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Frontier Article

Trophic status and metal bioaccumulation differences in multiple fish species exposed to coal ash-associated metals

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ABSTRACT

On December 22, 2008 a dike containing coal fly ash from the Tennessee Valley Authority Kingston Fossil Plant near Kingston Tennessee USA failed and resulted in the largest coal ash spill in U.S. history. Coal ash, a by-product of coal combustion, is known to contain multiple contaminants of concern, including arsenic and selenium. The purpose of this study was to investigate species differences in the bioaccumulation of arsenic and selenium and potential factors contributing to these differences (i.e., trophic dynamics and gut pH) in the vicinity of the Kingston coal ash spill. Elevated levels of arsenic and selenium were observed in various tissues of largemouth bass, white crappie, bluegill and redear sunfish from sites associated with the Kingston coal ash spill. Highest concentrations of selenium were found in redear sunfish with liver concentrations as high as 24.83 mg/kg dry weight and ovary concentrations up to 10.40 mg/kg dry weight at coal ash-associated sites. Investigations into the gut pH and trophic dynamics of redear sunfish and bluegill demonstrated a large difference in the gut physiology between these two species. Redear sunfish stomach and intestinal pH was found to be 1.1 and 0.16 pH units higher than in bluegill, respectively. In addition, fish from coal ash-associated sites showed enrichment differences (^{15}N and ^{13}C) compared to no ash sites, indicating differences in food web dynamics between sites. These results imply the incorporation of coal ash-associated compounds into local food webs and/or a shift in diet at ash sites compared to the no ash reference sites. Based on these results, further investigation into a broader food web at ash-associated sites is warranted.

1. Introduction

On December 22, 2008 a dike containing coal fly ash from the Tennessee Valley Authority Kingston Fossil Plant near Kingston, Tennessee failed and released over 3.7 million cubic meters of wet coal ash (mixture of fly ash and bottom ash) into adjacent waterways and over terrestrial areas, making this release the largest coal ash spill in U.S. history (Ruhl et al., 2010; TVA, 2009; Bednar et al., 2010) (Fig. 1). Approximately 2.3 million cubic meters of ash was deposited in the Emory River, filling the channel with up to 9 m of fly ash (Bednar et al., 2010). In addition to the volume of the spill, the hydrologic dynamics of the river systems in the vicinity of the spill contributed to the dispersion of coal ash in the Emory River.

Beyond the immediate biotic and abiotic effects of the spill there are long-term toxicological concerns due to the relatively high concentrations of contaminants, such as arsenic and selenium, that are present in coal ash which have the potential to impact the aquatic ecosystem (Ruhl et al., 2009; Jankowski et al., 2006; Rowe et al., 2002; Sakulpitakphon et al., 2003; Yudovich and Ketris, 2005).

The bioavailability of arsenic and selenium from coal ash can be complex and influenced by coal type (Yudovich and Ketris, 2005; Yudovich and Ketris, 2006) and environmental factors such as pH and reduction-oxidation (redox) potential (Rowe et al., 2002). Previous studies at other coal ash-influenced sites have shown significant bioaccumulation of arsenic and/or selenium in various organisms including turtles (Nagle et al., 2001), caddisflies (Reash et al., 2006), crayfish (Nagle et al., 2001), tadpoles (Hopkins et al., 1999), largemouth bass (*Micropterus salmoides*) (Baumann and Gillespie, 1986), bluegill (*Lepomis macrochirus*) (Gillespie and Baumann, 1986; Lohner et al., 2001; Reash et al., 2006), green sunfish (*Lepomis cyanellus*) (Lohner et al., 2001), redear sunfish (*Lepomis microlophus*) (Sorenson (1988)), and snakes (Hopkins et al., 1999). Both arsenic and selenium have been cited as potential contaminants of concern which may pose ecological risk to aquatic and terrestrial ecosystems in the vicinity of the spill site (USEPA, 2009; Ruhl et al., 2009).

