Ecotoxicology and Environmental Safety 72 (2009) 1384–1391

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Accumulation of trace elements and growth responses in *Corbicula fluminea* downstream of a coal-fired power plant

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ARTICLE INFO

Article history:
Received 7 August 2008
Received in revised form
23 January 2009
Accepted 26 January 2009
Available online 9 March 2009

Keywords:
Corbicula fluminea
Coal-fired power plant
Selenium
Mercury
Glutathione
Condition index
Bioaccumulation

ABSTRACT

Lentic organisms exposed to coal-fired power plant (CFPP) discharges can have elevated trace element concentrations in their tissues, but this relationship and its potential consequences are unclear for lotic organisms. To explore these patterns in a lotic environment, we transplanted *Corbicula fluminea* from a reference stream to a stream receiving CFPP discharge. We assessed trace element accumulation and glutathione concentration in clam tissue, shell growth, and condition index at five sites along a contamination gradient. Clams at the most upstream and contaminated site had the highest growth rate, condition index, glutathione concentrations, and concentrations of arsenic (7.85 ± 0.25 μg/g [dry mass]), selenium (17.75 ± 0.80 μg/g), and cadmium (7.28 ± 0.34 μg/g). Mercury concentrations declined from 4.33 ± 0.83 to 0.81 ± 0.11 μg/g [dry mass] in clams transplanted into the selenium-rich environment nearest the power plant, but this effect was not as evident at less impacted, downstream sites. Even though dilution of trace elements within modest distances from the power plant reduced bioaccumulation potential in clams, long-term loading of trace elements to downstream depositional regions (e.g., slow moving, silty areas) is likely significant.

1. Introduction

Over half of the electricity in the United States is produced by coal combustion (NRC, 2006; Rowe et al., 2002). Between 1993 and 2004, consumption of coal for energy generation increased from 847 million tons to over 1 billion tons (NRC, 2006). In 2003, approximately 126 million short tons of coal combustion waste was produced, of which 58% was disposed in surface impoundments and landfills (NRC, 2006). By 2030, coal usage is projected to increase by 50% under reference conditions and by 70% under high oil and natural gas markets (NRC, 2007).

Coal combustion waste contains metals and metalloids such as arsenic, selenium, cadmium, and others (NRC, 2006; Rowe et al., 2002); these are hereafter referred to as trace elements for brevity. A third of coal combustion waste is disposed into aquatic basins and is a significant source of trace element inputs into adjacent rivers and streams (Rowe et al., 2002). Most research has documented the effects of coal combustion wastes disposed in lentic environments (Cherry et al., 1984; Lemly, 1993, 1997, 2002; Rowe et al., 2002). For example, coal-fired power plant (CFPP) discharges have been associated with species extirpations, population declines, decreased individual growth rates, and diminished offspring viability in fish and amphibians (Crutchfield, 2000; Hopkins et al., 2006, 1998, 2000, 2004b; Lemly, 2002).

Coal combustion waste is also released into lotic systems; however, little is known about its impacts in rivers and streams (but see Lohner et al., 2001; Peltier, 2006; Peltier et al., 2008; Rowe et al., 2002; Stepanauskas et al., 2005; Wright et al., 2006). Dilution likely plays an important role in reducing the detectable impact of contaminated wastes in lotic environments, but aquatic organisms living immediately downstream of the discharges may be negatively affected. Likewise, areas such as oxbows, slow moving silty areas, and estuaries are subjected to long-term deposition of trace elements due to continual loading from power plant sites. Invertebrates likely play key roles in cycling trace elements from sediments in adjacent sites and deposition zones, resulting in opportunities for transfer of trace elements to higher trophic levels. For example, we previously documented elevated trace element concentrations in the tissues of *Corbicula fluminea* downstream of discharges from CFPPs in an urban river (Peltier, 2006; Peltier et al., 2008).

Assessing accumulation of trace elements in resident aquatic organisms such as *C. fluminea* is not sufficient in many cases to determine whether the health of the organisms is compromised.

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doi:10.1016/j.ecoenv.2009.01.011

Thus, evaluating molecular and physiological responses (e.g., biomarkers and growth) in combination with contaminant concentrations is needed to determine if accumulation in tissues is leading to measurable responses in an organism. The biomarker glutathione (GSH) is an important antioxidant that may protect against metal toxicity associated with oxidative stress (Ciocan and Rotchell, 2004; Stohs and Bagchi, 1995). GSH response to contaminant exposure has been variable. For example, GSH concentrations in the gills and digestive glands of a mussel (*Mytilus galloprovincialis*) decreased with 7-day exposure to copper and methylmercury (Canesi et al., 1999). Similarly GSH decreased in the digestive glands of *Unio tumidus* after exposure to contaminated sediments (Cossu et al., 2000). In contrast, GSH concentrations in the digestive gland increased in the green mussel (*Perna viridis*) after exposure to mercury and lead (Yan et al., 1997) and in three bivalves (*M. galloprovincialis*, *Scapharca inaequivalvis*, *and Tapes philippinarum*) living in metal-polluted sediments (Irato et al., 2003). *C. fluminea* are less commonly used in biomarker studies (see Vidal et al., 2002a, b), especially those measuring the response of GSH (see Lehmann et al., 2007).

