to population effects using adverse outcome pathway framework (Ankley et al. 2010).

ACKNOWLEDGMENTS

This research was sponsored by the Tennessee Valley Authority (TVA) and performed at Oak Ridge National Laboratory (ORNL). ORNL is managed by UT-Battelle, for the US Department of Energy under contract DE-AC05-00OR22725. Special thanks to C. Brandt, J. Smith, K. McCracken, C. Dunn, R. Vitale, K. Abbot, E. Rodgers, B. Rogers, M. Cagley, T. Baker, and N. Carriker.

LITERATURE CITED

Adams, S. M., & Greeley, M. S. (2000). Ecotoxicological indicators of water quality: using multi-response indicators to assess the health of aquatic ecosystems. *Water, Air, and Soil Pollution*, *123*(1-4), 103-115.

Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., ... & Serrrano, J. A. (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry*, *29*(3), 730-741.

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

Barwick, M., & Maher, W. (2003). Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. *Marine Environmental Research*, *56*(4), 471-502.

Beck, M. L., Hopkins, W. A., Hallagan, J. J., Jackson, B. P., & Hawley, D. M. (2014). Exposure to residual concentrations of elements from a remediated coal fly ash spill does not adversely influence stress and immune responses of nestling tree swallows. *Conservation Physiology*, *2*(1), doi: 10.1093/conphys/cou018.

Bevelhimer, M. S., Adams, S. M., Fortner, A. M., Greeley, M. S., & Brandt, C. C. (2014). Using ordination and clustering techniques to assess multi-metric fish health response following a coal ash spill. *Environmental Toxicology and Chemistry, 33*(8), 1903-1913.

Bryan Jr., A. L., Hopkins, W. A., Parikh, J. H., Jackson, B. P., & Unrine, J. M. (2012). Coal fly ash basins as an attractive nuisance to birds: Parental provisioning exposes nestlings to harmful trace elements. *Environmental Pollution*, *161*, 170-177.

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

Etnier, D.A. and W.C. Starnes. 1994. Fishes of Tennessee. University of Tennessee Press, Knoxville. 696pp.

Gillespie, R. B., & Baumann, P. C. (1986). Effects of high tissue concentrations of selenium on reproduction by bluegills. *Transactions of the American Fisheries Society*, *115*(2), 208-213.

Greeley Jr, M. S., Adams, M., & McCracken, K. (2012). *Evaluating the Effects of the Kingston Fly Ash Release on Fish Reproduction: Spring 2009-2010 Studies* (No. ORNL/TM-2012/142). Oak Ridge National Laboratory (ORNL).

Greeley Jr, M. S., Elmore, L. R., McCracken, M. K., & Sherrard, R. M. (2014). Effects of Sediment Containing Coal Ash from the Kingston Ash Release on Embryo-Larval Development in the Fathead Minnow, *Pimephales promelas* (Rafinesque, 1820). *Bulletin of Environmental Contamination and Toxicology*, *92*(2), 154-159.

Hammerschmidt, C. R., & Sandheinrich, M. B. (2005). Maternal diet during oogenesis is the major source of methylmercury in fish embryos. *Environmental Science & Technology*, *39*(10), 3580-3584.

Jacobasch, C., Völker, C., Giebner, S., Völker, J., Alsenz, H., Potouridis, T., Heidenreich, H., Kayser, G., Oehlmann,. & Oetken, M. (2014). Long-term effects of nanoscaled titanium dioxide on the cladoceran Daphnia magna over six generations. *Environmental Pollution*, *186*, 180-186.

King, K. A., Custer, T. W., & Weaver, D. A. (1994). Reproductive success of barn swallows nesting near a selenium-contaminated lake in east Texas, USA. *Environmental Pollution*, *84*(1), 53-58.

Lemly, A. D. (1993). Teratogenic Effects of Selenium in Natural Populations of Fresh Water Fish. *Ecotoxicology and Environmental Safety*, *26*(2), 181-204.

Lemly, A. D. 2002. Symptoms and implications of selenium toxicity in fish: The Belews Lake case example. Aquatic Toxicology 57: 39-49.

Lemly, A. D. (2014). Teratogenic effects and monetary cost of selenium poisoning of fish in Lake Sutton, North Carolina. *Ecotoxicology and Environmental Safety*, *104*, 160-167.

Lemly, A. D., & Skorupa, J. P. (2013). Wildlife and the coal waste policy debate: proposed rules for coal waste disposal ignore lessons from 45 years of wildlife poisoning. *Environmental Science & Technology*, *46*(16), 8595-8600.

Lohner, T. W., Reash, R. J., Willet, V. E., & Fletcher, J. (2001). Assessment of tolerant sunfish populations (Lepomis sp.) inhabiting selenium-laden coal ash effluents: 3. Serum chemistry and fish health indicators. *Ecotoxicology and Environmental Safety*, *50*(3), 225-232

Mathews, T. and Fisher, N.S. (2009) Dominance of dietary intake of metals in marine elasmobranch and teleost fish. *Science of the Total Environment*, 407(18), 5156-5161.

Mathews, T. J., Fortner, A. M., Jett, R. T., Morris, J., Gable, J., Peterson, M. J., & Carriker, N. (2014). Selenium bioaccumulation in fish exposed to coal ash at the Tennessee Valley Authority Kingston spill site. *Environmental Toxicology and Chemistry*, *33*(10), 2273-2279.

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

Peterson, M. J., Southworth, G. R., & Crumby, W. D. (1996). Monitoring mercury in fish in a stream system receiving multiple industrial inputs. *Environmental Monitoring and Assessment*, *40*(1), 91-105.

Pracheil, B. M., Hogan, J. D., Lyons, J., & McIntyre, P. B. (2014). Using Hard-Part Microchemistry to Advance Conservation and Management of North American Freshwater Fishes. *Fisheries*, *39*(10), 451-465.

Rose, K. A. (2000). Why are quantitative relationships between environmental quality and fish populations so elusive?. *Ecological Applications*, *10*(2), 367-385.

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

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

Sackett, D. K., Aday, D. D., Rice, J. A., Cope, W. G., & Buchwalter, D. (2010). Does proximity to coal-fired power plants influence fish tissue mercury?. *Ecotoxicology*, *19*(8), 1601-1611.

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

Southworth, G. R., Peterson, M. J., & Ryon, M. G. (2000). Long-term increased bioaccumulation of mercury in largemouth bass follows reduction of waterborne selenium. *Chemosphere*, *41*(7), 1101-1105.

Stewart, R., Grosell, M., Buchwalter, D., Fisher, N., Luoma, S., Mathews, T., Orr, P. and Wang, W. (2010) Bioaccumulation and trophic transfer of selenium. SETAC Pellston Workshop. CRC Press, pp 93-139.

Tsui, M. T., & Wang, W. X. (2005). Influences of maternal exposure on the tolerance and physiological performance of Daphnia magna under mercury stress. *Environmental Toxicology and Chemistry*, *24*(5), 1228-1234.

Tennessee Valley Authority (TVA). 2009. Continuing Investigation of the Nature and Extent of Ash in the Emory, Clinch and Tennessee River Bottoms; Tennessee Valley Authority: Knoxville, TN, 2009

US Environmental Protection Agency (USEPA). 2012. Hazard Ranking System (HRS) Documentation Record for Clinch River Corporation, Atlanta, GA. [cited 2014 March 1]. Available from: http://www.epa.gov/superfund/sites/docrec/pdoc1873.pdf.

Völker, C., Oetken, M., & Oehlmann, J. (2013). The biological effects and possible modes of action of nanosilver. In *Reviews of Environmental Contamination and Toxicology 223* (pp. 81-106). Springer New York.

Table 1. Description of the physiologic relevance of bioindicator metrics (abbreviation) of fish health and condition by functional response group.

Functional Response Group	Bioindicator	Physiologic Indication
Bioenergetics	Amylase (AMY)	Converts starch into sugars
Organ Function	Alanine transferase (ALT)	Liver function
	Blood urea nitrogen (BUN)	Kidney and gill function
	Creatinine (CREAT)	Kidney function
	Total bilirubin (TBIL)	Liver function
	Alkaline phosphatase (ALP)	Bone formation
Carbohydrate-protein metabolism	Glucose (GLU)	Metabolic efficiency
	Blood protein (BPRO)	Liver and general inflammation
	Globulin (GLOB)	Liver and kidney function
	Albumin (ALB)	Liver and kidney function
	Phosphorus (PHOS)	Indicator of kidney, liver, bone disease
Electrolyte homeostasis	Calcium (Ca), Sodium (Na)	Function of most organs including liver and kidney
Fish Condition	Condition factor (C_f)	Index of plumpness
	Gonadosomatic index (GSI)	Index of reproductive potential
	Hepatosomatic index (LSI)	Index of energy reserves
	Spleen somatic index (SSI)	Index of immune response
	Visceral somatic index (VSI)	Index of overall condition
Reproductive health	Vitellogenic oocytes (VO)	Number of oocytes with yolk
	Atresia (ATR)	Number of atretic oocytes
	Batch fecundity (FEC)	Number of eggs produced per clutch

Table 2. Results for MANCOVAs examining the response of metal and metalloid contaminant (in this table, hereafter, metal) concentrations to effects of collection site (hereafter, spill, denoting reference or ash-affected site), year, metal x spill, metal x year, year x spill, and metal x site x year. Overall model results are shown in the row with effect "metal" and all results are given as numerator degrees of freedom, denominator degrees of freedom, F-value. and P-value. Significant P-values (α=0.05) for each covariate from type III sum-of-squares are shown in bold.

Species	Effect	Num df	Denom df	F	P
Bluegill	**Metal**	**2**	**421**	**2369.08**	**<0.0001**
	Spill	**1**	**421**	**185.95**	**<0.0001**
	Year	4	421	2.39	0.0503
	Metal*Spill	**2**	**421**	**198.39**	**<0.0001**
	Metal*Year	**8**	**421**	**4.96**	**<0.0001**
	Year*Spill	**4**	**421**	**3.33**	**0.0106**
	Metal*Spill*Year	**8**	**421**	**3.53**	**0.0006**
Redear	**Metal**	**2**	**379**	**1695.48**	**<0.0001**
	Spill	**1**	**379**	**121.31**	**<0.0001**
	Year	**3**	**379**	**7.28**	**<0.0001**
	Metal*Spill	**2**	**379**	**105.25**	**<0.0001**
	Metal*Year	**6**	**379**	**10.33**	**<0.0001**
	Year*Spill	3	379	1.27	0.2860
	Metal*Spill*Year	**6**	**379**	**2.58**	**0.0184**
Lg.mouth Bass	**Metal**	**2**	**484**	**521.56**	**<0.0001**
	Spill	**1**	**484**	**16.27**	**<0.0001**
	Year	4	484	1.48	0.2072
	Metal*Spill	**2**	**484**	**37.10**	**<0.0001**
	Metal*Year	**8**	**484**	**12.02**	**<0.0001**
	Year*Spill	4	484	0.34	0.4417
	Metal*Spill*Year	**8**	**484**	**2.41**	**0.0148**

Table 3. Results of Tukey's post-hoc tests (completed following the MANCOVA shown in Table 2) comparing metal and metalloid concentrations of least square means (LS Means and the SE of the LS Means) at reference and spill sites (P-value Diff LS Means). Significant P-values (α=0.05) are shown in bold.

Species	Metal/ Metalloid	Site Type	LS Means	SE	P-value Diff LS Means
Bluegill	As	Reference	0.0426	0.0132	0.7671
		Spill	0.0654	0.0108	
	Hg	Reference	0.1036	0.0100	0.9032
		Spill	0.0895	0.0091	
	Se	**Reference**	**0.4998**	**0.0100**	**<0.0001**
		Spill	**0.8407**	**0.0091**	
Redear	As	Reference	0.1341	0.0151	0.1460
		Spill	0.1832	0.0133	
	Hg	Reference	0.1091	0.0138	0.7913
		Spill	0.0844	0.0133	
	Se	**Reference**	**0.6375**	**0.0138**	**<0.0001**
		Spill	**0.9842**	**0.0132**	
Largemouth Bass	As	Reference	0.1785	0.0127	0.3936
		Spill	0.2110	0.0112	
	Hg	**Reference**	**0.2382**	**0.0116**	**0.0059**
		Spill	**0.1811**	**0.0112**	
	Se	**Reference**	**0.4548**	**0.0116**	**<0.0001**
		Spill	**0.5943**	**0.0112**	

Table 4. Results for MANCOVAs examining the response of blood chemistry concentrations to effects of functional response group (hereafter, function: bioenergetics, carbohydrate-protein metabolism, electrolyte balance, hematology, organ function,), arsenic (As), mercury (Hg), selenium (Se), fish length (Length), spill (reference or ash-affected sites), year, function x spill, function x year, year x spill, and function x site x year. Overall model results are shown in the row with effect "function" and all results are given as numerator degrees of freedom, denominator degrees of freedom, F-value. and P-value. Significant P-values (α=0.05) for each covariate from type III sum-of-squares are shown in bold.