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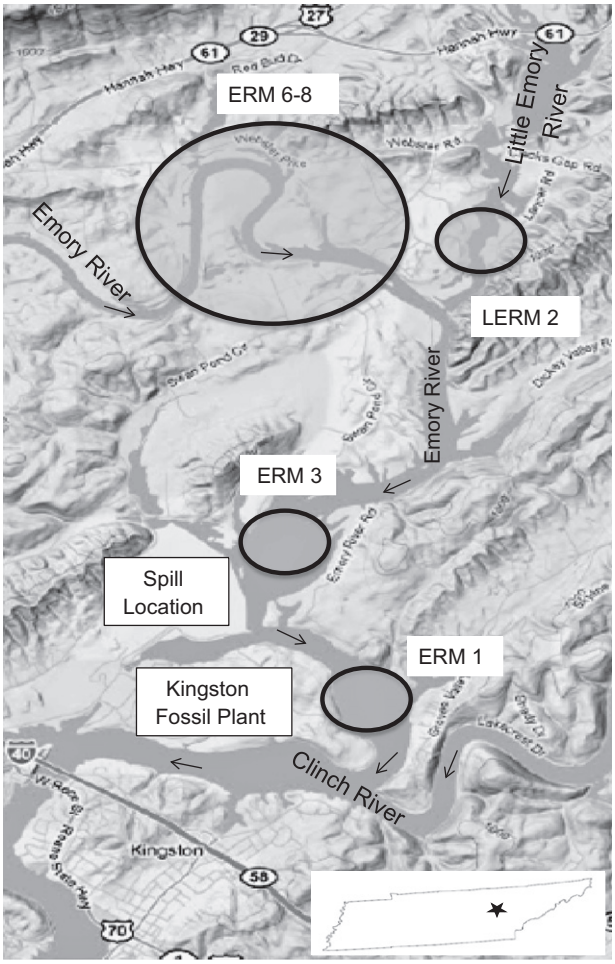

Fig. 1. Map of spill area and sampling locations. Sampling locations are labeled by river mile marker on the Emory and Little Emory River (ERM & LERM). Circles represent fish collection areas. Arrows indicate water flow direction. (base map provided by Google maps).

Arsenic is known to cause deleterious effects in fish under certain environmental conditions (Neff, 1997). Datta et al. (2007, 2009) showed that exposure to arsenic at sublethal concentrations can be hepatotoxic and immunotoxic to the walking catfish (*Clarias batrachus*). Additionally, arsenic exposure has been shown to affect antioxidant responses in zebrafish (*Danio rerio*) (Ventura-Lima et al., 2009). Recent studies have linked deleterious reproductive and developmental effects in fish to arsenic exposure, including catfish (*Pangasiandon hypophthalus*) (Yamaguchi et al., 2007), zebrafish (Boyle et al., 2008; Li et al., 2009; Gonzalez et al., 2006) and mummichog (*Fundulus heteroclitus*) (Gonzalez et al., 2010).

Selenium has been implicated at many contaminated field sites as the primary contaminant of concern because of its propensity to bioaccumulate within the food chain (Hamilton, 2004). Sorenson (1988) observed chronic bioaccumulation of selenium as well as histopathological and reproductive effects in redear sunfish (*Lepomis microlophus*) collected near a power plant discharge containing selenium. Gillespie and Baumann (1986) concluded that selenium exposure was responsible for deformities and reduced survival of larval bluegill inhabiting cooling reservoirs of coal-fired power plants. Significant bioaccumulation in the gonads and carcasses was also found in bluegill and largemouth bass residing in the same reservoirs (Baumann and Gillespie, 1986).

Trophic dynamics and food chain relationships have a major influence on the bioaccumulation of many contaminants including polychlorinated biphenyls (Walters et al., 2008), mercury (Chumchal et al., 2001), cadmium (Croteau et al., 2005) and selenium (Stewart et al., 2010). A commonly used method for determining trophic structure dynamics in wildlife species is the measurement of the stable isotopes $\delta^{15}N$ and $\delta^{13}C$ (Kelly, 2000; Herwig et al., 2004; Bergfur et al., 2009). The analysis of stable nitrogen isotope ratios in multiple organisms provides an integrated measure (spatially and temporally) of trophic position in a food web because $\delta^{15}N$ increases with increasing trophic level. This increase is due to the heavy isotope, ^{15}N, becoming enriched (elevated) relative to known standards because of preferential accumulation compared to the lighter isotope, ^{14}N, as the trophic level increases. Stable carbon isotope ratios show little to no enrichment with trophic increase but are useful in characterizing different food chains if the bases of the food chains possess a distinct isotopic signature, such as carbon originating from aquatic or terrestrial sources (Stewart et al., 2004).

The purpose of this study was to investigate the bioaccumulation of arsenic and selenium and to identify possible differences in trophic dynamics in feral fish at various sites in the vicinity of the Kingston coal ash spill. The specific objectives were to: (1) determine species differences in arsenic and selenium bioaccumulation in the liver, ovary, and muscle tissues of feral largemouth bass, white crappie (*Pomoxis annularis*), bluegill and redear sunfish; (2) investigate the potential role of species differences in stomach and intestine pH on the bioaccumulation of arsenic and selenium; and (3) investigate the potential role of species differences in trophic dynamics on arsenic and selenium bioaccumulation using stable carbon and nitrogen isotope values.

2. Material and methods

2.1. Site locations and fish collection

Fish were collected from two sites affected by the ash spill: Emory River mile 1.0 (ERM 1) and ERM 3 (Fig. 1). Because of the hydrologic complexity of the spill location fish were also collected from two references sites: ERM 6–8 and Little Emory River mile 2.0 (LERM 2). All fish were collected by boat electroshocking, measured for total length (cm) and weight (g), affixed with a unique individual identification number, placed in a live well, and transported to the lab alive for processing within 12 h of capture. The reference sites will be referred to collectively in this paper as "no ash sites" and the coal ash-associated sites as "ash sites". All fish size, weight, and pH data are reported as mean $\pm$ SE for the studies below.