To explore the temporal pattern and consequences of trace element accumulation in *C. fluminea*, we conducted a transplant study in a lotic system receiving input from a single point-source discharge from the ash ponds of a CFPP. The objectives of the study were to quantify the responses of *C. fluminea* transplanted from a reference stream to stream sites along a gradient of coal-ash contamination by analyzing: (1) accumulation of trace elements in soft tissues of the clams, (2) shell growth rates and condition index, and (3) GSH concentrations in clam digestive glands. We predicted that trace element concentrations in clams would be the highest at the site closest to discharges from the CFPP and that these clams would have the lowest growth rates and highest concentrations of GSH.

2. Methods

2.1. Site description

The five study sites were located on the US Department of Energy's Savannah River Site (SRS) near Aiken, SC (Fig. 1). The contaminated study stream, Beaver Dam Creek, is located near a CFPP that has been in operation since 1952. Discharge from the CFPP is the only known point source of pollution to the stream. Fly and bottom ash from the plant travel through two settling basins, which empty into a 2 ha lentic habitat that forms the headwaters of Beaver Dam Creek, which ultimately empties into the Savannah River. Combined water residence time in the series of basins is approximately 2 months (Sandhu et al., 1993). Four sites were along Beaver Dam Creek at varying distances from the settling basins and are referred to as Sites A–D. Site A was immediately downstream (< 5 m) of the outfall from the lentic habitat; Site B was approximately 400 m downstream from the outfall; Site C was 1400 m downstream from the outfall; and Site D was approximately 3.2 km downstream from the outfall. The fifth site was a reference stream, Meyers Branch, a historically unimpacted blackwater stream on the SRS set-aside for ecological research; it drains approximately 5085 ha of predominately forested land (McArthur and Tuckfield, 2000).

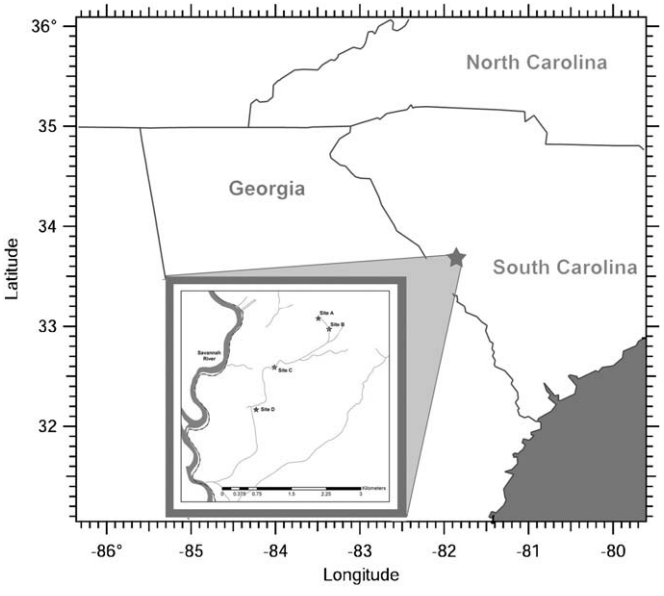

Fig. 1. Map of study sites in Beaver Dam Creek.

2.2. Experimental design

On the day before the transplant experiment began, approximately 400 clams (*C. fluminea*) were collected from the reference site, Meyers Branch. Clams were kept in aerated site water, returned to the laboratory, and each was labeled with a unique glue-on shellfish tag (Hallprint, Adelaide, South Australia). Shell length, width, depth, and total wet weight measurements were recorded. Initial trace element and GSH concentrations were determined using 16 clams (17.49 ± 0.37 mm length) from this collection. At each site, four plastic boxes ($31 \times 18 \times 11$ cm^3) with holes drilled along the sides were secured with aluminum poles. Sediment surrounding the cages at each site was used to fill the plastic boxes and provide a habitat for the clams. Twenty clams from the reference site were added to each cage at all five study sites. After adding sediment from the site and clams from the reference site, cages were covered on the top with netting (1 cm mesh) to prevent loss of clams and sediment. Cages were used to contain clams and allow repeated sampling over the study period and were not designed as units of replication. Cage locations were chosen to minimize differences in sediment particle size, depth, and flow conditions among the five study sites.

Clams were placed in cages from May 19 to 20, 2004, and on these dates, we collected resident clams to quantify the differences in trace element concentrations among sites at the beginning of the experiment. Clam collection dates occurred 28, 56, and 84 days after transplantation. On each date, we randomly selected two clams from each of the four cages for a total of eight clams from each site for trace element analysis. At Site A and Meyers Branch, we collected an additional two clams from each cage for a total of eight clams at each date for GSH analysis. We chose to conduct the GSH analysis on clams from the two sites with the greatest differences in trace element concentrations. Clams were weighed and measured (length, width, and depth). Using the formula for an ellipsoid, we calculated shell volume using the length, width, and depth measurements. The relationship between tissue (wet and dry) mass and calculated volume was significant ($p = 0.0000$) and is presented in the Supplemental Information. We then calculated condition index (g dry tissue mass/cm^3 volume) in transplanted clams among the study sites on all dates (Lawrence and Scott, 1982). Condition index is a measure of physiological health with high values reflecting good physiological health (Lawrence and Scott, 1982). On each sampling date, we also collected water and sediment from all sites, and resident clams from Site A. These clams provided a comparison to the accumulation patterns, growth, and biomarker responses in transplanted clams at the site closest to the power plant.