Species	Effect	Num df	Denom df	F	P
Bluegill	**Function**	**4**	**1585**	**80.97**	**<0.0001**
	Spill	1	1585	0.14	0.7118
	Year	4	1585	2.01	0.0913
	Arsenic	1	1585	0.43	0.5134
	Mercury	1	1585	0.42	0.5193
	Selenium	1	1585	0.14	0.7067
	Length	1	1585	0.33	0.5662
	Function*Spill	4	1585	0.10	0.9828
	Function*Year	16	1585	0.56	0.9137
	Year*Spill	4	1585	0.14	0.9666
	Function*Spill*Year	16	1585	0.15	1.0000
Redear	**Function**	**4**	**1658**	**124.32**	**<0.0001**
	Spill	1	1658	0.06	0.8046
	Year	3	1658	2.00	0.3884
	Arsenic	1	1658	0.74	0.9512
	Mercury	1	1658	0.00	0.6332
	Selenium	1	1658	0.23	0.6388
	Length	1	1658	0.22	0.1119
	Function*Spill	4	1658	0.29	0.8836
	Function*Year	12	1658	1.26	0.2348
	Year*Spill	3	1658	0.74	0.5288
	Function*Spill*Year	12	1658	0.46	0.9395
Lg.mouth Bass	**Function**	**4**	**2142**	**645.77**	**<0.0001**
	Spill	1	2142	0.22	0.6371
	Year	4	2142	2.23	0.0636
	Arsenic	1	2142	0.28	0.5994
	Mercury	1	2142	0.07	0.7913
	Selenium	1	2142	0.45	0.5024
	Length	1	2142	0.32	0.5702
	Function*Spill	4	2142	0.51	0.7282
	Function*Year	**16**	**2142**	**1.66**	**0.0477**
	Year*Spill	**4**	**2142**	**4.25**	**0.0020**
	Function*Spill*Year	**16**	**2142**	**2.31**	**0.0022**

Table 5. Results for MANCOVAs examining the response of fish condition values to effects of fish condition (hereafter, condition: condition factor, gonado-somatic index, hepato-somatic index, spleno-somatic index, viscero-somatic index), arsenic (As), mercury (Hg), selenium (Se), fish length (Length), spill (reference or ash-affected site type), year, condition x spill, condition x year, year x spill, and condition x site x year. Overall model results are shown in the row with effect "condition" and all results are given as numerator degrees of freedom, denominator degrees of freedom, F-value. and P-value. Significant P-values (α=0.05) for each covariate from type III sum-of-squares are shown in bold.

Species	Effect	Num df	Denom df	F	P
Bluegill	**Condition**	**4**	**551**	**16815.9**	**<0.0001**
	Spill	1	**551**	0.02	0.8784
	Year	**4**	**551**	**2.56**	**0.0375**
	Arsenic	1	**551**	0.13	0.7179
	Mercury	1	**551**	0.18	0.6689
	Selenium	1	**551**	1.87	0.1716
	Length	1	**551**	0.56	0.4532
	Condition*Spill	4	**551**	0.92	0.4504
	Condition*Year	**16**	**551**	**1.83**	**0.0244**
	Year*Spill	4	**551**	1.28	0.2750
	Condition*Spill*Year	16	**551**	0.59	0.8948
Redear	**Condition**	**4**	**581**	**13666.0**	**<0.0001**
	Spill	**1**	**581**	**4.68**	**0.0309**
	Year	**3**	**581**	**6.84**	**0.0002**
	Arsenic	1	**581**	1.93	0.1651
	Mercury	1	**581**	0.62	0.4325
	Selenium	1	**581**	2.27	0.1321
	Length	1	**581**	0.23	0.6289
	Condition*Spill	4	**581**	1.51	0.1965
	Condition*Year	**12**	**581**	**7.30**	**<0.0001**
	Year*Spill	3	**581**	0.98	0.4038
	Condition*Spill*Year	12	**581**	0.51	0.9066
Lg.mouth Bass	**Condition**	**4**	**775**	**5540.29**	**<0.0001**
	Spill	1	**775**	1.19	0.2755
	Year	4	**775**	1.61	0.1701
	Arsenic	1	**775**	0.26	0.6133
	Mercury	1	**775**	0.60	0.4380
	Selenium	1	**775**	0.21	0.6433
	Length	1	**775**	2.89	0.0893
	Condition*Spill	4	**775**	1.95	0.0996
	Condition*Year	16	775	1.42	0.1251
	Year*Spill	**4**	**775**	**2.82**	**0.0241**
	Condition*Spill*Year	**16**	**775**	**2.17**	**0.0049**

Table 6. Results for MANCOVAs examining fish reproduction to effects of reproductive metrics (hereafter, metric: atretic oocytes, batch fecundity, vitellogenic oocytes), arsenic (As), mercury (Hg), selenium (Se), fish length (Length), spill (reference or ash-affected sites), year, metrics x spill, metrics x year, year x spill, and metrics x site x year. Overall model results are shown in the row with effect "metric" and all results are given as numerator degrees of freedom, denominator degrees of freedom, F-value, and P-value. Significant P-values (α=0.05) for each covariate from type III sum-of-squares are shown in bold.

Species	Effect	Num df	Denom df	F	P
Bluegill	**Metric**	**2**	**317**	**538.03**	**<0.0001**
	Spill	1	317	0.42	0.5196
	Year	4	317	1.74	0.1401
	Arsenic	1	317	2.31	0.1295
	Mercury	1	317	1.54	0.2149
	Selenium	**1**	317	**11.82**	**0.0007**
	Length	**1**	317	**76.34**	**<0.0001**
	Metric*Spill	2	317	2.68	0.0698
	Metric*Year	8	317	0.83	0.5754
	Year*Spill	**4**	317	**4.58**	**0.0013**
	Metric*Spill*Year	**8**	317	**3.06**	**0.0025**
Redear	**Metric**	**2**	**341**	**411.39**	**<0.0001**
	Spill	**1**	**341**	**8.64**	**0.0035**
	Year	3	341	8.34	0.0611
	Arsenic	**1**	**341**	**10.24**	**0.0015**
	Mercury	1	341	1.79	0.1823
	Selenium	**1**	**341**	**10.99**	**0.0010**
	Length	**1**	**341**	**79.66**	**<0.0001**
	Metric*Spill	2	341	0.21	0.8093
	Metric*Year	**6**	**341**	**5.68**	**<0.0001**
	Year*Spill	3	341	2.43	0.0650
	Metric*Spill*Year	**6**	**341**	**2.40**	**0.0278**
Lg.mouth Bass	**Metric**	**2**	**434**	**208.49**	**<0.0001**
	Spill	1	434	0.09	0.7614
	Year	4	434	2.60	0.0354
	Arsenic	1	434	0.12	0.7316
	Mercury	1	434	0.22	0.6430
	Selenium	1	434	0.11	0.7398
	Length	**1**	434	**52.92**	**<0.0001**
	Metric*Spill	2	434	0.09	0.9146
	Metric*Year	8	434	1.28	0.2519
	Year*Spill	**4**	434	**3.33**	**0.0105**
	Metric*Spill*Year	8	434	1.05	0.3978

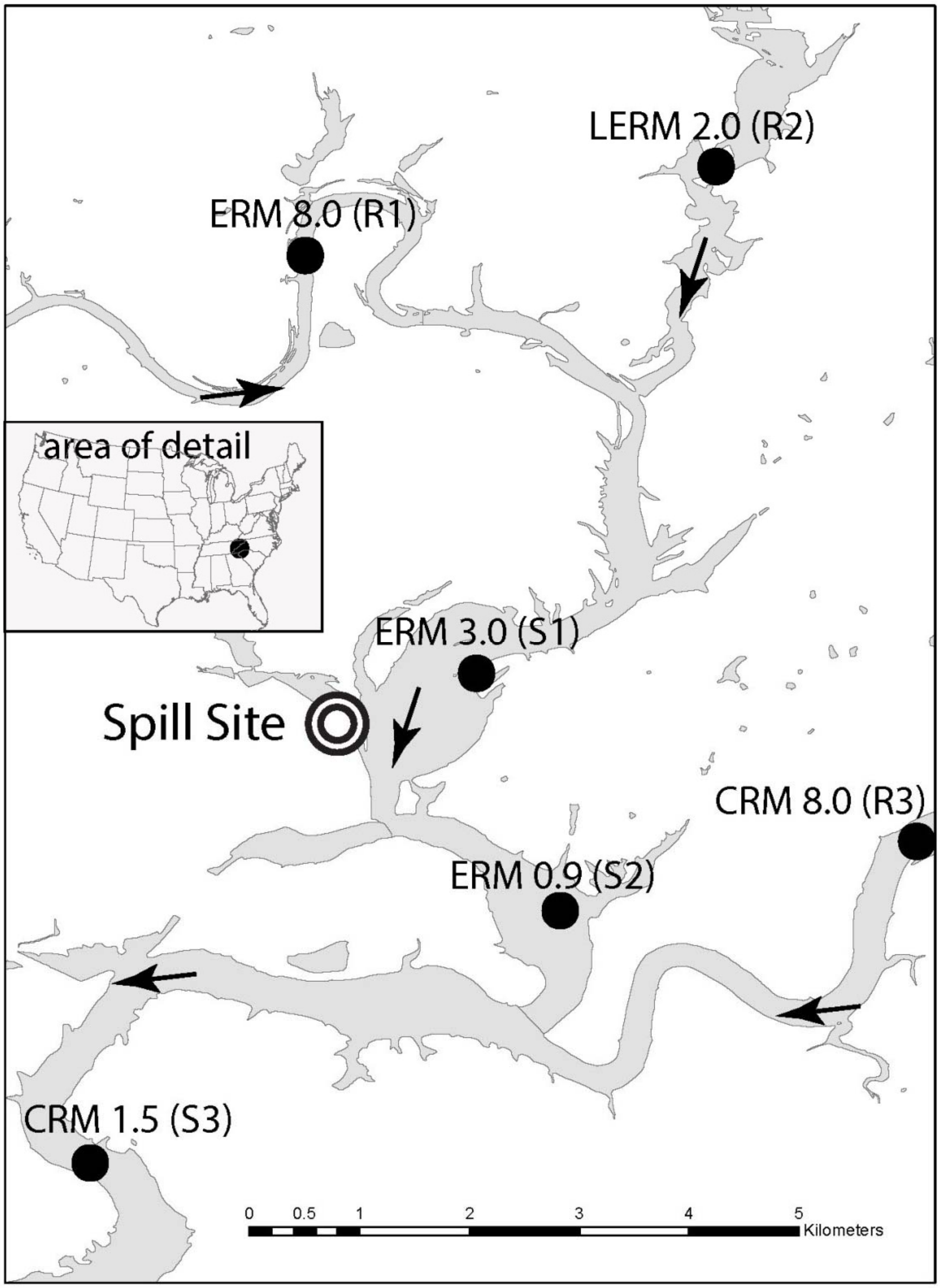

Figure 1. Map of study area showing locations of ash-affected (S) and reference (R; not affected by ash) monitoring sites where fish were collected including ash-affected sites Emory River mile 3.0 (ERM 3.0, S1), Emory River mile 0.9 (ERM 0.9, S2), and Clinch River mile 1.5 (CRM 1.5, S3) and reference sites Emory River mile 8.0 (ERM 8.0, R1), Little Emory River mile 2.0 (LERM 2.0, R2), and Clinch River mile 8.0 (CRM 8.0, R3). Arrows indicate the direction of flow.