2.2. Bioaccumulation study

Fish collection occurred between 4/12/2010 and 5/13/2010. Six adult females were collected from each of the four sampling locations for each of the following species: largemouth bass (43.3 ± 1.9 cm and $1{,}256.9 \pm 179.8$ g), white crappie (31.8 ± 0.9 cm and 447.5 ± 40.9 g), bluegill (14.4 ± 0.4 cm and 52.8 ± 4.2 g) and redear sunfish (18.6 ± 0.5 cm and 98.2 ± 7.0 g). From each fish, samples of liver, ovary and muscle were analyzed for total selenium and arsenic (dry weight) according to USEPA method 6020 using inductively coupled plasma-mass spectrometry (ICP-MS). Quality control measures consisted of blanks, duplicates, spikes, and the analysis of reference standard material every 20 samples.

2.3. Gut physiology and trophic dynamics

Based on bioaccumulation results for arsenic and selenium observed in redear sunfish two related studies referred to as the "pH and trophic dynamics studies" with bluegill and redear sunfish were conducted to determine if gut physiology and trophic relationships played important roles in the bioaccumulation of these contaminants. Sampling for these studies occurred between 4/25/2011 and 5/10/2011. Bluegill were used as a comparison species to redear sunfish, since both species are known to occupy similar habitats and are comparable in size yet display large differences in contaminant bioaccumulation. Stomach and intestine pH of the two fish species were measured as a basic comparison of gut physiology, and trophic dynamic differences were examined using stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) with fish collected from the same sites as the bioaccumulation study.

2.3.1. Bluegill and redear pH study

Between 8 and 16 adult (male and female) bluegill (14.2 ± 0.2 cm and 50.3 ± 1.9 g) and redear sunfish (18.4 ± 0.4 cm and 103.0 ± 6.2 g) were collected from each of the four sampling locations. Stomach and intestine pH analysis was performed on each fish using an Orion 2-Star benchtop pH meter with an Orion 9862 micro pH electrode (Thermo Fisher Scientific, Inc.). Stomach pH was measured in a single location, while intestinal measurements were made in the foregut and hindgut. No significant differences in pH values were found between the foregut and hindgut of either species; therefore, for an individual fish, these intestinal pH values were averaged. Care was taken to consistently measure pH in areas of both the stomach and intestine where food was not present.

2.3.2. Bluegill and redear trophic dynamics study

A subset of fish used in the pH study ($n=6$ for each species at each location; all female) were also used for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis. Briefly, a small piece of muscle tissue was excised from each fish (< 2 g), oven dried and analyzed using a NC 2500 elemental analyzer (Carlo Erba Milan, Italy) interfaced to a Delta Plus isotope ratio mass spectrometer (Thermo Finnigan Bremen, Germany). Raw isotope values were normalized to respective scales by the use of certified standards. All fish $\delta^{15}N$ values from ash and no ash sites were normalized to account for the variability at the base of the food chain by subtracting the corresponding $\delta^{15}N$ values of periphyton at ash and no ash sites (range of periphyton ^{15}N values: 3.24–6.55). Muscle filet samples from each fish were analyzed for total selenium and arsenic concentrations (dry weight) by the USEPA method 6020 using ICP-MS. Quality control measures consisted of blanks, duplicates, spikes, and the analysis of reference standard material every 20 samples.

2.4. Statistical analysis

Fish species and sample site differences (including year-to-year comparisons) in selenium and arsenic concentrations, and stable isotopes were investigated using two-way ANOVAs with Tukey's post hoc tests (variables of species and site). Analysis of pH data (sex, site, and species) was performed via one-way ANOVA. All analyses were performed using JMP Pro 9 software (Cary, NC). Linear regression modeling was performed to determine the relationship of arsenic and selenium bioaccumulation to $\delta^{15}N$ and $\delta^{13}C$ values. Significant differences for linear regressions and for one-way and two-way ANOVA's were defined by p-values < 0.05.

3. Results

3.1. Bioaccumulation study

The bioaccumulation of arsenic and selenium varied depending on sample site, type of tissue, and fish species (Tables 1a and 1b). Within a tissue type and species, there were no significant differences in arsenic or selenium bioaccumulation between the two no ash sites or between the two ash sites. However, there were significant differences in arsenic bioaccumulation between the ash and no ash sites, most notably at the ash site ERM 1 for the liver, ovary, and muscle of multiple species when compared to the no ash site LERM 2. Differences in selenium concentrations between ash and no ash sites were only observed in redear sunfish and bluegill. Interestingly, redear sunfish always had the highest levels of selenium in all tissues at all ash and no ash sites (except bluegill muscle at ERM 6–8).