2.3. Water quality and sediment analyses

In addition to trace element analyses of water and sediment (described below), we also measured general water quality and sediment characteristics. Temperature, conductivity, pH, and dissolved oxygen were measured with a YSI sonde (Yellow Springs, OH). Water samples were filtered in the field with pre-ashed (1 h at 500 °C) glass fiber filters (Whatman GFF). The filtrate was analyzed for dissolved organic carbon (DOC), which was measured using a total carbon analyzer (Sievers Model 800 Turbo, Boulder, CO). Unfiltered water samples were refrigerated prior to analysis of alkalinity, hardness, and turbidity with Hach© titration kits and a Hach© turbidimeter (HACH Company, Loveland, CO).

Three 1-liter sediment samples were collected from each study site at the completion of the experiment to quantify the particle size distribution at each site. Sediments were dried (5 days, 60 °C) and sieved to separate particle size classes. All particles that passed through the 2 mm sieve were further separated into sand, silt, and clay size classes using the hydrometer method (Gee and Bauder, 1986). Additional dried sediment samples from the 28, 56, and 84 sampling dates at each study site were ashed at 460 °C for 8 h to determine their organic matter content.

2.4. Trace element sampling and analysis

Water samples were filtered in the field into acid-washed polyethylene bottles using GHP Acrodisc GF 25 mm syringe filters with GF/0.45 μm GHP membranes (Pall Life Sciences, East Hills, NY, USA) and acidified with trace-metal grade nitric acid prior to freezing. Field blanks using deionized–distilled water were treated in the same manner. Three replicate grab samples of sediment from each site were

stored in whirl-pak bags, frozen at −70 °C, and freeze dried. Clams being analyzed for trace elements were held in aerated site water for 24 h before freezing at −70 °C. After thawing, tissues were dissected, freeze dried, and homogenized.

Approximately 20–60 mg dry mass (DM) of tissues and 100 mg of sediments were used for digestion. Trace-metal-grade nitric acid (2.5 ml for tissues and 5 ml for sediments) was added to the samples prior to digestion in a microwave (CEM Corporation, Matthews, NC, USA) with heating steps of 60%, 60%, 70%, and 80% microwave power for 10, 10, 15, and 20 min, respectively. After digestion with nitric acid, 0.5 ml of trace-metal-grade hydrogen peroxide was added to the samples, which were microwaved for the same duration as the nitric acid digestion. Samples were brought to a final volume of 10 ml (tissues) or 25 ml (sediments) with deionized–distilled water. Trace element analyses of Ni, Cu, Zn, As, Se, Cd, and Hg were performed using an inductively coupled plasma mass spectrometer (ICP-MS; Perkin Elmer, Norwalk, CT) following previously published methods (Hopkins et al., 2004a). Certified reference materials with similar matrices (Riverwater, Tort 2, MESS; NRC, Ottawa, Canada) and blanks were included in the digestion and analysis procedure. Mean percent recoveries for trace elements in tissues and water reference material ranged from 100 to 125%, whereas sediment recoveries ranged from 77 to 124%. Sediment recoveries exceeded 125% for Se and Hg; so concentrations are not reported for these two elements. Concentrations were expressed as μg/L for water samples and μg/g DM for tissues and sediments.

2.5. Glutathione assay

Digestive glands from transplanted and resident clams were immediately dissected after returning from the field and stored at −70 °C until analysis. Total GSH concentrations in transplanted clams from Site A and Meyers Branch were measured using the glutathione reductase recycling assay (Anderson, 1985). The glands were weighed, homogenized in 5% sulfosalicyclic acid, and centrifuged (14,000 rpm, 4 °C, 5 min). A 25 μL subsample of the supernatant was added to 700 μL of sodium phosphate buffer (which contained β-NADPH), 100 μL of 10 mM 5,5′–dithio-bis(2-nitrobenzoic acid), and 175 μL of deionized–distilled water for a total volume of 1 mL. Samples were vortexed and warmed to 30 °C in a water bath for 10 min. Glutathione reductase (15 μL at 50 units/ml) was added to initiate the enzymatic reaction and the formation of 5-thionitrobenzoic acid was measured spectrophotometrically at 412 nm every 30 s for a total of 120 s. GSH concentrations were expressed as nmol/g wet mass.

2.6. Statistical analyses

All tissue trace element and GSH concentrations are reported as means from eight individuals with standard errors. We initially compared tissue concentrations of each trace element in transplanted *C. fluminea* among the five study sites using analysis of variance (ANOVA) testing of the effect of site, cage, time, and interactions among these variables. Because cage effects were not significant at any of the sites ($p>0.20$ in all cases), we report and compare mean tissue concentrations of eight clams at each study site rather than cage means. We used one-way ANOVAs followed by Bonferroni and Scheffe tests to compare differences in mean trace element concentrations in tissues among all study sites as well as comparing tissue concentrations in resident and transplanted clams from Site A. One-way ANOVAs followed by Bonferroni and Scheffe tests were also used to compare mean GSH concentrations in clams between Meyers Branch and Site A at 28, 56, and 84 days. For both the trace element and GSH analysis, when the Bonferroni and Scheffe results differed, we report the significance level at which they agree. Differences in shell growth rate of clams among the five study sites were determined using analysis of covariance (ANCOVA), where the length versus time relationship was compared among sites. All analyses used a p-value <0.05 and were run using JMP 5.0.1 (SAS Institute, Cary, NC, USA), SAS 9.1 (SAS Institute, Cary, NC, USA), or STATA Release 10 (College Station, TX, USA).