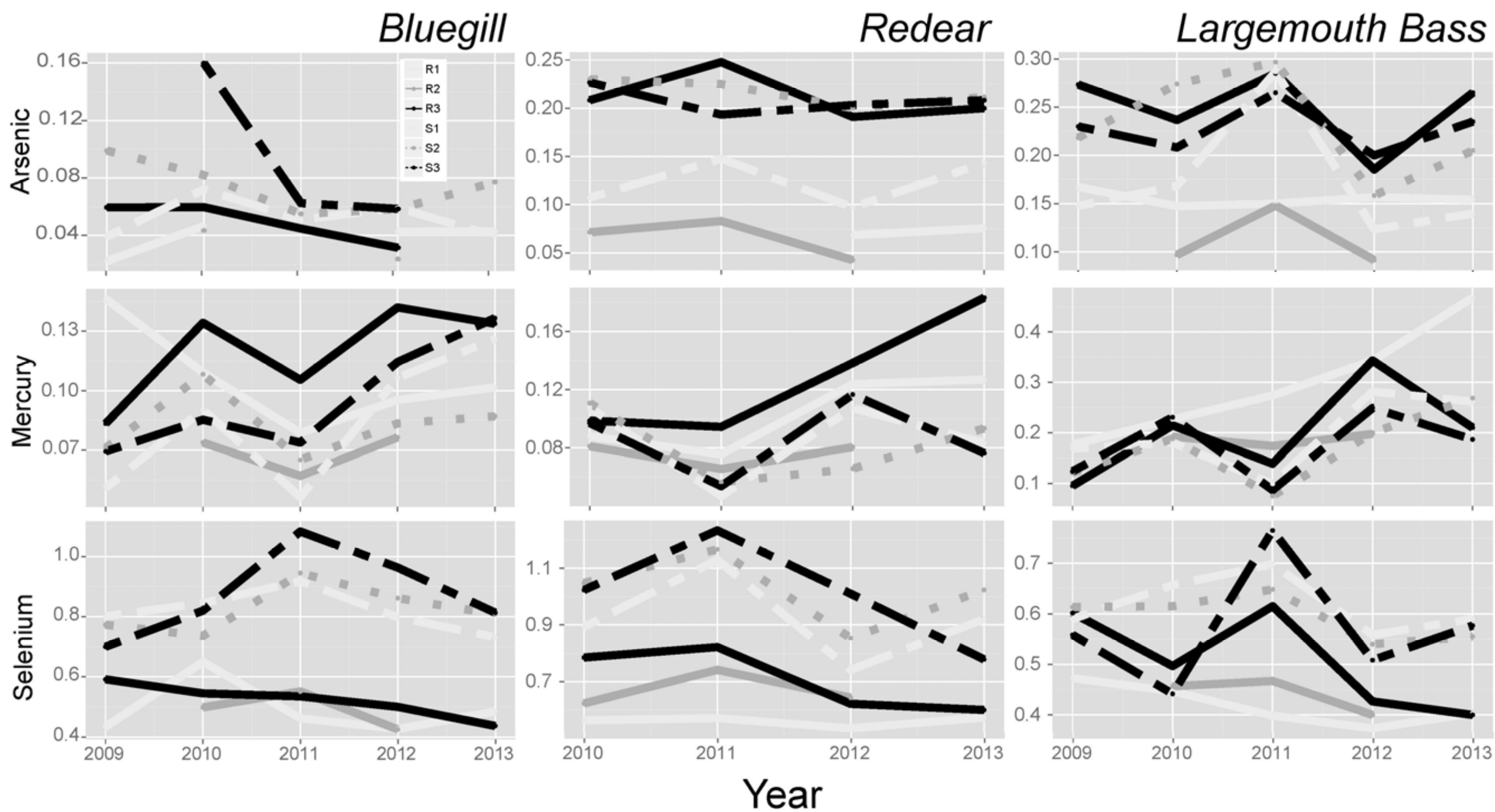

Figure 2. Time-series of mean arsenic, mercury and selenium fillet concentrations (mg/kg wet-weight) by site and species**.** Error bars are omitted for clarity. Sites abbreviations are as shown in Figure 1.

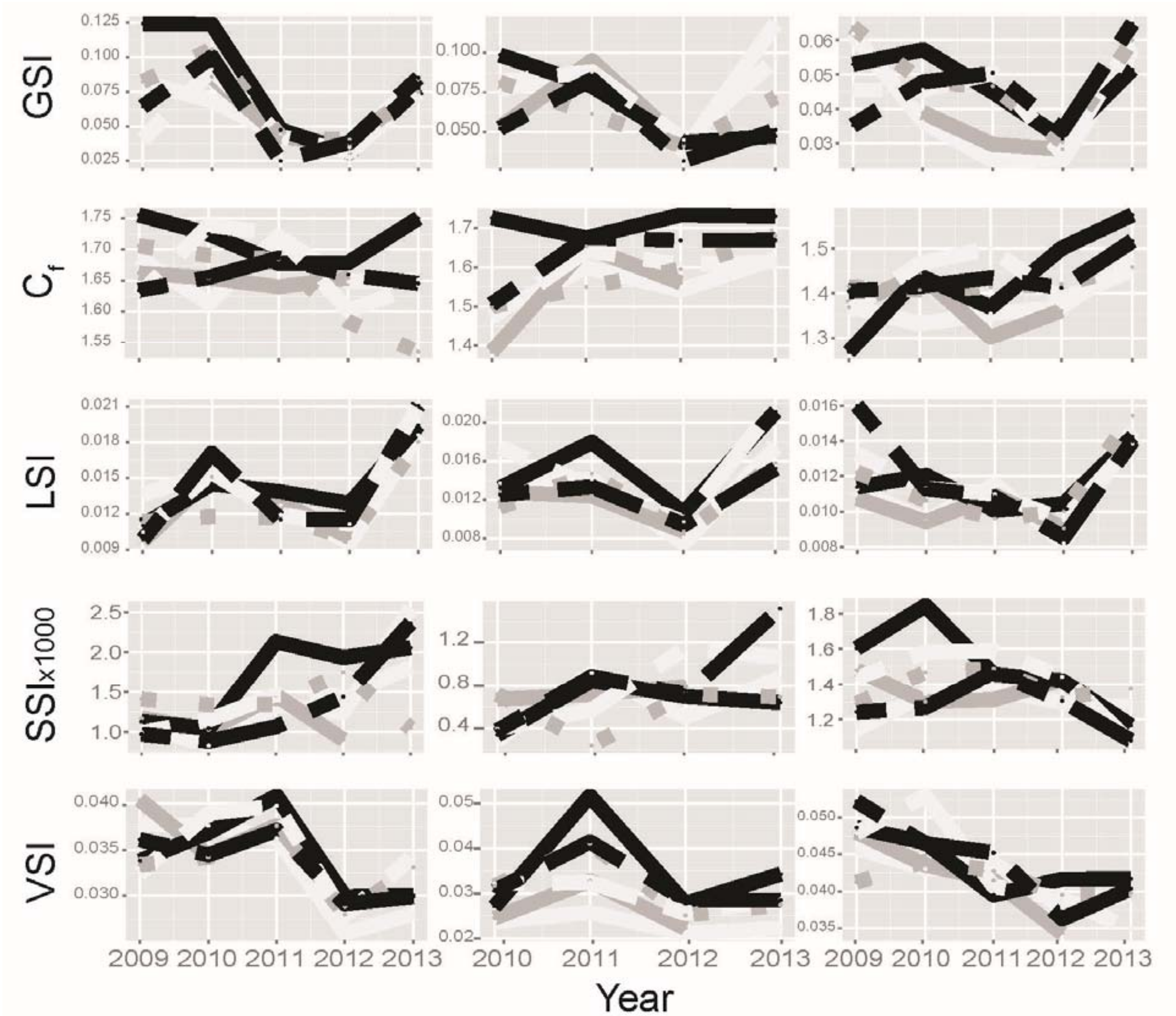

Figure 3. Time-series of gonodosomatic index (GSI), condition factor (C_f), hepatosomatic index (LSI), splenosomatic index (SSI), viscerosomatic index (VSI). Error bars are omitted for clarity. Legend is as in Figure 2 and sites are as in Figure 1.

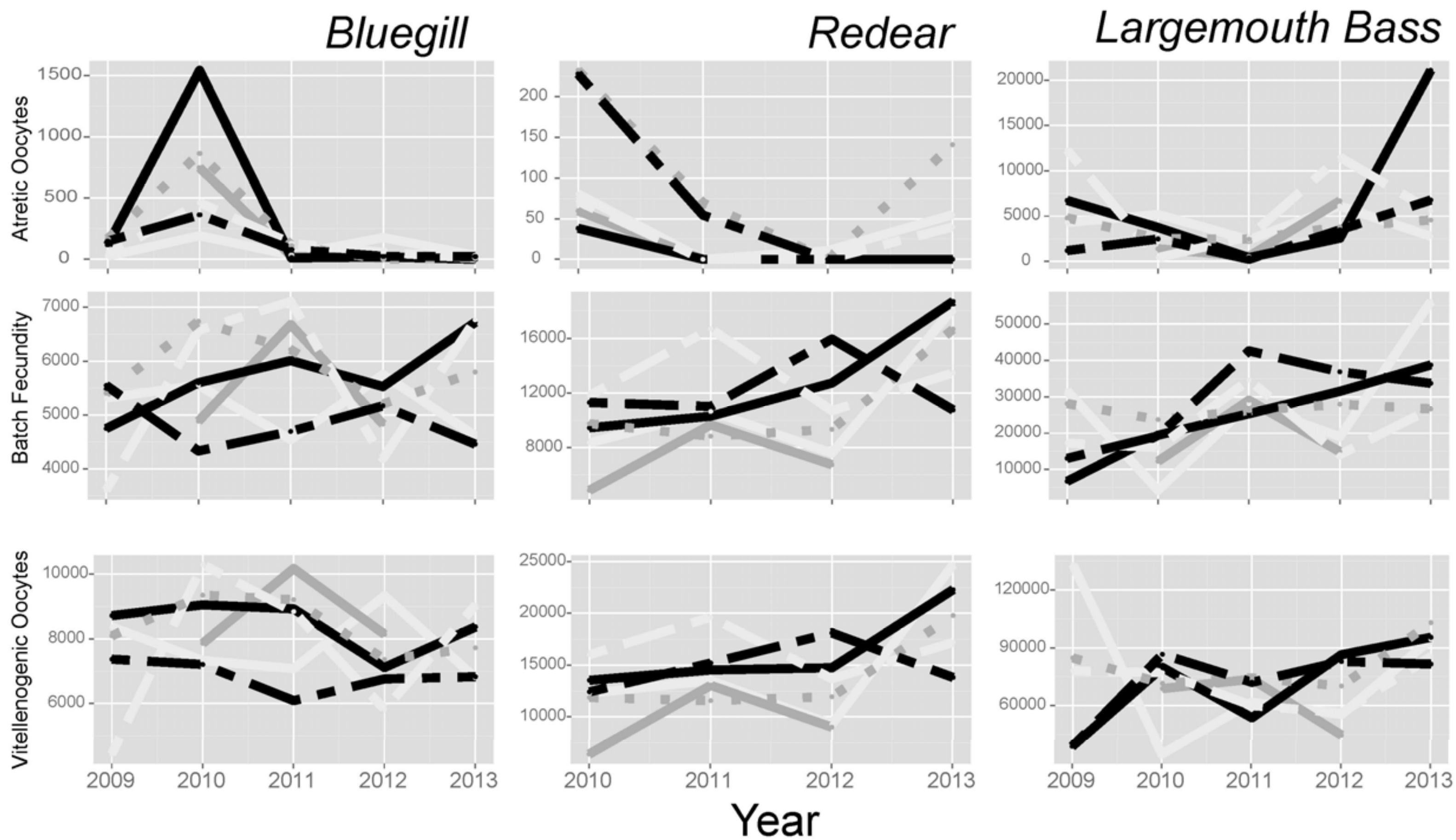

Figure 3. Time-series of mean number atretic oocytes, mean number vitellogenic oocytes, and mean batch fecundity by site and species. Largemouth bass are not shown because analysis of covariance results showed no significant effect of any metal/ metalloid or of site. Error bars are omitted for clarity. Legend is as in Figure 2 and sites are as in Figure 1.