The bioaccumulation of arsenic and selenium in the bluegill and redear sunfish collected for the trophic study (April–May 2011) was not significantly different from the concentrations measured for the same species during the bioaccumulation study conducted one year earlier (April–May 2010) (, 2). Each species at each site in the trophic study was statistically similar and showed the same general trends as the results of the bioaccumulation study, with elevated concentrations of selenium in bluegill and elevated levels of selenium and arsenic in redear sunfish.

3.2. Bluegill and redear pH study

No significant differences in pH of the intestine and stomach were found between sexes or among sites for either bluegill or redear sunfish. Therefore, pH data for both sexes and all sites were combined for each species for further analysis. The pH values (mean ± SE) for

Table 1a
Mean (± SE) selenium in the liver, ovaries, and muscle of largemouth bass (*Micropterus salmoides*), white crappie (*Pomoxis annularis*) bluegill (*Lepomis macrochirus*) *and redear sunfish* (*Lepomis microlophus*).

	Liver **Selenium (mg/kg dry weight)** ERM 1	ERM 3	ERM 6–8	LERM 2
Largemouth bass	11.42 ± 1.90	8.72 ± 0.69	9.04 ± 1.45	8.73 ± 0.57
White crappie	6.15 ± 0.98	10.15 ± 0.69	6.34 ± 0.63	8.41 ± 0.63
Bluegill	10.00 ± 1.25*	11.80 ± 1.45*	5.83 ± 0.44	8.33 ± 0.44
Redear sunfish	22.00 ± 2.5*	24.83 ± 6.28#,*	12.17 ± 0.92	15.17 ± 1.76
	Ovary **Selenium (mg/kg dry weight)**			
	ERM 1	ERM 3	ERM 6–8	LERM 2
Largemouth bass	5.44 ± 0.35	4.72 ± 0.15	3.86 ± 0.19	4.03 ± 0.16
White crappie	5.05 ± 0.44	5.53 ± 0.48	4.26 ± 0.74[a]	4.66 ± 0.35
Bluegill	6.36 ± 0.26*	5.65 ± 0.96*	4.40 ± 0.34	5.54 ± 0.17
Redear sunfish	9.49 ± 0.69#,*	10.40 ± 0.97#,$,*	6.24 ± 0.66	7.78 ± 0.44
	Muscle filet **Selenium (mg/kg dry weight)**			
	ERM 1	ERM 3	ERM 6–8	LERM 2
Largemouth bass	3.03 ± 0.20	3.08 ± 0.26	2.15 ± 0.08	2.25 ± 0.11
White crappie	1.93 ± 0.31	3.05 ± 0.17	1.80 ± 0.06	2.20 ± 0.09
Bluegill	3.64 ± 0.42*	4.41 ± 0.25$	3.43 ± 0.07[b]	2.68 ± 0.11
Redear Sunfish	5.08 ± 0.38#,$,*	4.91 ± 0.37#,$	3.15 ± 0.11	3.65 ± 0.10

Liver: $n=3$; Ovary: $n=5$; Muscle filet: $n=5$, for all species unless otherwise noted.

[#] Significantly different from ERM 6 to 8 for a specific species (two-way ANOVA; post hoc Tukey's test; $\alpha=0.05$).

[$] Significantly different from LERM 2 for a specific species (two-way ANOVA; post hoc Tukey's test; $\alpha=0.05$).

[*] Significant difference between bluegill and redear at a specific site (two-way ANOVA; post hoc Tukey's test; $\alpha=0.05$).

[a] $n=3$.

[b] $n=4$.

Table 1b
Mean (±SE) arsenic in the liver, ovaries, and muscle of largemouth bass (*Micropterus salmoides*), white crappie (*Pomoxis annularis*) bluegill (*Lepomis macrochirus*) and redear sunfish (*Lepomis microlophus*).