3. Results

3.1. Water quality and sediment analyses

Among all the study sites, the greatest differences in mean conductivity, temperature, alkalinity, and hardness were between Meyers Branch (reference) and Site A (upstream, contaminated; Table 1). Site A had the highest specific conductance (0.293 ± 0.003 mS/cm), hardness (38.00 ± 1.83 mg $CaCO_3$/L), and temperature (30.94 ± 1.64 °C), and Meyers Branch had the lowest specific conductance (0.060 ± 0.002 mS/cm) and temperature (22.48 ± 0.61 °C) (Table 1). Sediment particle size was dominated by <2 mm size class at all study sites, and sand comprised >90% of this size class. Sediment organic matter content was 1.76% in Meyers Branch and ranged from 0.36% at Site A to 2.32% at Site D in Beaver Dam Creek.

Mean dissolved concentrations of trace elements showed distinct differences among the study sites (Table 2 and Supplemental Information), but these differences were not evident in the sediments (Table 3 and Supplemental Information). Dissolved concentrations of Ni, As, Se, and Cd were significantly higher at Site A, and concentrations were the lowest at Meyers Branch (Table 2). Concentrations of Cu at all sites in Beaver Dam Creek

Table 3
Sediment concentrations of trace elements (mean ± 1SE) at each study site.

Site	Ni	Cu	Zn	As	Cd
Meyers	3.98 ± 2.96	4.36 ± 3.17	58.20 ± 12.50	1.33 ± 0.93	0.02 ± 0.02
Site A	2.46 ± 0.48	3.70 ± 0.56	17.62 ± 1.26	1.41 ± 0.27	0.08 ± 0.04
Site B	10.39 ± 3.11	14.12 ± 5.08	39.09 ± 5.74	4.44 ± 1.38	0.09 ± 0.03
Site C	3.59 ± 0.64	5.01 ± 1.29	52.82 ± 8.60	1.21 ± 0.65	0.11 ± 0.09
Site D	2.02 ± 0.16	2.33 ± 0.33	33.56 ± 15.50	0.56 ± 0.05	0.02 ± 0.00

Concentrations expressed as μg/g dry mass. $N=4$ sampling dates. Significant differences ($p<0.05$) are denoted by letters.

Table 1
Mean water quality parameters ($N=4$ dates) at the five study sites over the 3-month study period.

Study site	Temperature (°C)	pH	Specific conductance (mS/cm)	Dissolved oxygen (mg/L)	Alkalinity (mg $CaCO_3$/L)	Hardness (mg $CaCO_3$/L)	Turbidity (NTU)
Meyers Branch	22.48 ± 0.61	7.28 ± 0.25	0.06 ± 0.00	7.48 ± 0.36	23.63 ± 1.56	23.63 ± 0.99	3.59 ± 0.92
Site A	30.94 ± 1.64	7.43 ± 0.22	0.29 ± 0.00	7.29 ± 0.48	16.80 ± 1.88	38.00 ± 1.83	1.91 ± 0.22
Site B	27.76 ± 0.63	7.25 ± 0.16	0.14 ± 0.01	7.02 ± 0.44	26.50 ± 2.87	20.50 ± 0.65	4.56 ± 0.72
Site C	27.40 ± 0.43	7.27 ± 0.19	0.14 ± 0.01	7.07 ± 0.53	24.03 ± 1.14	20.19 ± 0.94	6.74 ± 1.68
Site C	27.08 ± 0.47	7.33 ± 0.21	0.14 ± 0.01	7.00 ± 0.38	26.20 ± 3.59	21.19 ± 0.90	7.00 ± 1.60

Table 2
Dissolved concentrations of trace elements (mean ± 1SE) at each study site.

Study site	Ni	Cu	Zn	As	Se	Cd
Meyers	BDL	0.35^{b} ± 0.11	26.96 ± 2.12	0.21^{b} ± 0.04	0.27^{b} ± 0.05	BDL^{b}
Site A	2.68^{a} ± 0.39	2.53^{a} ± 0.40	18.72 ± 3.79	18.31^{a} ± 3.14	2.76^{a} ± 0.15	0.13^{a} ± 0.03
Site B	0.58^{b} ± 0.12	1.91^{a} ± 0.24	28.49 ± 3.07	1.81^{b} ± 0.44	0.56^{b} ± 0.09	BDL^{b}
Site C	0.58^{b} ± 0.16	1.84^{a} ± 0.23	29.05 ± 3.86	1.69^{b} ± 0.33	0.57^{b} ± 0.07	0.04^{b} ± 0.02
Site D	0.58^{b} ± 0.16	1.52^{a} ± 0.10	25.98 ± 1.17	1.66^{b} ± 0.40	0.51^{b} ± 0.11	0.04^{b} ± 0.02

Concentrations expressed as μg/L. $N=4$ sampling dates. Significant differences ($p<0.05$) are denoted by letters. BDL = below detection limit.