	Site	Year	N	As	Hg	Se
Bluegill						
	R1	2009	6	0.02 ± 0.14	0.43 ± <0.01	0.03 ± 0.03
	R1	2010	4	0.05 ± 0.11	0.65 ± 0.01	0.02 ± 0.15
	R1	2011	5	--	--	0.016 ± 0.01
	R1	2012	6	0.04 ± 0.10	0.43 ± 0.01	0.01± 0.06
	R1	2013	6	0.043 ± 0.10	0.49 ± 0.01	0.01 ± 0.09
	R2	2010	6	0.04 ± 0.08	0.50 ± 0.01	0.01 ± 0.03
	R2	2011	7	--	0.55	<0.01 ± 0.02
	R2	2012	6	0.02 ± 0.08	0.43 ± <0.01	0.01 ± 0.02
	R3	2009	6	0.06 ± 0.09	0.59 ± <0.001	0.01 ± 0.03
	R3	2010	6	0.06 ± 0.13	0.55 ± 0.01	0.01 ± 0.02
	R3	2011	6	0.05 ± 0.10	0.54 ± <0.01	0.01 ± 0.03
	R3	2012	6	0.03 ± 0.14	0.50 ± 0.01	0.01 ± 0.03
	R3	2013	6	--	0.44	0.01 ± 0.02
	S1	2009	6	0.04 ± 0.06	0.80 ± <0.01	0.01 ± 0.04
	S1	2010	6	0.07 ± 0.09	0.85 ± 0.01	0.01 ± 0.06
	S1	2011	6	0.05 ± 0.06	0.92 ± 0.01	<0.01 ± 0.04
	S1	2012	6	0.06 ± 0.11	0.80 ± 0.01	0.01 ± 0.08
	S1	2013	6	0.04 ± 0.12	0.73 ± 0.01	0.02 ± 0.04
	S2	2009	6	0.10 ± 0.08	0.77	0.01 ± 0.03
	S2	2010	5	0.08 ± 0.11	0.74 ± 0.02	0.03 ± 0.06
	S2	2011	6	0.06 ± 0.07	0.95 ± 0.01	<0.01 ± 0.03
	S2	2012	6	0.06 ± 0.09	0.86 ± 0.01	0.01 ± 0.06
	S2	2013	6	0.08 ± 0.09	0.81 ± 0.01	0.01 ± 0.05
	S3	2009	6	--	0.70	0.01 ± 0.07
	S3	2010	6	0.16 ± 0.09	0.82 ± 0.04	0.01± 0.06
	S3	2011	6	0.06 ± 0.08	1.08 ± 0.01	<0.01 ± 0.06
	S3	2012	6	0.06 ± 0.11	0.96 ± 0.01	0.01 ± 0.08
	S3	2013	6	--	0.81	0.02± 0.06
Redear						
	R1	2010	6	0.07 ± 0.09	0.56 ± 0.01	0.02 ± 0.02
	R1	2011	7	--	0.57	0.01± 0.04
	R1	2012	6	0.07 ± 0.12	0.54 ± 0.02	0.01 ± 0.04
	R1	2013	6	0.08 ± 0.13	0.573 ± 0.01	0.02± 0.11
	R2	2010	6	0.07 ± 0.08	0.62 ± 0.01	0.01 ± 0.02
	R2	2011	7	0.08 ± 0.07	0.74± 0.02	<0.01 ± 0.05
	R2	2012	6	0.04 ± 0.08	0.64 ± 0.01	0.01 ± 0.05
	R3	2010	6	0.21 ± 0.10	0.79 ± 0.03	0.02 ± 0.04
	R3	2011	6	0.25 ± 0.09	0.82 ± 0.06	0.02 ± 0.07
	R3	2012	6	0.19 ± 0.14	0.62 ± 0.03	0.01 ± 0.02
	R3	2013	6	0.20 ± 0.18	0.6 ± 0.02	0.02 ± 0.03
	S1	2010	6	0.11 ± 0.11	0.89 ± 0.01	0.02 ± 0.07

	Site	Year	N	As	Hg	Se
	S1	2011	6	0.15 ± 0.05	1.13 ± 0.02	0.01 ± 0.04
	S1	2012	6	0.10 ± 0.11	0.74 ± 0.02	0.02 ± 0.07
	S1	2013	6	0.14 ± 0.08	0.92 ± 0.03	0.02 ± 0.06
	S2	2010	5	0.23 ± 0.11	1.05 ± 0.02	0.01 ± 0.07
	S2	2011	6	0.23 ± 0.06	1.17 ± 0.04	0.01 ± 0.031
	S2	2012	6	0.20 ± 0.07	0.85 ± 0.02	0.01 ± 0.04
	S2	2013	6	0.21 ± 0.09	1.02 ± 0.02	0.02 ± 0.11
	S3	2010	6	0.23 ± 0.10	1.02 ± 0.03	0.01 ± 0.12
	S3	2011	6	0.19± 0.05	1.23 ± 0.02	0.01 ± 0.11
	S3	2012	6	0.20 ± 0.12	1.01 ± 0.02	0.01± 0.08
	S3	2013	6	0.21 ± 0.08	0.78 ± 0.02	0.01 ± 0.02
LMBass						
	R1	2009	11	0.17 ± 0.03	0.18± 0.02	0.477 ± 0.04
	R1	2010	6	0.15 ± 0.01	0.23 ± 0.02	0.457 ± 0.02
	R1	2011	7	0.15 ± 0.02	0.27 ± 0.04	0.40 ± 0.01
	R1	2012	6	0.16 ± 0.01	0.34 ± 0.09	0.37± 0.02
	R1	2013	6	0.15 ± 0.02	0.47 ± 0.07	0.40 ± 0.01
	R2	2010	6	0.10 ± 0.02	0.19 ± 0.04	0.46 ± 0.03
	R2	2011	7	0.15 ± 0.02	0.17 ± 0.02	0.47 ± 0.03
	R2	2012	6	0.09 ± 0.01	0.20± 0.04	0.40 ± 0.02
	R3	2009	6	0.27 ± 0.02	0.10 ± 0.01	0.60 ± 0.07
	R3	2010	6	0.24 ± 0.03	0.21± 0.02	0.50 ± 0.03
	R3	2011	6	0.29 ± 0.03	0.14 ± 0.02	0.62 ± 0.06
	R3	2012	6	0.19 ± 0.03	0.34 ± 0.05	0.43 ± 0.03
	R3	2013	6	0.27 ± 0.01	0.21 ± 0.02	0.40 ± 0.05
	S1	2009	5	0.15 ± 0.02	0.17± 0.05	0.59 ± 0.05
	S1	2010	6	0.17 ± 0.01	0.18± 0.03	0.66 ± 0.06
	S1	2011	6	0.29 ± 0.02	0.10 ± 0.02	0.70 ± 0.07
	S1	2012	6	0.12 ± 0.04	0.28 ± 0.07	0.56 ± 0.04
	S1	2013	6	0.14 ± 0.01	0.26 ± 0.04	0.59 ± 0.06
	S2	2009	6	0.22 ± 0.03	0.12 ± 0.02	0.61 ± 0.04
	S2	2010	6	0.27 ± 0.05	0.19 ± 0.06	0.62 ± 0.03
	S2	2011	6	0.30 ± 0.04	0.08 ± 0.02	0.65 ± 0.10
	S2	2012	6	0.16 ± 0.01	0.2 ± 0.02	0.54 ± 0.07
	S2	2013	6	0.21 ± 0.03	0.27 ± 0.06	0.56 ± 0.07
	S3	2009	6	0.23 ± 0.02	0.12 ± 0.02	0.56 ± 0.03
	S3	2010	6	0.21 ± 0.04	0.23 ± 0.09	0.447 ± 0.02
	S3	2011	6	0.27 ± 0.03	0.09 ± 0.01	0.77 ± 0.05
	S3	2012	6	0.20 ± 0.03	0.25 ± 0.04	0.513 ± 0.07
	S3	2013	6	0.24 ± 0.02	0.19 ± 0.01	0.58 ± 0.04

	Site	Year	N	alb	alp	amy	bpro	bun	ca	creat	glob	glu	na	phos	tbil
Bluegill															
	R1	2009	30	15.89 ± 29.96	30.36 ± 37.01	2.48 ± 14.49	0.08 ± 21.85	65.04 ± 149.64	10.21 ± 0.49	1.69 ± 0.08	0.011 ± <0.01	0.04 ± 1.45	2.26 ± 4.10	2.67 ± 0.22	0.79 ± 0.02
	R1	2010	15	20.47 ± 29.20	38.6 ± 40.67	2.87 ± 17.31	0.03 ± 20.13	60.67 ± 153.53	11.12 ± 0.73	1.61 ± 0.07	0.02 ± <0.01	0.04 ± 1.09	3.83 ± 2.84	1.26 ± 0.24	0.80 ± 0.01
	R1	2011	16	21.36 ± 24.07	48.143 ± 40.93	2.86 ± 15.82	0.04 ± 19.50	49.57 ± 148.14	10.09 ± 0.78	1.72 ± 0.04	0.01 ± <0.01	0.04 ± 1.04	7.96 ± 7.72	1.37 ± 0.23	0.75 ± 0.02
	R1	2012	17	20.88 ± 17.29	30.24 ± 39.71	1.76 ± 15.78	0.01 ± 18.82	46.53 ± 149.29	9.93 ± 0.69	1.65 ± 0.03	0.01 ± <0.01	0.03 ± 0.74	1.26 ± 2.95	1.06 ± 0.22	0.78 ± 0.01
	R1	2013	9	24.89 ± 29.22	54.89 ± 46.78	3.00 ± 19.36	--	56.00 ± 149.11	14.46 ± 0.63	1.65 ± 0.08	0.02 ± <0.01	0.03 ± 0.96	4.47 ± 2.71	1.19 ± 0.37	0.25
	R2	2009	10	16.7 ± 22.2	21.40 ± 35.60	2.20 ± 12.19	0.12 ± 18.9	39.10 ± 149.10	6.39 ± 0.70	1.66	0.012 ± <0.01	0.04 ± 0.50	0.98 ± 2.08	0.6 ± 0.25	0.33 ± 0.02
	R2	2010	18	20 ± 26.41	45.65 ± 39.65	2.59 ± 17.41	0.08 ± 19.94	45.94 ± 148.59	9.85 ± 0.52	1.65 ± 0.09	0.02 ± <0.01	0.03 ± 0.64	3.34 ± 5.72	1.05 ± 0.15	0.86 ± 0.02
	R2	2011	18	24.33 ± 15.83	47.33 ± 41.78	2.17 ± 16.70	0.01 ± 17.56	54.67 ± 150.67	10.42 ± 0.73	1.64 ± 0.04	0.01 ± <0.01	0.04 ± 0.83	2.26 ± 5.40	1.29 ± 0.19	0.75 ± 0.01
	R2	2012	17	19.82 ± 20.59	33.35 ± 38.65	2.24 ± 15.75	0.01 ± 18.65	50.06 ± 152.00	9.95 ± 0.7	1.65 ± 0.04	0.01 ± <0.01	0.03 ± 0.76	2.04 ± 2.57	0.84 ± 0.16	0.72 ± 0.01
	R3	2009	23	15.33 ± 31.81	34.19± 38.48	3.19 ± 16.59	0.04 ± 23.45	62.24 ± 151.43	11.10 ± 0.44	1.76 ± 0.12	0.01 ± <0.01	0.03 ± 0.70	1.75 ± 3.19	1.09 ± 0.18	0.73 ± 0.01
	R3	2010	17	19.5 ± 25.06	45.0625 ± 40.1875	2.25 ± 17.93	0.06 ± 20.75	44.38 ± 150.31	10.68 ± 0.44	1.72 ± 0.12	0.01 ± <0.01	0.04 ± 0.74	1.54 ± 4.35	1.19 ± 0.21	1.11 ± 0.02
	R3	2011	19	19.21 ± 24.05	45.00 ± 39.63	1.74 ± 16.6	0.01 ± 20.42	52.42 ± 153.16	10.91 ± 0.65	1.68 ± 0.05	0.01 ± <0.01	0.04 ± 0.89	2.48 ± 6.40	0.74 ± 0.17	0.78 ± 0.01
	R3	2012	18	21.22 ± 21.722	37.44 ± 40.50	2.17 ± 14.92	0.01 ± 19.28	48.50 ± 151.06	10.19 ± 0.64	1.68 ± 0.03	0.01 ± <0.01	0.03 ± 0.79	2.67 ± 3.55	0.98 ± 0.20	0.66 ± 0.01
	R3	2013	9	22.78 ± 25.44	56.33 ± 46.22	2.67 ± 19.28	--	60.44 ± 149.00	13.14 ± 0.54	1.75 ± 0.08	0.02 ± <0.01	0.03 ± 1.26	3.05 ± 4.06	1.84 ± 0.24	0.39
	S1	2009	19	14.75 ± 25.88	36.63 ± 35.56	2.57 ± 14.11	0.05 ± 21.13	47.19 ± 148.00	8.56 ± 0.41	1.63 ± 0.04	0.01 ± <0.01	0.03 ± 0.66	1.11 ± 5.06	1.20 ± 0.26	0.83 ± 0.02
	S1	2010	20	19.80 ± 27.40	39.10 ± 40.35	2.35 ± 17.45	0.07 ± 20.65	67.60 ± 150.00	11.10 ± 0.50	1.74 ± 0.09	0.02 ± <0.01	0.04 ± 0.60	4.03 ± 3.86	0.95 ± 0.13	0.73 ± 0.02
	S1	2011	19	22.28 ± 13.33	37.22 ± 41.06	2.28 ± 16.70	0.03 ± 18.56	58.67 ± 151.33	11.64 ± 0.59	1.72 ± 0.04	0.01 ± <0.01	0.04 ± 0.51	2.87 ± 4.33	0.88 ± 0.18	0.69 ± 0.01
	S1	2012	16	18.00 ± 16.50	31.82 ± 37.44	2.50 ± 16.01	0.04 ± 18.31	57.19 ± 147.63	10.84 ± 0.67	1.60 ± 0.03	0.01 ± <0.01	0.03 ± 1.18	0.97 ± 2.97	1.13 ± 0.26	0.61 ± 0.02
	S1	2013	9	21.38 ± 24.50	41.63 ± 43.63	2.13 ± 19.28	0 ± 22.13	64.00 ± 147.63	12.63 ± 0.41	1.64 ± 0.07	0.02 ± <0.01	0.03 ± 1.05	1.94 ± 4.50	1.15 ± 0.13	0.39
	S2	2009	22	16.06 ± 32.81	37.56 ± 39.81	2.94 ± 16.68	0.08 ± 23.75	75.25 ± 155.44	11.84 ± 0.46	1.71 ± 0.08	0.01 ± <0.01	0.03 ± 0.58	2.15 ± 4.86	0.56 ± 0.27	0.69 ± 0.03
	S2	2010	17	19.75 ± 29.13	44.69 ± 39.88	1.81 ± 16.46	0.08 ± 20.19	52.88± 151.50	10.74 ± 0.38	1.69 ± 0.11	0.01 ± <0.01	0.03 ± 0.67	4.12 ± 5.98	0.82 ± 0.25	0.84 ± 0.02
	S2	2011	20	23.82 ± 19.06	45.41 ± 44.18	2.65 ± 17.47	0.04 ± 20.41	53.53 ± 153.65	10.81 ± 0.53	1.68 ± 0.04	0.01 ± <0.01	0.04 ± 0.69	3.89 ± 6.33	0.88 ± 0.28	0.61 ± 0.01
	S2	2012	17	19.06 ± 15.63	26.31 ± 38.31	2 ± 15.42	0.01 ± 19.44	43.19 ± 146.69	10.62 ± 0.48	1.58 ± 0.04	0.01 ± <0.01	0.03 ± 0.69	2.04 ± 3.20	1.00 ± 0.18	0.97 ± 0.01