	Liver **Arsenic (mg/kg dry weight)** ERM 1	ERM 3	ERM 6–8	LERM 2
Largemouth bass	1.39 ± 0.22	0.94 ± 0.03	1.39 ± 0.29	0.83 ± 0.19
White crappie	4.66 ± 0.82	5.09 ± 1.02	2.41 ± 0.19	3.39 ± 0.79
Bluegill	1.76 ± 0.21*	1.97 ± 0.31	0.59 ± 0.18	1.05 ± 0.21
Redear sunfish	6.83 ± 0.726#,$,*	3.53 ± 0.65	2.47 ± 1.52	1.85 ± 0.33
	Ovary **Arsenic (mg/kg dry weight)** ERM 1	ERM 3	ERM 6–8	LERM 2
Largemouth bass	0.95 ± 0.12	0.61 ± 0.07	0.67 ± 0.07	0.45 ± 0.05
White crappie	1.68 ± 0.16#,$	1.17 ± 0.13	0.81 ± 0.10[a]	0.78 ± 0.09
Bluegill	0.45 ± 0.59*	0.39 ± 0.01	0.23 ± 0.07	0.34 ± 0.07
Redear sunfish	1.66 ± 0.29#,$,*	0.81 ± 0.15	0.41 ± 0.09	0.32 ± 0.04
	Muscle filet **Arsenic (mg/kg dry weight)** ERM 1	ERM 3	ERM 6–8	LERM 2
Largemouth bass	1.33 ± 0.21#,$	0.78 ± 0.04	0.70 ± 0.06	0.48 ± 0.08
White crappie	1.33 ± 0.03#,$	0.86 ± 0.10	0.65 ± 0.05	0.69 ± 0.06
Bluegill	0.42 ± 0.11*	0.38 ± 0.02	0.25 ± [b]	0.24 ± 0.04
Redear sunfish	1.30 ± 0.09#,$,*	0.60 ± 0.08	0.39 ± 0.05	0.38 ± 0.07

Liver: $n=3$; Ovary: $n=5$; Muscle filet: $n=5$, for all species unless otherwise noted.

Significantly different from ERM 6 to 8 for a specific species (two-way ANOVA; post hoc Tukey's test; $\alpha=0.05$).

$ Significantly different from LERM 2 for a specific species (two-way ANOVA; post hoc Tukey's test; $\alpha=0.05$).

* Significant difference between bluegill and redear at a specific site (two-way ANOVA; post hoc Tukey's test; $\alpha=\alpha=0.05$).

[a] $n=2$.

[b] $n=3$.

Table 2
Mean (±SE) arsenic and selenium in the muscle of bluegill (*Lepomis macrochirus*) and redear sunfish (*Lepomis microlophus*) collected during the bluegill and redear trophic study.

	Muscle Filet **Arsenic (mg/kg dry weight)** ERM 1	ERM 3	ERM 6–8	LERM 2
Bluegill	0.28 ± 0.04*	0.27 ± 0.05*	0.14 ± 0.01	0.14 ± 0.01
Redear sunfish	1.08 ± 0.17#,$,*	0.72 ± 0.13#*	0.21 ± 0.06	0.38 ± 0.10
	Muscle filet **Selenium (mg/kg dry weight)** ERM 1	ERM 3	ERM 6–8	LERM 2
Bluegill	4.77 ± 0.19#,$,*	4.88 ± 0.21#,$	2.28 ± 0.05	2.85 ± 0.11*
Redear sunfish	5.60 ± 0.17#,$,*	5.50 ± 0.16#,$	2.49 ± 0.14$	3.75 ± 0.14#,*

$n=6$ for both species.

Significantly different from ERM 6 to 8 for a specific species (two-way ANOVA; post hoc Tukey's test; $\alpha=0.05$).

$ Significantly different from LERM 2 for a specific species (two-way ANOVA; post hoc Tukey's test; $\alpha=0.05$).

* Significant difference between bluegill and redear at a specific site (two-way ANOVA; post hoc Tukey's test; $\alpha=0.05$).

bluegill and redear sunfish stomachs were 4.39 ± 0.13 and 5.63 ± 0.14, respectively, and for intestines 7.12 ± 0.04 and 7.36 ± 0.04, respectively. When bluegill and redear sunfish were compared, there were significant differences in pH values in both the stomach ($F_{(1,94)}=27.42$, $p<0.001$) and intestine ($F_{(1,94)}=4.89$, $p=0.029$) (Fig. 2). Although significant differences between bluegill and redear sunfish did exist for both stomach and intestine pH, differences were not similar, with the mean stomach pH difference being 1.1 pH units higher in redear sunfish and the mean intestine pH difference being only 0.16 pH units higher in redear sunfish.

3.3. Bluegill and redear trophic dynamics study

Bluegill and redear sunfish showed no significant differences in $\delta^{15}N$ or $\delta^{13}C$ between the two no ash sites or the two ash sites. Therefore, data from the two ash sites were pooled and data from the two no ash sites were also pooled for each species for further comparisons. No significant site-species interactions were found for $\delta^{15}N$ ($F_{(1,43)}=0.406$, $p=0.527$) (Fig. 3). Across species, $\delta^{15}N$ was significantly enriched at no ash sites relative to ash sites ($F_{(1,43)}=26.445$, $p<0.001$). Across sites, significant enrichment of ^{15}N was observed in redear sunfish compared to bluegill ($F_{(1,43)}=15.257$, $p<0.001$). No significant site-species interactions were found for $\delta^{13}C$ ($F_{(1,43)}=0.077$, $p=0.783$) (Fig. 3). Across species $\delta^{13}C$ was significantly enriched at ash sites relative to no ash sites ($F_{(1,43)}=16.834$, $p<0.001$). Across sites $\delta^{13}C$ was not significantly enriched in either species ($F_{(1,43)}=3.936$, $p=0.053$). Within species, significant ^{13}C enrichment at ash sites relative to no ash sites was found for both bluegill ($p=0.019$) and redear sunfish ($p=0.043$).