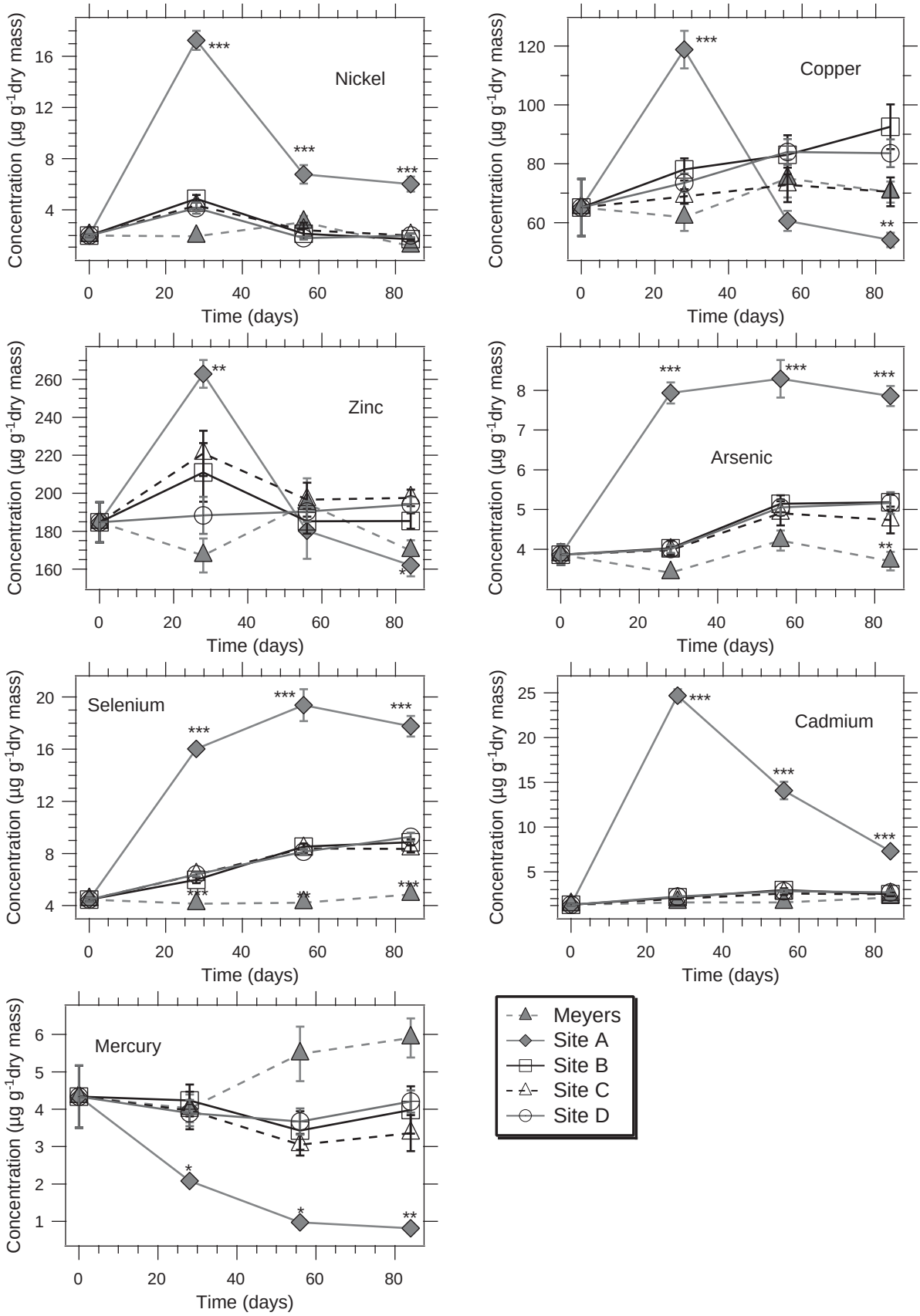

Fig. 2. Tissue concentrations expressed as µg/g dry mass (mean ± 1SE) of Ni, Cu, Zn, As, Se, Cd, and Hg in transplanted *Corbicula fluminea*. Filled triangles represent the Meyers Branch (reference) site, filled diamonds represent Site A, and open symbols represent sites B–D. Symbols are often larger than standard error bars. Results of the ANOVA comparison at each time point are shown: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