	S2	2013	8	19.13 ± 18.00	51.88 ± 39.63	2.75 ± 17.49	0.04 ± 20.50	56.63 ± 146.38	11.60 ± 0.38	1.53 ± 0.07	0.02 ± <0.01	0.03 ± 0.55	1.98 ± 2.73	0.89 ± 0.16	0.55 ± 0.02
	S3	2009	29	14.68 ± 30.21	24.14 ± 35.57	2.04 ± 14.85	0.08 ± 21.00	70.18 ± 150.04	9.53 ± 0.45	1.63 ± 0.06	0.01 ± <0.01	0.04 ± 1.00	2.43 ± 2.60	1.28 ± 0.20	0.65 ± 0.015
	S3	2010	20	19.47 ± 31.05	42.95 ± 38.63	2.00 ± 17.26	0.09 ± 18.26	68.53 ± 151.11	12.05 ± 0.53	1.66 ± 0.10	0.02 ± <0.01	0.03 ± 0.94	3.00 ± 3.85	1.21 ± 0.17	0.68 ± 0.025
	S3	2011	18	21.67 ± 17.67	45.72± 41.06	1.89 ± 16.59	0.03 ± 19.50	59.617 ± 151.218	10.73 ± 0.50	1.69 ± 0.02	0.01± <0.01	0.04 ± 0.88	3.70 ± 6.04	1.06 ± 0.23	0.67 ± 0.01
	S3	2012	19	19.16 ± 26.21	35.32 ± 37.42	1.74 ± 15.06	0.02 ± 18.32	63.11 ± 148.58	10.53 ± 0.54	1.66 ± 0.04	0.01± <0.01	0.03 ± 0.61	6.43 ± 2.59	0.83 ± 0.23	0.66 ± 0.01
	S3	2013	9	19.78 ± 28.56	43.78 ± 43.33	2.44 ± 19.77	--	65.78 ± 151.67	15.30 ± 0.57	1.65 ± 0.08	0.02 ± <0.01	0.03 ± 1.12	2.281 ± 3.70	1.78 ± 0.24	0.21
Redear															
	R1	2010	13	11.33 ± 0.68	21.167 ± 2.55	75.75 ± 10.02	36.08 ± 0.77	6.25 ± 0.30	18.12 ± 0.34	0.09 ± 0.03	16.33 ± 3.53	67.58 ± 5.15	154.67 ± 1.78	10.43 ± 0.90	0.43 ± 0.04
	R1	2011	9	17.33 ± 1.04	5.67 ± 1.3	108.22 ± 15.03	40.22± 1.68	7.56 ± 0.60	18.06 ± 0.72	--	22.67 ± 0.87	74.56 ± 4.48	152.22 ± 1.35	10.79 ± 0.79	0.51 ± 0.21
	R1	2012	17	15.88 ± 0.62	11.47± 1.80	137.35 ± 21.12	38.18 ± 0.63	3.88 ± 0.33	15.65 ± 0.55	--	22.06 ± 0.40	73.35 ± 6.39	153.18 ± 1.11	10.34 ± 0.70	0.54 ± 0.06
	R1	2013	8	18.00 ± 0.85	13.88 ± 1.25	106.50 ± 12.49	42.75 ± 0.94	6.25 ± 0.25	18.44 ± 0.50	--	24.88 ± 0.64	61.63 ± 3.35	150.50 ± 1.50	10.38 ± 0.79	0.41 ± 0.02
	R2	2010	12	10.25 ± 0.88	25.13 ± 4.65	43.63 ± 5.07	35.75 ± 1.06	4.88 ± 0.79	18.94 ± 0.52	0.09 ± 0.02	12.63 ± 4.78	92.25 ± 12.54	155.50 ± 1.16	14.13 ± 1.69	0.46 ± 0.06
	R2	2011	10	16 ± 1.07	10.20 ± 0.94	89.00 ± 12.18	38.20 ± 0.98	6.20 ± 0.44	17.46 ± 0.52	--	22.20 ± 1.07	73.40 ± 8.98	153.40 ± 1.45	9.68 ± 0.84	0.32 ± 0.02
	R2	2012	17	14.76 ± 0.60	13.29 ± 1.48	179.828 ± 29.59	37.35 ± 0.85	4.59 ± 0.31	14.48 ± 0.60	0.01 ± 0.01	22.47 ± 0.55	61.41 ± 3.63	151.00 ± 1.45	9.23 ± 0.48	0.59 ± 0.07
	R3	2010	7	15.14 ± 1.74	12.43 ± 1.25	78.14 ± 11.72	41.00 ± 2.36	7.71 ± 0.92	20.49 ± 1.00	0.07 ± 0.03	25.86 ± 0.91	60.14 ± 4.04	156.29 ± 1.66	11.44 ± 1.02	0.33 ± 0.03
	R3	2011	9	19.44 ± 1.96	8.22 ± 2.01	102.56 ± 14.21	46.33 ± 2.69	6.44 ± 1.04	19.69 ± 0.30	--	26.67 ± 1.14	76.33 ± 8.55	163.56 ± 1.77	14.19 ± 0.74	0.81 ± 0.10
	R3	2012	17	19.06 ± 1.07	10.76 ± 1.52	146.59 ± 32.39	46.12 ± 1.87	5.47 ± 0.52	16.56 ± 0.68	0.02 ± 0.01	27.00 ± 0.84	63.18 ± 4.07	155.18 ± 1.86	10.65 ± 0.79	0.71 ± 0.07
	R3	2013	8	16.63 ± 2.40	11.75 ± 1.33	75.38 ± 5.45	45.13 ± 3.48	5.38 ± 0.75	19.75 ± 0.17	--	25.50 ± 3.76	87.38 ± 4.63	152.25 ± 2.17	10.89 ± 0.89	0.60 ± 0.11
	S1	2010	8	7.25 ± 1.19	9.88 ± 0.85	68.38 ± 8.40	32.75 ± 1.67	3.63 ± 0.26	18.21 ± 0.35	0.08 ± 0.04	9.63 ± 4.73	76.25 ± 3.70	151.38 ± 1.03	10.41 ± 0.60	0.21 ± 0.01
	S1	2011	8	19.5 ± 0.82	8.13 ± 0.69	76.88 ± 16.97	42.13 ± 1.32	4.75 ± 0.45	18.04 ± 0.47	--	22.75 ± 1.10	58.13 ± 5.23	153.25 ± 2.22	9.45 ± 0.90	0.44 ± 0.05
	S1	2012	17	15.18 ± 0.72	10.41 ± 1.13	142.59 ± 30.18	38.71 ± 0.79	4.94 ± 0.30	15.32± 0.33	0.01 ± 0.01	22.24 ± 1.50	67.71 ± 7.02	149.18 ± 0.85	10.66 ± 0.42	0.56 ± 0.08
	S1	2013	8	20.88 ± 2.75	12.38 ± 1.18	127.88 ± 18.24	48.63 ± 5.85	5.63 ± 0.89	19.03 ± 0.42	--	27.88 ± 3.07	75.63 ± 8.93	152.13 ± 4.34	12.74 ± 1.02	0.40 ± 0.02
	S2	2010	10	11.78 ± 1.10	12.89 ± 1.11	46.78 ± 3.93	38.11 ± 1.51	6.78 ± 0.55	18.96 ± 0.71	0.06 ± 0.02	20.44 ± 3.94	100.56 ± 9.65	153.11 ± 3.36	11.77 ± 1.45	0.48 ± 0.08
	S2	2011	9	17.78 ± 2.31	8.44 ± 0.96	86.11 ± 18.13	42.56 ± 3.54	6.67 ± 0.69	18.42 ± 1.25	0.02 ± 0.01	22.78 ± 3.04	55.89 ± 5.35	148.11 ± 6.09	9.61 ± 1.07	0.48 ± 0.06
	S2	2012	18	17.72 ± 0.56	11.56 ± 1.19	154.17 ± 20.36	42.11 ± 0.81	5.33 ± 0.29	16.12 ± 0.54	0.03 ± 0.01	24.33 ± 0.46	68.61 ± 4.18	154.11 ± 1.21	10.10 ± 0.54	0.58 ± 0.05