Since isotope enrichment as well as arsenic and selenium concentrations were different at ash sites compared to no ash sites, regression analysis was performed to determine if relationships existed between these two parameters. There were no significant relationships between $\delta^{15}N$ and arsenic for both bluegill ($r^2_{(24)}=0.025$, $P=0.4676$) and redear sunfish ($r^2_{(24)}=0.032$, $P=0.4051$) (Fig. 4a) and

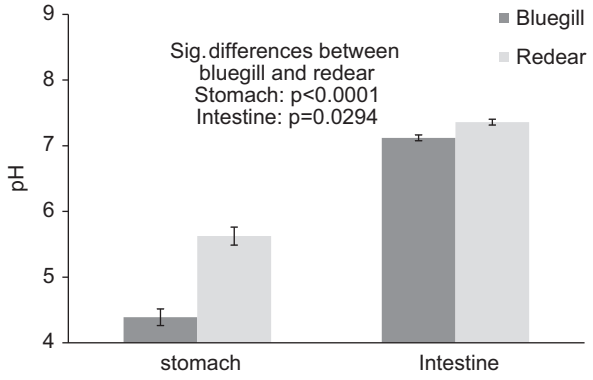

Fig. 2. Mean (±SE) stomach and intestine pH of bluegill (*Lepomis macrochirus*) and redear sunfish (*Lepomis microlophus*). Data from all sites were pooled for analysis. Significant differences determined by two-way ANOVA (post hoc Tukey's test, $p<0.05$).

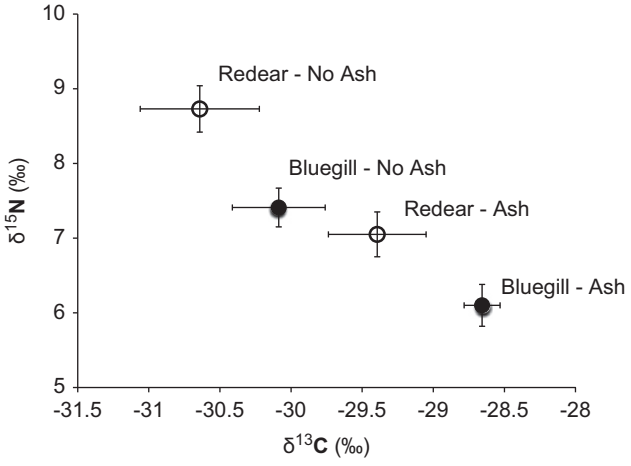

Fig. 3. $\delta^{15}N$ & $\delta^{13}C$ values (mean ± SE) for bluegill and redear sunfish.

between $\delta^{13}C$ and arsenic for bluegill ($r^2_{(24)}$= 0.11, P=0.1227) (Fig. 4b). Significant negative relationships were observed between $\delta^{15}N$ and selenium for bluegill ($r^2_{(24)}$=0.3592, P=0.0025) and redear sunfish ($r^2_{(24)}$=0.3850, P=0.0012) (Fig. 5a). Significant positive relationships were observed between $\delta^{13}C$ and selenium for bluegill ($r^2_{(24)}$=0.27, P=0.0116) and redear sunfish ($r^2_{(24)}$=0.36, P=0.0018) (Fig. 5b) and between $\delta^{13}C$ and arsenic for redear sunfish (Fig. 4b) ($r^2_{(24)}$=0.22, P=0.0190)

4. Discussion

4.1. Bioaccumulation study

Previous studies investigating the bioaccumulation of coal ash-associated metals in wildlife species have focused primarily on areas receiving coal ash effluent and not from an environmental spill, as was the case in this study. Arsenic concentrations found in bluegill livers and ovaries from ash sites in this study were substantially lower than concentrations reported for bluegill inhabiting coal ash effluent streams (~2-fold in liver; ~10-fold in ovary) (Reash et al., 2006; Lohner et al., 2001). Likewise, notably lower (~5-fold) selenium concentrations were found in bluegill liver (Lohner et al., 2001) and ovary (Reash et al., 2006) as well as largemouth bass ovaries (~2-fold) (Baumann and Gillespie, 1986) at ash sites in this study compared to previous coal ash effluent studies. In this study, concentrations of selenium in the livers and ovaries of redear sunfish collected at ash sites were elevated relative to the selenium concentrations measured in coal ash itself collected from the Emory River (ERM ~2.2) after the spill (6.97 mg/kg) (Bednar et al., 2010). In addition, redear sunfish liver selenium concentrations were the highest of all tissues tested across all species.