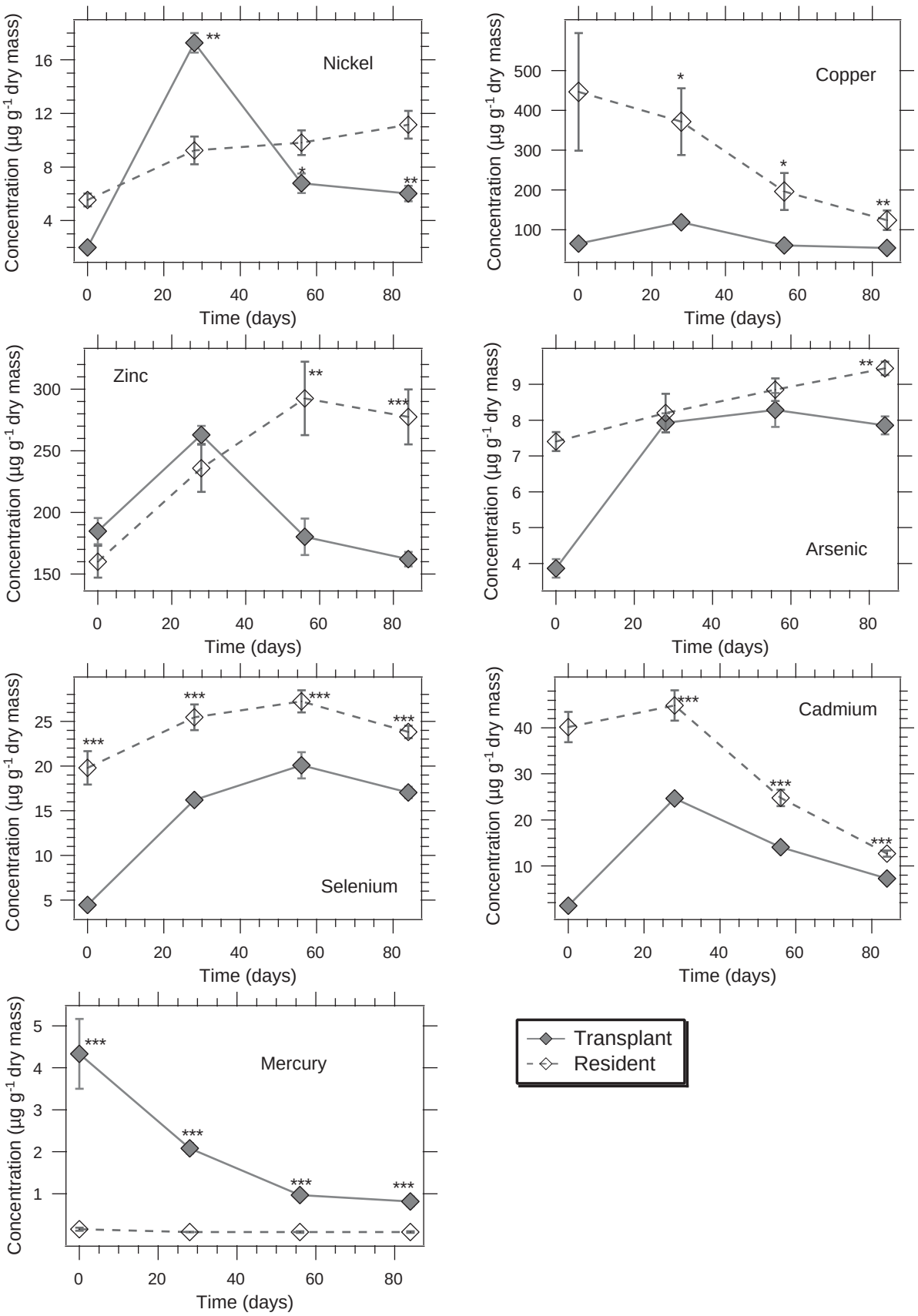

Fig. 3. Tissue concentrations expressed as µg/g dry mass (mean ± 1SE) of Ni, Cu, Zn, As, Se, Cd, and Hg in transplant and resident *Corbicula fluminea* at Site A. Filled diamonds represent transplanted clams and open diamonds represent resident clams. Symbols are often larger than standard error bars. Significance levels are shown: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

were significantly higher than concentrations in Meyers Branch (Table 2). Dissolved Zn concentrations did not differ among sites (Table 2).

3.2. Transplanted clams

Concentrations of Ni, As, Se, and Cd in the tissue of transplanted clams were significantly greater ($p<0.05$) at Site A compared to all other sites throughout the study (Fig. 2). Hg concentrations in the tissues of transplanted clams at Site A decreased during the experiment and, by the end of the study, were significantly lower ($p<0.01$) than at all other sites (Fig. 2). Very few significant differences in trace element concentrations were detected among transplanted clams at Sites B–D (Fig. 2). Ni, Cu, As, Se, and Cd tissue concentrations in transplanted clams were highly correlated with dissolved concentrations ($r>0.60$, $p<0.005$). However, these correlations were driven by elevated tissue and dissolved concentrations in clams at Site A; when this site was removed from the analysis, none of the correlations were significant. Graphs for As and Se are presented in the Supplemental Information section.

3.3. Comparison between transplanted and resident clams

Trace element concentrations in resident clams at Site A were significantly different than transplanted clams throughout the study (Fig. 3). Ni, Cu, Zn, As, Se, and Cd concentrations were significantly higher in resident clams at the completion of the study (Fig. 3). In contrast, despite significant declines, Hg concentrations in transplanted clams at Site A were significantly higher than resident clams at the same site throughout the study (Fig. 3). Even though there were differences in total concentrations at Site A, temporal trends in tissue concentrations of As, Se, and Cd in resident and transplanted clams were similar within this site (Fig. 3). For example, Cd concentrations in both transplanted and resident clams spiked at day 28, although the total concentration was 20 μg/g DM higher in the residents (Fig. 3).

3.4. Growth and stress responses

Clam mortality was less than 1% during the study. Clams from Site A had the highest growth rate (0.040 mm/day) and condition index (0.006 g DM/cm^3, day 84). Clam growth rate was significantly higher at Site B (0.011 mm/day) than at the reference site (−0.001 mm/day), but significantly lesser than at Site A (0.040 mm/day). However, water temperatures varied significantly among sites ($p=0.0001$) and log[growth rate] increased with mean temperature across all sites ($r^2=0.68$, $p=0.0001$). The graph of log[growth rate] and mean temperature is provided in the Supplemental Information. GSH concentrations in transplanted clams were significantly higher at Site A than at Meyers Branch at the completion of the study (Fig. 4).