	S2	2013	9	20.33 ± 0.71	12.22 ± 1.14	103.56 ± 11.30	46.22 ± 0.81	5.56 ± 0.444	19.71± 0.18	--	25.89 ± 0.26	74.44± 4.32	158.44 ± 1.65	11.40± 0.57	0.5 ± 0.06
	S3	2010	9	9.67 ± 1.38	23.78 ± 3.85	45.22 ± 6.77	37.33 ± 1.46	6.33 ± 0.41	19.93 ± 0.17	0.10 ± 0.03	12.44 ± 4.93	102.33 ± 8.09	155.67 ± 1.55	12.36 ± 1.22	0.6 ± 0.06
	S3	2011	8	12.75 ± 2.14	12.38 ± 0.94	68.25 ± 11.13	40.75 ± 3.01	5.50 ± 0.57	18.25 ± 0.72	--	21.00 ± 4.80	60.63 ± 9.73	151.75 ± 1.86	11.00 ± 0.75	0.38 ± 0.04
	S3	2012	16	17.06± 1.12	12.81 ± 1.65	212.88 ± 53.71	41.50 ± 1.27	4.50 ± 0.37	15.762 ± 0.502	0.01 ± 0.01	22.94 ± 1.61	68.75 ± 5.54	153.94 ± 1.47	9.90 ± 0.50	0.54 ± 0.05
	S3	2013	8	18.00 ± 1.51	9.13 ± 1.49	65.00 ± 9.68	44.63 ± 1.85	5.88 ± 0.40	19.54 ± 0.23	--	26.38 ± 0.80	86.25 ± 10.66	159.50 ± 2.10	13.29± 1.49	0.61 ± 0.07
LMBass															
	R1	2009	26	20.50 ± 37.83	182.17 ± 50.33	1.83 ± 17.48	0.18 ± 29.83	57.83 ± 156.50	7.98 ± 0.30	1.28 ± 0.02	0.01 ± <0.01	0.04 ± 1.54	4.634 ± 39.27	3.57 ± 0.17	0.87 ± 0.06
	R1	2010	16	19.64 ± 32.72	210.08 ± 47.96	2.12 ± 15.40	0.10 ± 28.29	65.60 ± 153.40	9.46 ± 0.36	1.37 ± 0.06	0.01 ± <0.01	0.05 ± 0.94	1.36 ± 15.95	1.47 ± 0.25	0.64 ± 0.02
	R1	2011	16	20.00 ± 24.88	215.25 ± 46.75	2.44 ± 16.13	0.11 ± 26.88	50.50 ± 151.13	8.40 ± 0.36	1.33 ± 0.04	0.01 ± <0.01	0.04 ± 0.94	1.70 ± 25.42	1.84 ± 0.22	0.96 ± 0.04
	R1	2012	17	21.20 ± 23.73	201.20 ± 48.27	2.40 ± 17.05	0.14 ± 27.13	61.00 ± 150.40	8.71 ± 0.35	1.35 ± 0.02	0.01 ± <0.01	0.04 ± 1.05	2.06 ± 17.40	1.75 ± 0.21	0.83 ± 0.04
	R1	2013	8	22.82 ± 28.06	228.88 ± 48.41	1.94 ± 14.78	0.07 ± 25.59	55.47 ± 151.35	7.77 ± 0.36	1.37 ± 0.02	0.01 ± <0.01	0.04 ± 0.69	2.43 ± 18.69	1.36 ± 0.39	0.59 ± 0.02
	R2	2009	8	22.13 ± 20.75	200.63 ± 50.75	2.75 ± 17.98	0.23 ± 28.88	58.37 ± 150.13	7.23 ± 0.33	1.48 ± 0.06	0.01 ± <0.01	0.04 ± 1.16	1.08 ± 18.48	1.81 ± 0.31	0.45 ± 0.07
	R2	2010	16	18.75 ± 26.63	204.50 ± 44.50	2.00 ± 13.23	0.09 ± 25.75	36.00 ± 148.88	7.13 ± 0.48	1.37	0.01 ± <0.01	0.05 ± 0.98	1.57 ± 30.32	2.16	0.91 ± 0.03
	R2	2011	17	20.81 ± 24.69	191.31 ± 47.75	2.38 ± 16.67	0.11 ± 27.00	60.13 ± 152.81	8.76 ± 0.35	1.44 ± 0.04	0.01 ± <0.01	0.04 ± 0.96	1.23 ± 15.85	1.52 ± 0.22	1.03 ± 0.04
	R2	2012	16	22.00 ± 29.06	234.00 ± 48.71	3.24 ± 15.86	0.09 ± 26.82	64.41 ± 151.35	7.83 ± 0.33	1.30 ± 0.03	0.01 ± <0.01	0.04 ± 0.90	1.04 ± 23.12	1.52 ± 0.33	1.24 ± 0.034
	R3	2009	15	23.80 ± 26.53	210.33 ± 50.73	1.80 ± 15.43	0.02 ± 26.67	61.00 ± 152.53	8.473 ± 0.373	1.36 ± 0.03	0.01 ± <0.01	0.03 ± 0.72	1.94 ± 14.76	1.46 ± 0.34	0.71 ± 0.01
	R3	2010	16	16.17 ± 35.25	154.83 ± 47.83	3.00 ± 14.9	0.08 ± 32.27	60.58 ± 151.83	7.53 ± 0.32	1.27 ± 0.05	0.01 ± <0.01	0.05 ± 1.01	1.90 ± 14.10	1.98 ± 0.43	0.99 ± 0.04
	R3	2011	17	21.13 ± 38.94	221.75 ± 54.13	3.00 ± 16.84	0.23 ± 33.13	78.50 ± 154.81	10.60 ± 0.35	1.44 ± 0.06	0.01 ± <0.01	0.05 ± 0.96	2.48 ± 18.32	2.18 ± 0.29	0.71 ± 0.03
	R3	2012	17	18.75 ± 26.75	195.25 ± 44.63	2.44 ± 14.11	0.09 ± 25.88	44.81 ± 151.38	7.43 ± 0.29	1.37 ± 0.04	0.01 ± <0.01	0.04 ± 0.684	1.88 ± 13.51	1.22 ± 0.33	0.41 ± 0.03
	R3	2013	8	21.24 ± 24.76	199.47 ± 48.88	1.82 ± 15.07	0.12 ± 27.47	51.12 ± 151.65	8.24 ± 0.35	1.50 ± 0.03	0.01 ± <0.01	0.044± 0.74	1.62 ± 15.52	1.15 ± 0.27	0.68 ± 0.03
	S1	2009	16	21.88 ± 27.88	229.88 ± 54.50	1.88 ± 17.53	0.07 ± 32.75	70.88 ± 150.25	7.93 ± 0.33	1.58 ± 0.05	0.01 ± <0.01	0.044 ± 0.77	2.78 ± 22.65	2.60 ± 0.30	0.79 ± 0.03
	S1	2010	16	18.40 ± 33.07	162.73 ± 48.73	3.00 ± 16.29	0.05 ± 30.27	77.07 ± 154.00	9.13 ± 0.31	1.36 ± 0.05	0.01 ± <0.01	0.05 ± 0.82	2.10 ± 14.22	1.85 ± 0.20	0.94 ± 0.02
	S1	2011	17	22.14 ± 30.14	194.93 ± 53.43	2.07 ± 17.25	0.07 ± 31.29	109.14 ± 154.86	10.68 ± 0.38	1.47 ± 0.05	0.01± <0.01	0.05 ± 1.00	2.79 ± 14.67	1.92 ± 0.16	0.94 ± 0.03
	S1	2012	17	21.59 ± 26.41	224.76 ± 49.24	4.47 ± 16.39	0.12 ± 27.47	87.18 ± 152.71	7.96 ± 0.33	1.50 ± 0.05	0.01 ± <0.01	0.04 ± 0.66	2.29 ± 13.33	1.17 ± 0.36	0.61 ± 0.03
	S1	2013	8	22.38 ± 29.50	182.63 ± 49.82	2.31 ± 15.79	0.11 ± 27.50	58.44 ± 151.94	8.81 ± 0.40	1.39 ± 0.03	0.01 ± <0.01	0.04 ± 0.88	3.07 ± 20.51	1.66 ± 0.27	0.73 ± 0.03

S2	2009	21	21.714 ± 26.86	218.29 ± 50.71	2.29 ± 17.27	0.06 ± 28.86	57.00 ± 145.00	7.46 ± 0.31	1.45 ± 0.06	0.01 ± <0.01	0.03 ± 1.38	4.99 ± 13.68	2.58 ± 0.36	0.43 ± 0.03
S2	2010	16	19.32 ± 35.47	207.11 ± 52.58	3.37 ± 16.48	0.18 ± 33.16	98.68 ± 157.95	10.53 ± 0.32	1.41 ± 0.06	0.01 ± <0.01	0.04 ± 0.85	1.61 ± 13.06	1.49 ± 0.16	0.50 ± 0.031
S2	2011	16	20.56± 34.81	259.06 ± 54.25	2.69 ± 16.12	0.20 ± 29.13	90.38 ± 154.50	8.52 ± 0.41	1.41 ± 0.05	0.01 ± <0.01	0.04 ± 1.67	2.14 ± 28.97	2.50 ± 0.24	1.24± 0.04
S2	2012	16	22.25 ± 26.44	259.00 ± 50.25	3.50 ± 16.31	0.10 ± 27.88	70.50 ± 150.88	9.39 ± 0.35	1.41 ± 0.05	0.01 ± <0.01	0.04 ± 0.77	1.83 ± 22.334	1.34 ± 0.43	0.72 ± 0.03
S2	2013	10	21.56 ± 24.25	186.63 ± 47.88	2.19 ± 15.42	0.08 ± 26.13	52.13 ± 149.88	8.16 ± 0.44	1.42 ± 0.03	0.01 ± <0.01	0.04 ± 0.76	1.67 ± 14.19	1.42± 0.16	0.68 ± 0.03
S3	2009	26	25.56 ± 31.11	309.44 ± 59.33	2.78 ± 18.3	0.16 ± 33.67	89.67 ± 154.56	9.67 ± 0.38	1.46 ± 0.06	0.02 ± <0.01	0.04 ± 1.72	1.40 ± 43.82	3.46 ± 0.32	0.74 ± 0.05
S3	2010	15	18.12 ± 29.33	189.38 ± 50.21	2.20 ± 16.00	0.11 ± 31.36	60.68 ± 152.38	9.34 ± 0.34	1.40 ± 0.04	0.02 ± <0.01	0.05 ± 1.20	2.82 ± 20.19	1.86 ± 0.29	0.75 ± 0.02
S3	2011	16	21.50 ± 34.58	257.33 ± 52.00	2.58 ± 16.85	0.17 ± 30.42	102.67 ± 155.08	11.95 ± 0.34	1.42 ± 0.05	0.01 ± <0.01	0.05 ± 1.28	1.64 ± 28.31	2.25 ± 0.29	1.18 ± 0.06
S3	2012	17	22.56 ± 29.13	237.44 ± 52.06	3.81 ± 16.36	0.17 ± 29.63	77.00 ± 150.50	7.15 ± 0.34	1.43 ± 0.05	0.01 ± <0.01	0.05 ± 0.66	2.25 ± 22.25	1.26 ± 0.36	0.81 ± 0.04
S3	2013	9	21.53 ± 25.20	177.53 ± 47.67	2.40 ± 14.67	0.09 ± 26.13	54.47 ± 150.60	6.99 ± 0.32	1.41 ± 0.03	0.01 ± <0.01	0.04 ± 1.06	1.50 ± 20.60	1.88 ± 0.41	0.83 ± 0.02
			23.33 ± 26.89	231.78 ± 53.33	3.00 ± 18.14	0.03 ± 30.00	59.22 ± 151.33	7.79 ± 0.33	1.52 ± 0.06	0.01 ± <0.01	0.04 ± 0.73	2.96 ± 17.43	1.35 ± 0.33	0.37 ± 0.02

cf	gsi	lsi	ssi	vsi
1.67 + 5.20	1.69 + 0.75	0.0514198405 + 0.0311851775	0.0080790974 + 0.0010373031	0.0000908616 + 0.0010879156
0.56 + 11.26	1.73 + 0.54	0.1388615846 + 0.0393596471	0.004360654 + 0.0018746539	0.0001177914 + 0.0029337136
0.53 + 3.45	1.54 + 0.85	0.1105324632 + 0.0322207969	0.0126250385 + 0.0014063943	0.0003028121 + 0.0014215348
0.72 + 4.37	1.31 + 0.81	0.0669077245 + 0.0254847325	0.0085147165 + 0.0008355596	0.0001447257 + 0.0007344819
0.85 + 7.04	1.45 + 1.27	0.0552770798 + 0.0411849473	0.0113945824 + 0.0012679708	0.0002079944 + 0.0014992817
0.41 + 1.32	1.56 + 0.33	0.2097617696 + 0.0426146283	+ 0.0008003847	0.0003518192 + 0.0025876959
0.61 + 2.54	1.29 + 0.51	0.0681883713 + 0.0376930225	0.0088577721 + 0.001696328	0.0000722765 + 0.0013805341
0.66 + 3.19	1.95 + 0.59	0.0866235019 + 0.0462936917	0.0100264954 + 0.0014084264	0.0002250784 + 0.0018743371
0.53 + 4.45	1.27 + 0.70	0.0804399667 + 0.0295894752	0.0085730945 + 0.0008306322	0.0001025242 + 0.0017388376
0.61 + 5.49	1.16 + 0.61	0.0439542124 + 0.0312753728	0.0082543654 + 0.0007212738	0.0001564305 + 0.0016484741
0.70 + 2.31	1.34 + 0.93	0.0455292946 + 0.0339857654	0.0055170721 + 0.0016122053	0.0001758619 + 0.0007869291
0.57 + 3.3789127542	1.11 + 0.65	0.0762300385 + 0.0275762619	0.0104593991 + 0.0013632913	0.0002052645 + 0.0021285389
0.47 + 3.62	1.84 + 0.66	0.0600774131 + 0.0294896618	0.0074544405 + 0.0012312621	0.0002752462 + 0.0008956656
0.71 + 8.87	1.20 + 1.05	0.0293972368 + 0.0467174973	0.0124862414 + 0.0010520999	0.0001428303 + 0.0019803116
0.68 + 3.22	2.79 + 0.66	0.0280902563 + 0.029304156	0.0052774417 + 0.0007970338	0.0001634111 + 0.0017031283
0.56 + 5.94	1.31 + 0.57	0.0656946685 + 0.0365091464	0.0056327189 + 0.0014921382	0.0001230362 + 0.0020527583