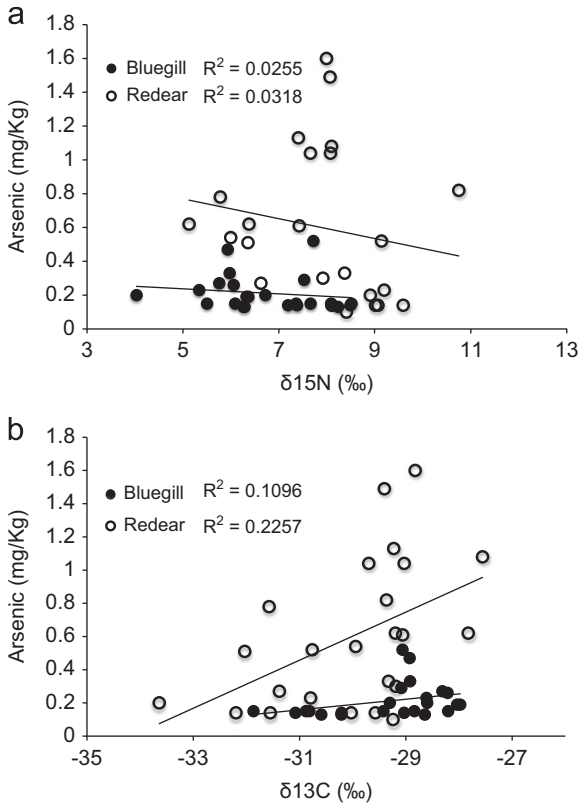

Fig. 4. Relationship between arsenic concentration (dry weight) in bluegill and redear sunfish muscle tissue and (a) $\delta^{15}N$ values and (b) $\delta^{13}C$ values. All circles represent individual fish.

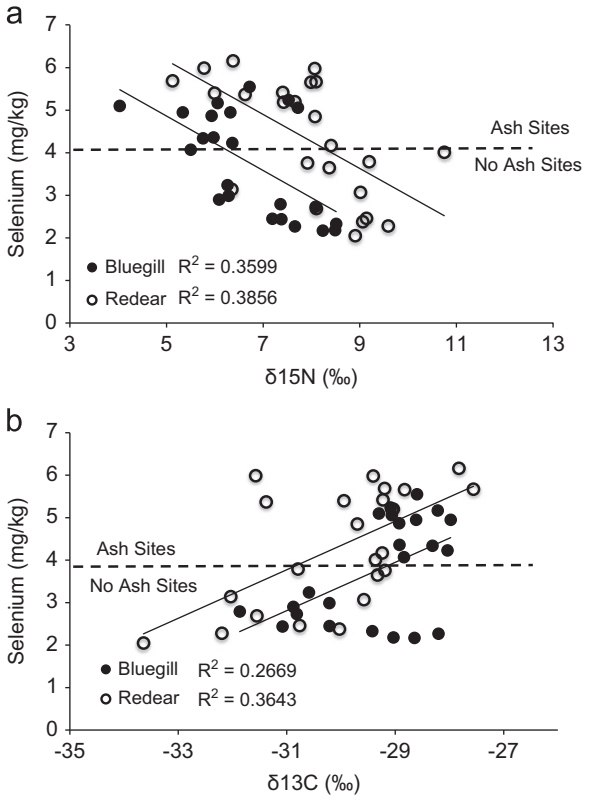

Fig. 5. Relationship between selenium concentration (dry weight) in bluegill and redear sunfish muscle tissue and (a) $\delta^{15}N$ values and (b) $\delta^{13}C$ values. All circles represent individual fish.

The striking differences observed in selenium concentrations between redear sunfish and bluegill in this study were the impetus for the pH and trophic dynamics study, which had as its primary objective to determine a possible physiological basis for these large differences in selenium levels. One possible explanation could be related to the trophic status of these two species. Redear sunfish are primarily molluscivores (Huckins, 1997; Wainwright, 1996), feeding on gastropods and bivalves, hence their alternative name "shellcracker". In contrast, bluegill are considered to be primarily omnivores. Bivalves are known to have a ~10-fold lower depuration rate for selenium than other invertebrates (e.g., mysid shrimp) which results in a much higher risk of selenium accumulation and toxicity to predators that feed primarily on mollusks (Schlekat et al., 2004; Stewart et al., 2010). Such differences in the body burdens of selenium in prey indicate that food web analysis is critical to the understanding of selenium bioaccumulation and toxicity to consumers through the food web.