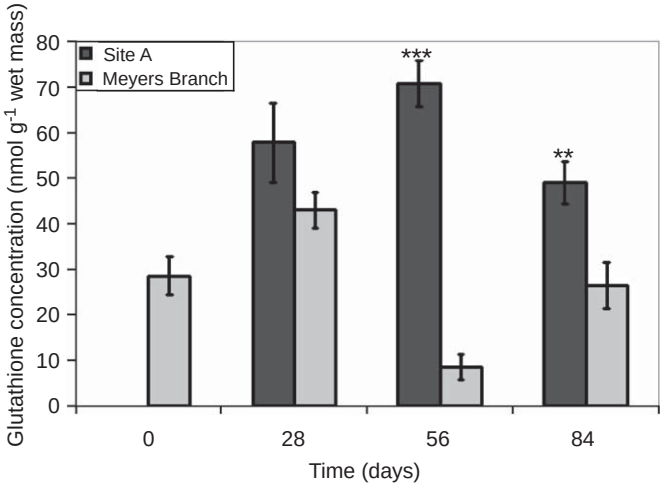

Fig. 4. Glutathione concentration (mean ± 1SE) in transplanted clams from Meyers Branch and Site A. Black bars represent Site A and light gray bars represent Meyers Branch. Significance levels are shown: $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

4. Discussion

4.1. Patterns in dissolved concentrations

Dissolved trace element concentrations differed significantly between Site A in Beaver Dam Creek and Meyers Branch. However, both water and tissue concentrations at the three other sites in Beaver Dam Creek (B–D) were significantly lower than at Site A (Table 2 and Fig. 2), which suggests that rapid dilution of contaminants is occurring downstream from the power plant. The highest dissolved concentrations of Ni, Cu, Zn, and Cd at Site A occurred on day 0, which suggests increased delivery of trace elements to the site at the beginning of the experimental period. Strong correlations between tissue and dissolved concentrations were driven by elevated concentrations at the most contaminated site and were not significant when the site was removed from the analysis. We suspect that dietary sources of trace elements likely contributed more than dissolved sources to accumulation in whole body tissue as demonstrated by copper uptake in *Corbicula sp.* (Croteau and Luoma, 2005; Croteau et al., 2004).

4.2. Patterns in tissue concentrations

Tissue concentrations of Ni, Cu, Cd, and Zn reached maximum levels during the first 28 days of the experiment (Fig. 2), suggesting that rapid accumulation occurred after introduction into the contaminated environment. After Cu and Cd concentrations peaked at day 28, they declined for the remainder of the experiment in both transplanted and resident clams at Site A (Fig. 3), which contrasted with the consistent elevated concentration demonstrated by As and Se concentrations (Figs. 2 and 3). The spike in concentration may be a result of trace elements entering the food web upstream of Site A before day 28 because of increased discharges of trace elements associated with a period of elevated precipitation and stream discharge preceding this sampling date (Wright et al., 2006).

Clams from the reference site had high tissue concentrations of total Hg. When these clams were transplanted into the Se-rich environment of Site A, Hg concentrations declined while Se concentrations increased (Fig. 2). Hg concentration in clams transplanted to Sites B–D also declined, but not to the extent observed at Site A (Fig. 2). The declining Hg and increasing Se concentration in the transplanted clams may be simple depuration of Hg over the course of the experiment, but it is more likely a demonstration of the antagonistic relationship between Se and Hg that has been observed in other studies (e.g., Chen et al., 2001; Hamilton, 2004; Southworth et al., 2000). The ameliorating effects of Se in Hg-contaminated environments have been documented since the early 1980s (Rudd et al., 1980; Turner and Rudd, 1983). However, Se amelioration of Hg contamination has limited utility when Se water concentrations exceed 3 μg/L. Above this concentration, Se can bioaccumulate in the food web and become toxic to aquatic organisms (Hamilton, 2004). Several mechanisms may explain the Se–Hg relationship: increased competition for binding

Table 4
Mean trace element concentrations (μg/g dry weight) in *Corbicula fluminea* from Savannah River Site (day 84, resident and transplant, $N = 8$) and three Chattahoochee River coal-fired power plant sites* ($N = 9$).

	Ni	Cu	Zn	As	Se	Cd	Hg
SRS-Resident	$11.15^{a} \pm 1.04$	$123.66^{a} \pm 24.31$	$277.48^{c} \pm 22.34$	$9.45^{a} \pm 0.19$	$23.81^{a} \pm 0.68$	$12.64^{a} \pm 0.65$	$0.09^{b} \pm 0.02$
SRS-Transplant	$6.02^{b} \pm 0.58$	$54.13^{bc} \pm 2.45$	$161.99^{d} \pm 5.77$	$7.85^{b} \pm 0.25$	$17.75^{b} \pm 0.80$	$7.28^{b} \pm 0.34$	$0.81^{a} \pm 0.11$
Plant#1	$1.30^{c} \pm 0.05$	$61.24^{bc} \pm 3.34$	$509.52^{a} \pm 35.73$	$2.26^{e} \pm 0.10$	$4.53^{e} \pm 0.19$	$2.04^{c} \pm 0.23$	$0.12^{b} \pm 0.01$
Plant#2	$1.85^{c} \pm 0.26$	$40.39^{c} \pm 1.93$	$543.96^{a} \pm 17.65$	$5.03^{c} \pm 0.17$	$9.80^{d} \pm 0.26$	$2.30^{c} \pm 0.09$	$0.13^{b} \pm 0.00$
Plant#3	$1.33^{c} \pm 0.09$	$87.73^{ab} \pm 7.99$	$380.75^{b} \pm 27.67$	$3.50^{d} \pm 0.12$	$13.60^{c} \pm 0.92$	$3.61^{c} \pm 0.91$	$0.17^{b} \pm 0.01$