0.60 + 5.24	1.20 + 0.75	0.0774971595 + 0.0299399963	0.0096840877 + 0.0011243309	0.0001776761 + 0.0017646277
1.39 + 6.25	1.20 + 0.74	0.0820156235 + 0.0411044812	0.0073124069 + 0.0009008282	0.0001837666 + 0.0011951144
0.91 + 10.92	1.32 + 0.56	0.0440677239 + 0.0250785993	0.007670067 + 0.0008542013	0.0003621226 + 0.0012394717
0.50+ 6.35	1.08 + 0.77	0.1052279272 + 0.0299269678	0.005132726 + 0.0008557771	0.0005262771 + 0.0011157531
0.56 + 4.84	1.36 + 0.79	0.025 + 0.0363975323	0.005757346 + 0.001237825	0.0001823304 + 0.0014037998
0.51 + 4.53	1.93 + 0.86	0.063457203 + 0.0231033757	0.0088361911 + 0.0009179858	0.0002249425 + 0.0009434817
0.94 + 2.06	1.55 + 0.72	0.0378800189 + 0.0197643109	0.0087220474 + 0.0006914983	0.0002207917 + 0.0011192259
0.57 + 7.07	0.78 + 0.31	0.0163663418 + 0.0465035988	0.0050295252 + 0.0011647935	0.0001478619 + 0.000606573
0.73 + 13.47	2.01 + 0.62	0.0422577127 + 0.0242923534	0.0046209683 + 0.0005736081	0.0000905334 + 0.0014307663
1.08 + 18.44	1.32 + 1.28	0.0670349936 + 0.0267690906	0.0068809821 + 0.0012868661	0.0000968254 + 0.0017051453
0.52 + 6.45	1.19 + 0.69	0.0560112034 + 0.0275944818	0.005927657 + 0.0009304856	0.0001986491 + 0.0016144588
0.66 + 11.89	1.21 + 0.57	0.05364521 + 0.0329458077	0.0088659799 + 0.0010764484	0.0002083039 + 0.001009613
0.91 + 10.25	1.26 + 0.92	0.040824829 + 0.0398185558	0.0066168558 + 0.0009857411	0.000169134 + 0.001716774
1.48 + 0.02	0.08 + 0.01	0.0137418225 + 0.0006320598	0.0002908866 + 0.0000556346	0.0237052242 + 0.0010215366
1.60 + 0.03	0.09 + 0.0	0.0141659563 + 0.0011864187	0.0009166628 + 0.0001145112	0.0257782915 + 0.0026115327
1.54 + 0.03	0.04 + 0.01	0.0075548781 + 0.0008263314	0.0005059874 + 0.0000747687	0.0211462076 + 0.0006780738
1.62 + 0.04	0.12 + 0.01	0.0174425798 +	0.000875333 +	0.0222798164 +

		0.0009233511	0.0001077062	0.0007008804
1.38 + 0.03	0.05 + 0.01	0.0128576869 + 0.0005942357	0.0006692727 + 0.000068078	0.0245524941 + 0.0013501137
1.65 + 0.04	0.20 + 0.01	0.0124132955 + 0.0006117832	0.0007168164 + 0.0000882576	0.0327918482 + 0.0027521939
1.56 + 0.02	0.04 + 0.01	0.0087995534 + 0.0007919866	0.0007883609 + 0.0001135188	0.0222597074 + 0.001248892
1.73 + 0.06	0.10 + 0.01	0.0132443163 + 0.0007823854	0.0003419272 + 0.0000721904	0.0270688561 + 0.0021310982
1.67 + 0.04	0.08 + <0.01	0.0179823757 + 0.0011289111	0.000843317 + 0.000169421	0.0516590496 + 0.0026732993
1.73 + 0.03	0.042 + 0.01	0.0106831603 + 0.0009427461	0.0006960064 + 0.0001469818	0.0277791463 + 0.0011278727
1.73 + 0.07	0.05 + 0.01	0.0211273303 + 0.00065225	0.0006380767 + 0.0000497118	0.0342695776 + 0.0017317641
1.47 + 0.05	0.08 + 0.01	0.0174492974 + 0.001704999	0.0003975025 + 0.0000899634	0.0321635587 + 0.0022858079
1.64 + 0.06	0.09 + 0.02	0.0133541287 + 0.0006558167	0.0005704619 + 0.0000894605	0.0324578864 + 0.0027912359
1.61 + 0.02	0.04 + 0.01	0.009736271 + 0.0009602309	0.0011500833 + 0.0001594918	0.0256161843 + 0.001274093
1.61 + 0.05	0.10 + 0.01	0.0217425203 + 0.0012844307	0.0010629149 + 0.0002770104	0.0287144153 + 0.0010854344
1.50 + 0.03	0.08 + 0.01	0.0112571163 + 0.0005325621	0.0007171868 + 0.0000765368	0.0316459751 + 0.002259957
1.55 + 0.03	0.06 + 0.01	0.0147265607 + 0.000948005	0.0002431525 + 0.000100515	0.0410615081 + 0.0020291013
1.60 + 0.02	0.04 + 0.01	0.008498382 + 0.0007588681	0.000721518 + 0.0000742157	0.0251463217 + 0.0008131643
1.68 + 0.04	0.07 + 0.01	0.0158297241 + 0.0007155499	0.0006921604 + 0.0001122083	0.0274477199 + 0.0008325346
1.51 + 0.04	0.05 + 0.01	0.0125595454 + 0.0004561014	0.0003808637 + 0.0000413598	0.0305871144 + 0.001788346
1.68 + 0.07	0.08 + 0.01	0.0133775171 +	0.0008833153 +	0.0414488938 +

		0.0004904775	0.0001249213	0.0044050217
1.67 + 0.03	0.03 + 0.01	0.009454344 + 0.0009553966	0.000718171 + 0.0000942699	0.0285634966 + 0.0007745024
1.67 + 0.05	0.05 + <0.01	0.0152549285 + 0.0007114228	0.0015141917 + 0.000150293	0.0284970643 + 0.0022053705
2.26 + 7.84	1.77 + 1.20	0.025819889 + 0.0269395512	0.0037498736 + 0.0012309221	0.000127687 + 0.0028490636
0.88 + 9.09	1.33 + 0.74	0.0245492702 + 0.028153566	0.0074910875 + 0.0007304857	0.0000587032 + 0.0016565308
1.16 + 3.20	1.04 + 0.79	0.042787021 + 0.0230403808	0.0058624737 + 0.0008797403	0.0001251907 + 0.0025168736
1.05 + 6.45	1.37 + 0.76	0.0350056685 + 0.0378077414	0.0049632206 + 0.0010818674	0.0001575927 + 0.0026348559
0.93 + 3.71	1.20 + 0.52	0.0382918134 + 0.0353675003	0.0063895404 + 0.0005861827	0.000143499 + 0.000975933
1.01 + 8.03	0.58 + 0.69	0.0163663418 + 0.06572168	0.0054313294 + 0.0007179032	0.0002255833 + 0.0015577334
1.47 + 1.71	0.69 + 0.60	0.0526104281 + 0.0622867702	+ 0.0013720083	0.0001343597 + 0.0039003008
0.788 + 4.67	0.97 + 0.51	0.0418330013 + 0.0838846919	0.0050164386 + 0.0008893482	0.000150849 + 0.0022608526
1.82 + 6.77	0.92 + 0.45	0.0294117647 + 0.0400996216	0.0060813431 + 0.0010547975	0.0001376921 + 0.0020978031
1.08 + 7.67	1.13 + 0.61	0.0371184291 + 0.0211079517	0.0065838898 + 0.0005647628	0.0001047175 + 0.002445197
1.57 + 6.99	3.67+ 0.45	0.03217691 + 0.056926109	0.0062892872 + 0.0013005009	0.0001466293 + 0.0044782672
1.61 + 15.92	1.30 + 0.67	0.0273861279 + 0.0466881865	0.0046303216 + 0.0015150089	0.0003677304 + 0.0018396257
0.80 + 1.42	0.73 + 0.36	0.0295363477 + 0.031827934	0.0110535049 + 0.0011240654	0.0001187279 + 0.0015733419
0.80 + 4.44	1.04 + 0.43	0.032218974 + 0.112741376	0.0070753069 + 0.0004816292	0.0001129782 + 0.0017079578

2.11 + 10.26	0.88 + 0.55	0.0163663418 + 0.0457432403	0.0054900327 + 0.0008334346	0.0000793165 + 0.0009685844
1.43 + 13.69	1.35 + 0.55	0.025572803 + 0.0375633657	0.0063628246 + 0.0014676442	0.000116312 + 0.0019326767
1.28 + 20.24	1.34 + 0.82	0.0470895469 + 0.0344335918	0.0046050677 + 0.0007383563	0.0001982819 + 0.0025769088
0.87 + 10.91	0.97 + 0.41	0.0498266893 + 0.1177485788	0.0119117404 + 0.0010147212	0.0001808844 + 0.002245701
1.15 + 7.02	1.51 + 0.56	0.0508265023 + 0.0303767012	0.0052070367 + 0.0006769587	0.0001957985 + 0.0017505817
1.84 + 10.19	5.45 + 0.47	0.0142857143 + 0.0331272116	0.0044517616 + 0.0010017467	0.0001299873 + 0.0021859336
0.83 + 9.56	0.95 + 0.74	0.0344235384 + 0.0404878241	0.0038114051 + 0.0012474416	0.0000952665 + 0.0016544992
2.35 + 11.16	1.02 + 0.86	0.0543666181 + 0.0367825229	0.0039105514 + 0.000985633	0.0001528414 + 0.0030718983
0.76 + 7.33	1.55 + 0.93	0.0540061725 + 0.0495563585	0.0111133736 + 0.0010307185	0.0001577464 + 0.00204843
0.78 + 2.62	1.09 + 0.30	0.0618928846 + 0.0357082762	0.0069300329 + 0.0005924784	0.0001280367 + 0.0015001723
2.26 + 16.93	2.00 + 1.49	0.0222222222 + 0.0376242705	0.0054863534 + 0.0005738005	0.0001410464 + 0.0015253355
1.12 + 9.54	2.23 + 0.58	0.0293711877 + 0.0248306357	0.0032973526 + 0.0013700966	0.0001026239 + 0.001996418
1.12+ 20.62	1.23 + 1.32	0.0287579589 + 0.0280868309	0.0049524679 + 0.0012141927	0.0001301728 + 0.0024183456
0.94 + 11.65	1.40 + 0.41	0.041801864 + 0.0574356266	0.0115577942 + 0.0013331872	0.0001370293 + 0.0022458437
0.93 + 5.00	0.77 + 0.20	0.0279455252 + 0.0400681076	0.0070606251 + 0.000636449	0.0001492483 + 0.0015023038
0.94 + 10.17	0.85 + 0.66	0.0166666667 + 0.05362473	0.0047453464 + 0.0007435198	0.0000895233 + 0.0013323897

COMMENTS TO THE AUTHOR:

Comments from the Associate Editor:

Dear Dr. Pracheil,

Please find appended below the reports from the two reviewers for your revised manuscript. While the comments are generally positive, both reviewers agree that the manuscript should still benefit from several amendments. After examining the revised manuscript and the response to review, I must agree with this appraisal. Therefore, I call you and your co-authors to prepare a second revised manuscript and its respective response-to-review letter. In particular, I urge to consider seriously the issues raised by Reviewer #1, with which I concur. Seemingly, there are many questions that may have not been entirely addressed or clarified during the revision, even if mentioned in the response letter.

On my behalf, I acknowledge effort to render the work of interest outside its area of origin, among other issues. Still, I suggest that, in Discussion and Conclusions, the remarks on the novelty of the study and the need for future work is reduced to a minimum, or even removed, in order to keep the manuscript objective and unbiased.

We have removed the last paragraph of the conclusions to address this comment.

Also, I find the changes to the statistical approach to have improved the manuscript. However, some measure of deviation should be given in the charts. I suggest reducing the thickness of the lines and perhaps offsetting the data series to better allocate error bars.

We have reconstructed our figures to accommodate error bars.

We look forward to receive your revised manuscript.

Pedro M. Costa
Associate Editor, Ecotoxicology
(Aquatic Ecotoxicology)

Reviewer #1

General Comments
There remains quite a bit of revision needed for this manuscript, some of which was pointed out in previous comments.
The title should be changed to say "Contaminant" instead of "Metal" for reasons given in comments below.

Changed as recommended.

My previous comment said "Also, Hg is a metal, while As and Se are metalloids, rather than metals. This distinction should be maintained throughout the paper, or possibly explained early in the Introduction (first paragraph) if the generic term "metal" is to be used for convenience when meaning is general for all three elements. But As and Se should not be called metals when referring specifically to them."
Authors' response says "We have added text in Line 73 to address this concern "...coal ash can contain high concentrations of metals such as arsenic (As), mercury (Hg), and selenium (Se) (hereafter, generally, as metals)""
However, text at 68-69 (not 73) says "coal ash can contain high concentrations of metals such as arsenic (As), mercury (Hg), and 69 selenium (Se), all can be toxic to biota and can bioaccumulate. These metals

can then be . . ." This is not responsive to my previous comment about the metalloids and revision is required. Throughout, the word "metal" is not used correctly; if used it should be only when meaning Hg.

We apologize for this oversight in our submission of the final revision. This comment has been now been addressed recommended in this revision.

The word "only" is frequently misplaced, as noted in previous comments. It should be placed just before what is/was limited.

Changed as recommended.

In Tables 2, 4, 5, and 6, the wording "site type" should be used instead of "spill" throughout the table titles and body, matching word usage in text.

"Spill" was used for brevity and is defined in the caption. No change made.

Lines in Figures 2 through 4 (one of which is called a second "Figure 3") are difficult to see and relate to the particular sites; they should be done much more clearly.

Figures have been reconstructed for clarity.