4.2. Bluegill and redear pH study

Differences in pH can have a major effect on metal, metalloid, and non-metal element speciation (Masscheleyn et al.,1990; Masscheleyn et al., 1991) and bioaccumulation (Van Dorst and Peterson, 1984). For example, selenium can be taken up differentially in organisms depending on the selenium species (e.g., selenite, selenate) present (Maher et al., 2010; Bailey et al., 1995) and the pH of the medium (Johnsson, 1991). In this study, pH values in the stomach and intestine did not differ between ash and no ash sites, indicating that the ash spill did not have an influence on gut pH in either species. However, pH values differed significantly between species in both the stomach and the intestine (Fig. 2), which may indicate that the physiological

mechanisms for the digestion and assimilation of food and associated contaminants are different between these two species. Given that pH is known to affect the speciation (e.g., selenite, selenate) and therefore availability of selenium for the transport and assimilation through the gut wall, differences in gut pH between these two species may be a partial explanation for the higher selenium bioaccumulation observed in redear sunfish. In addition to differences in digestive track pH and physiology, other factors also could influence digestive uptake of selenium such as redox of the gut and amount and type of food present in the digestive tract (Deguara et al., 2003; Nikolopoulou et al., 2011).

4.3. Bluegill and redear trophic study

The observed $\delta^{15}N$ and $\delta^{13}C$ values as well as the arsenic and selenium concentrations in fish indicate that there are differences in the trophic or food web dynamics for both species between the ash and no ash sites. For example, the significant ^{13}C enrichment for both species at ash sites shows that a dietary carbon source at ash sites may be different than at no ash sites. Since fly ash from this spill is known to have high concentrations of arsenic, and selenium and a substantial amount of organic carbon ($\sim$4.5 percent) (Bednar et al., 2010), it is possible that the differences observed in $\delta^{13}C$ values could be due to incorporation of coal ash-related carbon compounds into the food web at ash sites. If this incorporation is occurring, it may be from fish ingesting coal ash either directly or indirectly (consuming prey items that have ingested coal ash) and thereby accumulating arsenic and selenium associated with the coal ash.

Another possibility to explain differences in food web dynamics between ash and no ash sites is that the ash from this spill is causing a shift in the normal types of dietary items for bluegill and redear sunfish at ash sites compared to no ash sites. A shift in the availability of normal dietary items is further supported by the enrichment of ^{15}N observed at no ash sites compared to ash sites (Fig. 3). In a previous study conducted by Vizzini et al. (2010) a change in diet resulted in a shift in ^{15}N enrichment in the muscle tissue of two populations of the same species of bluefin tuna (*Thunnus thynnus*) when one population was fed a controlled diet and the other was wild tuna consuming natural prey.

Arsenic concentrations are known to decrease with increasing trophic level (Mason et al. 2000; Chen and Folt 2000; Culioli et al., 2009). The results from our trophic study did not show a relationship between $\delta^{15}N$ and arsenic concentrations, which may be an artifact of examining only one tissue type (muscle) from two fish species with overlapping habitats. However, results from our bioaccumulation study indicate a positive trend between arsenic bioaccumulation and trophic position. Muscle filet concentrations of arsenic from largemouth bass to white crappie were consistently higher than those in bluegill and redear sunfish (Table 1b), which typically occupy a lower trophic position. A further examination into the broader food web at these spill sites, specifically lower trophic levels (e.g., macroinvertebrates), should provide additional insight on this phenomenon.

Selenium has been shown to bioaccumulate in aquatic ecosystems, sometimes leading to toxicity at higher trophic levels, especially in oviparous vertebrates (Hodson et al., 2010; Hamilton, 2004). Results from the present study showed a significant negative relationship between $\delta^{15}N$ and selenium in bluegill and redear sunfish (Fig. 5a). While this study was not designed to compare the community structure differences between ash and no ash sites, these trophic study results provide evidence that a shift in the normal types of dietary items for bluegill and redear sunfish may be occurring at ash sites. On the other hand, there is a significant positive relationship between selenium bioaccumulation and $\delta^{13}C$ in bluegill and redear sunfish (Fig. 5b). This relationship could be due, in part, to the incorporation of coal ash-associated organic carbon (and the selenium associated with it) into the local food web at ash sites.

5. Conclusions

Elevated levels of arsenic and selenium were observed in various tissues of largemouth bass, white crappie, bluegill and redear sunfish from sites associated with the Kingston coal ash spill. This was most notable for selenium in redear sunfish. To try to identify possible reasons for this difference in selenium levels between species, we investigated the potential roles of gut physiology and trophic dynamics in redear sunfish and bluegill, with the difference in digestive tract pH potentially playing a significant role in the higher propensity of redear sunfish to bioaccumulate selenium. In addition, fish from ash contaminated sites showed enrichment of ^{13}C compared to no ash sites, and fish from no ash sites had enriched ^{15}N compared to ash sites, indicating differences in trophic dynamics. These results suggest that coal ash-associated compounds may be incorporated into local food webs and/or a shift in diet at ash sites compared to the no ash reference sites. Based on these results, further investigations into a broader food web dynamics at ash-associated sites is warranted.

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