Significant differences ($p < 0.05$) from ANOVA tests are denoted by letters.
* From Peltier (2006) and Peltier et al. (2008).

sites between Se and Hg, formation of Hg–Se complexes, or increased activity of glutathione peroxidase (Belzile et al., 2006). We observed increased GSH concentration in clams from Site A.

4.3. Growth and stress responses

The difference in growth rate among the study sites was unanticipated. We expected that the lowest growth rate and condition index would occur at the site with the highest concentrations of trace elements as has been observed in other freshwater bivalves (Couillard et al., 1995). However, unanticipated warm temperatures at site A confounded the comparison among sites. Elevated temperatures stimulate growth rates in bivalves (Cataldo and Boltovskoy, 1998; McMahon, 1991). In 1-year-old bivalves, a 17 °C decrease in water temperature was associated with a seven-fold decrease in growth rate (Cataldo and Boltovskoy, 1998). Comparisons of growth and condition could have further been confounded because clams at Site A may also have had more plentiful food resources than the other study sites. *C. fluminea* downstream of a swamp, a situation similar to the swamp that precedes Beaver Dam Creek, had higher condition indices than clams from upstream environments likely because of higher seston concentrations (Kesler, 2004).

GSH concentrations can signal that oxidative stress is occurring in response to metal or organic contaminant exposures (Stohs and Bagchi, 1995). Differences in GSH concentration between Site A and Meyers Branch suggest that defense mechanisms were induced (Irato et al., 2003; Lehmann et al., 2007) in response to oxidative stress associated with metal exposure. Many studies show that GSH concentrations change in response to contaminant exposure (e.g., Canesi et al., 1999; Hoffman, 2002; Irato et al., 2003; Lehmann et al., 2007; Ringwood et al., 1999; Yan et al., 1997). Trace element concentrations may be high enough to initiate detoxification processes as demonstrated by increased GSH concentrations, but not enough to reduce growth or reproduction. Other variables such as warmer water temperatures and increased food resource availability at Site A may have diminished the negative effects of elevated trace element concentrations.

4.4. Contamination at other coal-fired power plants

Trace element accumulation in aquatic organisms downstream of CFPP discharges is a function of several factors, including river discharge, proximity of other CFPP discharges in a river, and energy generation capacity. Trace element concentrations of Ni, As, Se, and Cd in the tissues of clams from Site A at the SRS were significantly higher than concentrations found in clams below CFPP discharges in the Chattahoochee River in Atlanta, GA (Table 4). As and Se concentrations in resident clams from this study were 9 and 24 μg/g DM, respectively, compared to 5 and 14 μg/g DM from one of the Chattahoochee sites (Table 4). Approximately 12 km downstream of the CFPP discharges in the Chattahoochee River and clam collection sites, dissolved concentrations of As and Se were still elevated compared to sites upstream of CFPP discharges (3.07 and 1.41 μg/L, respectively; Lesley and Froelich, 2003). This suggests that elevated concentrations of trace elements can extend far downstream from power plant discharges despite dilution in the Chattahoochee River, which receives much higher discharge from industry than the streams on the SRS. In Beaver Dam Creek, we observed evidence of dilution (Sites B–D); however the size of the CFPP was considerably smaller than those in the Chattahoochee River (70 versus 1730 MW).

5. Conclusion

This study clearly demonstrated that trace elements associated with coal combustion wastes are bioavailable to benthic organisms in a stream receiving discharge from coal-ash settling basins. As coal combustion continues to be among the most important sources of electricity generation, waste disposal methods that minimize the potential for human and environmental exposure are of paramount importance. This study suggests that the potential exists for trace element accumulation in lotic food webs receiving overflow or discharges from surface impoundments and that the ecological implications of this method of disposal, particularly in rivers and streams, need further study. Although dilution can occur rapidly in downstream sites, this depends on the generation capacity of the power plant and the hydrology of the site. The importance of long-term loading to lotic systems should not be underestimated, especially in downstream habitats with characteristics that favor deposition of trace elements.

Acknowledgments

We thank Heather Brant, Chuck Jagoe, Brian Jackson, and the Advanced Analytical Center for Environmental Sciences at the Savannah River Ecology Laboratory for assistance in ICP-MS analysis. Field assistance was provided by Sarah DuRant, Davis Harrelson, and John Peterson. We extend special thanks to Marsha Black and Deanna Conners for the use of field equipment and guidance in cage design. G.L.P. was partially funded by a Graduate Research Fellowship under Financial Assistance Award DE-FC09-96SR18-546 from the University of Georgia and the US Department of Energy.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2009.01.011.

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