As in previous comments, suggestions are provided to improve readability of the paper in addition to notations of non-grammatical/writing errors. I again suggest authors consider those corrections and suggestions and read their revised manuscript before submitting it to avoid obvious errors and problems such as those noted in this review.

Specific Comments
34: Change "metal" to "contaminant" or say "As, Hg, and Se." Wording of sentence should be changed because "were measured" applies only to tissue analyses for these constituents as stated now (no verb for the other metrics).

Changed as recommended.

44-45: This could be better stated as "at this site; these findings are relevant . . ."

This merely changes our words into the reviewer's words. No change needed or made.

50: Delete extra space and first comma. Suggest changing "the bulk of" to "most" to simplify wording.

We have deleted the comma.

This merely changes our words into the reviewer's words. No change needed or made.

68-69: My previous comment (in part) said "53-55: This part of the text should be rewritten to correct that error and clarify the metal/metalloid classification of the three elements (as well as deleting mention of "heavy"). The three elements should not be referred to collectively as "These metals . . ." because only Hg is a metal [emphasis added].
Authors' response says "Changed as recommended. See additional text in Line 73"
As noted in my response above relative to the similar previous General Comment, the revision is not responsive to the comment; it still calls all three "metals."

See above response.

72-73: Why is "(in what species??)" in the paper? This seems to be an internal note that should be resolved.

We have deleted this materrial.

76: The King (1988) reference is still missing from Literature Cited (omission noted in previous comments).

We have removed this citation.

97: Change "was" to "were" ("data" is plural term).

Changed as recommended.

98 and 101: Reword to say "contaminant" or "As, Hg, and Se;" they are not all metals.

Changed as recommended.

112-117: As noted in my previous comment on this text "In each of the five instances where "approx." is used, it would be preferable to say "about" (simpler wording is OK)."
Authors' response says "This was not incorrect. No change made."
That's true; it was not incorrect, but the suggestion was offered to make for easier reading and change is still recommended.

We disagree that this makes the wording simpler or the reading easier; this merely changes our words into the reviewer's words. No change needed or made.

119: Change "was" to "were" (subject is "4.1 million m3").

Changed as recommended.

135-136: The word "only" is out of place; reword as "elements showed consistent trends only with . . ."
Change "these metals" to "these contaminants" or some word other than "metals."
138 and 149: Again, they're not "metals."

Changed as recommended.

153: My previous comment said "126: Reference is missing or there is an error in year."
Authors' response says "Changed as recommended"
However, there was no change, and there is still a mis-match of year with Literature Cited section.

154-155, 157: Again, they're not "metals."

Changed as recommended.

173-176: The equation for condition factor shows "Mo" instead of "Mt." If the condition factor was "Cf" why does the equation say "K ="?

Changed as recommended

187: Fix spelling of "vitellinogenesis" to "vitellogenesis."

Changed as recommended.

189: Fix spelling of "vitellenogenic" to "vitellogenic."

Changed as recommended.

192-193: My previous comment on this text (at 160 in that draft) said "Reword as "estimates consider only the . . ."
Authors' response says "Information was removed from manuscript"
However, text was not removed or changed and still says "estimates only consider the . . ." Word order should be changed, as previously suggested, and comma after "season" should be a semicolon.

Changed as recommended.

195: Fix spelling of "vitellenogenic" to "vitellogenic."

Changed as recommended.

227: Reword as "analyses used only data . . ."

Changed as recommended.

241, 246, and 247: Reword "metals.

Changed as recommended.

250: Seems you should insert "x 3 species" after "years" based on first part of the sentence. 6 sites x 5 years x 3 species would = 90, not 720; what are other factors (e.g., As, Hg, Se?) to get the total of 720 combinations? Please indicate in parentheses the factors on which the number 720 is based.

We had calculated this through a permutation analysis, which was clearly not the right way of doing things. We have corrected this in our current draft to read 90.

258-264: To allow readers to easily see in Figure 2 the patterns described in text, the scale should be the same across species for each element. Varying the scale makes it difficult to see the similarities and differences among species. In the figure it is not apparent how the overall mean selenium concentration is higher in 2010 than in 2011, for example - what are the means, and are they significantly different? It does not look like they would differ. As noted below in reference to the figure, the lines in the figure do not stand out well, so it is difficult to see the patterns described in text.

While we agree that using the same axis range for these graphs would theoretically help to compare among values among species, the values are different enough among species that some element x species graphs have fairly low values that preclude comparisons among sites to be made. We were very careful in determining how to display these graphs and have also displayed these multiple ways and have found the current view the best for illustrating our points. We have, however, reduced the line size as recommended for clarity. While we did not mark significant differences among sites using letters or other common notations, we have redone our graphs at the suggestion of the AE to include standard error bars that can be used to infer significant differences among sites.

272-273: Table 2 does not show year to be significant for bluegill or bass; this is true only for redear, and nearly so for bluegill but clearly not so for bass.

Changed as recommended.

296: Fix "the most recent year data was collecte3d" to say "first year data were collected."

Changed as recommended.

306: Fix spelling of "vitellenogenic" to "vitellogenic."

Changed as recommended.

314: Change "this data" to "these data."

Changed as recommended.

320: Change "examining relationships metal concentration and site" to something like "examining relationships between As, Hg, or Se concentration and site."

Was changed to "examining relationships contaminant concentration and site"

328: Delete the comma.

Changed as recommended.

330: Reverse word order for "Selenium fillet" (intended meaning seems to be fillet concentrations of selenium, so word order is now incorrect).

Changed as recommended.

334: Fix spelling of "vitellenogenic" to "vitellogenic."

Changed as recommended.

338: Should Table 5 be referenced here for the GSI? It is not in Table 6 (which does show reproductive metrics). There is no Figure 5 - is that supposed to say "Figure 3?" Fix as needed.

Table and figure citations have been corrected and replaced here as needed.

343: Reword as "focuses only on . . ."

Changed as recommended.

347: Reword "data . . . was . . ." to "data . . . were . . ."

Changed as recommended.

349: Change "lack of effects" to "lack of long-term effects."

Changed as recommended.

350: Suggest changing "as after effects can take a decade or more to manifest" to something like "because such effects may not be measurable in short-term studies."

Given the direct regulatory and disciplinary implications of this project, our intent here was to provide a specific number of years so as to be unambiguous to regulators and power producers.

353: The meaning of "did not occur for 10 years following mitigation" does not seem consistent with the context of the previous two sentences; please clarify.

This sentence now reads "For instance, developmental deformities due to Se contamination in Belews Lake, NC did not manifest for 10 years following contamination by coal ash waste inputs as Se was transferred up the food chain from producers to consumers (Lemly 1993)."

361: Change "are due" to "is due" (the subject is "lack").

Changed as recommended.

377: Change "was" to "were."

Changed as recommended.

379: Fix wording of "sites is appears."

Changed as recommended.

390: Change "was" to "were" ("data" [to which this refers] is plural term).

Changed as recommended.

393: Insert "are" to say "or are of."

Changed as recommended.

407: Add period for "et al."

Changed as recommended.

412: Wording of "because they are uncontrolled phenomenon that . . ." might more properly stated as "because such impacts . . ." If not worded like that, some other wording should be used (at least, change to "phenomena" to match plural subject [which is "impacts"]).

This paragraph has been deleted at the suggestion of the AE.

417: Insert coma after "data."

This paragraph has been deleted at the suggestion of the AE.

419-420: The intended meaning of "contaminants and other environmental contaminants such as coal ash" is unclear; please clarify.

This paragraph has been deleted at the suggestion of the AE.

440: Fix initial caps of journal name.

Changed here and throughout Literature Cited

447-448: Should this say "10.1093/conphys/cou018?"

Yes. Corrected as suggested.

484: Fix initial caps of journal name.
522-523: Citation for this book chapter is incomplete.

Changed as recommended.

526: Fix initial caps of journal name.
534: Delete "Volume."

Changed as recommended.

Table 2: Fix "metal" in lines 539-542 of title and in table body to say "contaminant" or some other alternative, as it is not only Hg that is assessed.

Changed as recommended.

The meaning of "collection site (hereafter, spill, denoting reference or ash-affected site)" in line 540 and table body is unclear - suggest changing to just say "collection site type."

We have left this as is because "collection site type" is too long to fit into table.

Table 3: "shown in Table 3" is in error (it should say Table 2); this is Table 3.

Changed as recommended.

Table 4: Saying "Site Type" would be preferable to saying "Spill" (in title and table body) to designate spill vs. reference sites. Fix spacing in third line of title.

See above response.

Table 5: It would be preferable to say "site type" instead of "spill" throughout the table title and body, matching word usage in text at 288-292.

See above response.

Table 6, line 6-7: Fix spelling of "vitellogenic."

Changed as recommended.

Also, on this line it seems "hereafter, metric:" could be deleted without loss of clarity. It would be preferable to say "site type" instead of "spill" throughout the table title and body, matching word usage in text at 302-304.

See above response.

Figure 2: The lines for different sites do not show well in the figure; different kinds of lines are needed (especially for those other than R3 and S3). See also comments on figure at 258-264. Word order for "and selenium fillet concentrations (mg/kg wet-weight) by . . ." would be better as "and selenium concentrations (mg/kg wet-weight) in fillet by . . ."

We have split this figure into one that shows reference and spill sites on different graphs to address this comment. We have reconstructed this figure several different ways with several different line types, and this configuration was the most easily discernable without going to a color figure which is very expensive for a project with a limited budget. Unfortunately, this reconstruction comes at the cost of making easy comparisons between reference and spill sites.

Figure 3 (first one): Fix spelling of "indiex" in first line of title.

Changed as recommended.

As in Figure 2, the lines for different sites are not easily discerned; alternative types of lines would be helpful.

See above comment.

Figure 3 (second one): Fix spelling of "vitellenogenic" to "vitellogenic" in y-axis label. This should be Figure 4. Figure title says bass are not shown, but they are. Fix "metal" in the title if bass are kept in the figure.

Changed as recommended/ see above comments.

Reviewer #2

The authors have submitted a greatly improved manuscript. They should take further care to make minor revisions to improve punctuation and remove some "hang-overs" from earlier versions of the paper, as detailed below). There are also still two Figures titled "Figure 3"

I have one new criticism that I regret I did not spot at the initial review, and that is that the authors have omitted data that was below the detection limits (<DL) from their statistical analysis. Ignoring such data naturally biases the data to higher concentrations. Up to 40% of arsenic data were below the DL for bluegill (Appendix A) and, as it is not clear if there were more non-detects at the reference sites than a the spill sites, this could be a source of inaccuracy in estimates of the mean concentrations for this element in bluegill. I suggest to substitute values of DL/2 for results <DL and repeat the statistical analysis, although I doubt it will change the main findings of the paper.

We appreciate the reviewers sentiment here. As the reviewer states, the exclusion of values <DL would bias data to higher concentrations, so the estimates we present in this manuscript are effectively worst case scenario estimates. These WCS As values reported are well-below regulatory limits and biological thresholds, so really, even if it made a statistical difference among sites, it would not change the storyline that As values are very low. Furthermore, while it would be interesting to examine these relationships and we may find additional significance, given the regulatory and policy implications of this study and our need for conservatism in presenting only values above detection limits, we have not repeated this analysis as suggested.

Details
The abstract states (line 33) that the sites were samples bi-annually - this should be removed as data are only presented for one sampling per year.

Changed as recommended.

Please amend punctuation to remove extraneous commas from lines 50 and 64

Changed as recommended.

Line 69: suggest to change "all" to "which"

Changed as recommended.

Line 72/72: there is a comment in brackets that does not belong in the paper

Changed as recommended.

Lines 73-76 - this section is poorly worded and should be redrafted

*Changed to "*Although the exact mechanisms and toxic constituents of coal ash are not known, coal ash spills have been linked to reduced nest success in birds (King et al. 1994) and skeletal deformities in fish (Lemly 2002)."

Line 175: "MO" in this equation should be "MT"

Changed as recommended.

Line 190 is poorly worded and should be redrafted

*Changed to, "*From these measurements, batch fecundity, numbers of vitellogenic oocytes and numbers of atretic oocytes were estimated for each ovary."

Lines 212-214: why did only Se require this "sample specific adjustment"? Also, the presentation of units here (mg*kg-1) does not match that of the title of Fig 2 (mg/kg wet wt; which I prefer)

Changed as recommended.

Line 296: typo - "collecte3d"

Changed as recommended.

Line 320: should read "relationships BETWEEN metal concentration and site"

Changed as recommended.

Lines 327-328: punctuation should be corrected to make this sentence easier to read

We were unsure what punctuation should be corrected in this sentence, however, we have revised it to enhance clarity.

Line 338: a comma is required after "(Table 6; Figure 5)"

Changed as recommended.

Line 370: pls amend "sites is appears"

Changed as recommended.